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2014

NORM
NORM-VET

Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway

Norsk overvåkingssystem for antibiotikaresistens hos mikrober (NORM)

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CONTENTS

INTRODUCTION

Antimicrobial resistance is an emerging problem worldwide. It reduces the effectiveness of antimicrobial treatment of infectious diseases in humans and animals thereby leading to increased morbidity and mortality, as well as higher costs. It is well established that there is a strong association between the usage of antimicrobial agents and the occurrence of resistance. The selective pressure exerted following use of antimicrobial agents is a key issue in the epidemiology of resistance. Moreover, resistance can be disseminated through the spread of resistant pathogenic bacteria themselves or by horizontal transfer of resistance genes from one type of bacteria to another. Such transfer is not limited to closely related bacteria; it can also take place between bacteria of different evolutionary origins and/or ecological niches. Thus, antimicrobial drug usage and resistance in one ecological compartment can have consequences for the occurrence of resistance in another compartment. When addressing antimicrobial resistance – the occurrences, causes, consequences and preventive measures – a holistic approach is needed, encompassing both data on usage and resistance in human and veterinary medicine, as well as microbes in food production.

Several countries have implemented monitoring programmes for antimicrobial resistance and usage of antimicrobial agents in recent years. Some programmes focus primarily on human usage and resistance in human pathogens, whereas others also include data concerning veterinary medicine and food production. The EU supported this broad approach in 1998 through the Copenhagen recommendations and at later follow-up conferences. The World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Animal Health Organization (OIE) have through several expert consultations emphasised the importance of a One Health approach to monitoring of antimicrobial drug usage and resistance in both human and veterinary medicine. Several reports and recommendations have been published in this regard including the WHO Global Action Plan adopted at the World Health Assembly in May 2015.

In response to the growing concern about antimicrobial resistance, the Norwegian Ministry of Health and Social Affairs issued a national action plan against antimicrobial resistance in March 2000. Again, the importance of monitoring both the human and veterinary sectors, including food production, was emphasised. The action plan recognised the need for ongoing surveillance as a fundamental component of the strategy for containment of antimicrobial resistance. The NORM and NORM-VET programmes were consequently established in order to provide and present microbiologically and epidemiologically valid data on the occurrence and distribution of antimicrobial resistance over time. The national action plan formally expired by the end of 2004. However, the need for continued surveillance of both resistance and drug usage was emphasised at subsequent consultations and an integrated national strategy for prevention of infections in the health service and antibiotic resistance (2008-2012) was issued in the summer of 2008. Following the renewed effort of the WHO in recent years, a new national action plan for Norway is presently being developed.

The NORM surveillance programme for antimicrobial resistance in human pathogens was established in 1999 and is coordinated by the Department of Microbiology and Infection Control at the University Hospital of North Norway in Tromsø. The NORM-VET monitoring programme for antimicrobial resistance in the veterinary and food production sectors was established in 2000 and is coordinated by the Norwegian Zoonosis Centre at the Norwegian Veterinary Institute. The usage of antimicrobial agents is based on reporting of wholesalers' data for humans and animals, which was made mandatory from January 1st 2002, as well as human prescription data reported to the Norwegian Institute of Public Health. Data on the usage of feed additives, i.e. coccidiostatic growth promoters, are collated at the Norwegian Food Safety Authority.

This report, which is the fifteenth annual joint report from NORM and NORM-VET, presents data for 2014. In addition to resistance data, the NORM/NORM-VET reports present data on the usage of antimicrobial agents in humans and animals in Norway. The present report, together with earlier reports and reports from the years to come, form a basis for the detection, interpretation and evaluation of trends regarding usage of antimicrobial agents and occurrence of resistance in Norway. The NORM and NORM-VET programmes are valuable tools for setting policies, assessing risks and evaluating interventions.

The editors would like to thank all those who contributed to data collection and the writing of this report, for excellent work.

Tromsø / Oslo, September 2015

SAMMENDRAG

Dette er den femtende felles rapporten fra Norsk overvåkingssystem for antibiotikaresistens hos mikrober (NORM) og Norsk overvåkingsprogram for antibiotikaresistens i mikrober fra fôr, dyr og næringsmidler (NORM-VET). Rapporten presenterer data om forekomst av antibiotikaresistens og forbruk av antibiotika til mennesker og dyr i 2014. Data fra relevante prosjekter som ikke er med i de kontinuerlige overvåkingsprogrammene, presenteres også.

Både NORM og NORM-VET programmene ble etablert som deler av Regjeringens tiltaksplan mot antibiotikaresistens som ble offentliggjort i 2000. NORM koordineres av Avdeling for mikrobiologi og smittevern, Universitetssykehuset Nord-Norge i Tromsø. NORM-VET koordineres av Zoonosesenteret ved Veterinærinstituttet i Oslo. Programmene har et godt samarbeid og utgir en felles årsrapport.

Forbruk av antibiotika til dyr

Forbruket av antibiotika i norsk husdyrproduksjon og akvakultur er lavt. Totalsalget av veterinære antibiotika til terapeutisk bruk på landdyr i 2014 var 5927 kg. Fra 1995 til 2014 er salget av veterinære antibiotika til landdyr redusert med 38 %. For preparater som nesten utelukkende benyttes til produksjonsdyr (landdyr), er reduksjonen i denne perioden på 41 %, mens salget av veterinære antibakterielle preparater som kun brukes til kjæledyr, har økt med 19 % fra 467 til 548 kg.

Forbruksmønsteret til produksjonsdyr har utviklet seg i gunstig retning siden 1995 idet andelen av rene penicillinpreparater har økt fra 25 % i 1995 til 53 % i 2014. Årsaken til dette er redusert forbruk av kombinasjonspreparater med penicillin og dihydrostreptomycin. Siden det første penicillinpreparatet til smådyr kom på markedet i Norge i 1994 har bruk av veterinære penicillinpreparater, i kg, til smådyr økt fra 1 % til 63 % av totalsalget av slike preparater markedsført kun til kjæledyr. Nedgangen i antibiotikaforbruket til produksjonsdyr (landdyr) og endringene i forskrivningsmønsteret skyldes først og fremst at husdyrnæringen i andre halvdel av 1990-tallet gjennomførte systematiske kampanjer for å redusere forbruket av antibiotika til dyr samt for riktig bruk av antibiotika.

Totalsalget av veterinære antibiotika til terapeutisk bruk hos oppdrettsfisk i Norge var i 2014 på 511 kg aktiv substans, hvorav 79 % var amfenikoler. I 2013 var kinoloner den mest solgte antibakterielle klassen til bruk på oppdrettsfisk (76 % av total salget). Forbruket av antibiotika i oppdrettsnæringen er redusert med 99 % siden 1987. Denne betydelige reduksjonen kan tilskrives innføringen av effektive vaksiner til laks og ørret samt andre infeksjonsforebyggende tiltak, herunder bedrede miljøforhold.

Avoparcin ble brukt som antibakteriell vekstfremmer i norsk broiler- og kalkunproduksjon fra 1986 inntil det ble forbudt i 1995. Samme år innførte husdyrnæringene et selvpålagt forbud mot bruk av alle antibakterielle vekstfremmere. Koksidiostatika som fôrtilsetningsstoff brukes fremdeles i norsk fjørfeproduksjon. Narasin har i de senere årene utgjort hovedparten av forbruket. Salgstallene, i kg aktiv substans, er mer enn firedoblet siden forbudet mot bruk av avoparcin trådte i kraft, noe som kan forklares ved økt produksjon av broilere. Forbruksmønstret for koksidiostatika er endret fra monensin til narasin etter 1996.

Forbruk av antibiotika hos mennesker

I 2014 var humant forbruk av antibiotika til systemisk bruk 19,3 definerte døgndoser (DDD)/1000 innbyggere/dag. Dette var en reduksjon i forhold til 2013. Det samlede forbruket har vært forholdsvis stabilt gjennom mange år, men det har skjedd en gradvis økning og en forskyvning mellom de ulike antibakterielle undergruppene. Salget av smalspektret penicillin ble redusert i 2014. I de senere år har bruken av det urinveisantiseptiske middelet metenamin økt kraftig, i 2014 utgjorde metenamin 19 % av totalt salg målt i DDD.

I 2014 utgjorde penicillinene 42 % av det totale antibiotikaforbruket i Norge målt i DDD. Bruken av bredspektrede penicilliner er stabil mens bruk av beta-lactamase sensitive penicilliner går ned. Tetracykliner utgjorde 18 %, mens makrolider og linkosamider utgjorde 9 % av totalt salg i 2014. Salget av cefalosporiner, monobaktamer og karbapenemer utgjør 2 % av totalsalget. De siste to årene har salget av kinoloner vist en nedgang, og de utgjorde i 2014 3 % av totalsalget i DDD.

Rundt 85 % av DDD selges på resept i allmennpraksis. Bruken av antibakterielle midler varierer avhengig av kjønn, alder og bosted. I 2014 utgjorde salget til sykehus 8 % av totalt antibiotikasalg. I sykehus brukes penicilliner i stor grad (48 % av antibiotikasalget målt i DDD til sykehus). Tilsvarende tall for allmennpraksis er 41 %. Den viktigste andre gruppen i 2014 var på sykehus: cefalosporiner (17 %), og i allmennpraksis tetracykliner (20 %).

Resistens hos indikatorbakterier fra dyr

Forekomsten av ervervet antibiotikaresistens blant bakterier som er en del av den normale tarmfloraen, kan være indikator på selektivt antibiotikapress i ulike populasjoner.

I 2014 ble det resistenstestet 205 *Escherichia coli* isolater fra blindtarm hos kylling. Forekomsten av resistente *E. coli* var moderat med 85,4 % av isolatene følsomme for alle de undersøkte antibiotika. Bare 2,5 % av isolatene var multiresistente. Siden starten av NORM-VET i 2000, har det vært en reduksjon i forekomsten av resistens mot flere forskjellige typer antibiotika, og i et internasjonalt perspektiv er forekomsten av resistens i *E. coli* fra norske kyllinger relativt lav. Denne gunstige situasjonen skyldes trolig en svært begrenset bruk av antibiotika i norsk slaktekyllingproduksjon.

Ved bruk av en selektiv metodikk ble det påvist utvidet ekstendert spektrum beta-laktamase (ESBL) produserende *E. coli* i 35,5 % av blindtarmsprøvene og i 28,9 % av kyllingfiletene. Alle isolatene hadde en AmpC fenotype og var bærere av bla_{CMY-2} genet. Kvantifisering utført på samme prøvemateriale indikerer at andelen av *E. coli* som er ESBL/AmpC-produserende er svært lav i majoriteten av prøvene. Tilsvarende er det i de fleste tilfeller meget lav grad av kontaminering av ESBL/AmpC-produserende *E. coli* i de positive kyllingfiletene, selv om noe variasjon kan forekomme.

Ved bruk av selektiv metodikk ble det påvist kinolonresistente *E. coli* i 89,5 % av blindtarmsprøvene og

i 70,7 % av kyllingfiletene. Majoriteten av isolatene var kun resistente mot kinolonene nalidixinsyre og ciprofloxacin, mens hhv. 12,8 % og 18,6 % var resistente mot ytterligere ett antibiotikum, og 10,7 % og 9,3 % var resistente mot ytterligere to eller flere antibiotika. Alle de kinolonresistente isolatene hadde en resistensprofil som indikerer at resistensen sannsynligvis skyldes ikke overførbare kromosomale mutasjoner. Selv om man ved selektiv metodikk påviser kinolonresistente *E. coli* i majoriteten av prøvene, påvises det vanligvis bare noen få isolater ved ikke-selektiv metodikk. Dette indikerer at det generelt er en lav andel av kinolonresistente *E. coli* i prøvene. Resultatet var likevel noe overraskende, siden det ikke er selektivt press pga bruk av kinoloner i norsk broilerproduksjon. Det er ukjent når, hvorfor og hvordan denne kinolonresistensen har oppstått hos broilere og hvilken betydning den eventuelt har.

Enterococcus faecalis og *E. faecium* fra 65 kyllingflokker og 143 kyllingfileter ble resistenstestet. Det var en meget høy forekomst av resistens hos *E. faecalis* og en høy forekomst av resistens hos *E. faecium.* Totalt var 32,3 % av *E. faecalis* og 76,2 % av *E. faecium* følsomme for alle antibiotika inkludert i testpanelet. Resistens mot ett (hovedsakelig tetracyklin) og to antimikrobielle midler (hovedsakelig tetracyklin og erytromycin) ble påvist i hhv. 60 % og 6 % av *E. faecalis*. I tillegg var 1,5 % av *E. faecalis* resistente mot tre antimikrobielle midler. Blant *E. faecium* ble resistens mot ett (hovedsakelig tetracyklin eller erytromycin) og to antimikrobielle midler påvist i hhv. 21,7 % og 2,1 % av isolatene.

Ved bruk av selektiv metodikk ble det påvist vankomycinresistente enterokokker (VRE) i prøver fra 6,7 % av kyllingflokkene. Alle isolater ble identifisert som *E. faecium* og bærere av *vanA* genet. Dette er en betydelig reduksjon i forhold til forekomsten i 2011 og er på tilsvarende nivå som i 2009. Imidlertid har prøvetakings-metoden blitt endret og sammenligning av resultatene bør derfor gjøres med forsiktighet.

Resistens hos zoonotiske bakterier og andre enteropatogene bakterier

Zoonosebakterier isolert fra dyr

I 2014, ble det undersøkt totalt 25 *Salmonella* spp. isolater fra dyr. Av disse var 13 isolater fullt følsomme, seks isolater var resistente mot ett antibiotikum, ett for to antibiotika, og tre for tre antibiotika. Ett isolat fra en storfebesetning var resistent mot fem antibiotika, mens ett isolat fra en kyllingflokk var resistent mot syv av de antimikrobielle midlene det ble testet for.

I løpet av våren 2014, ble totalt 986 svinebesetninger undersøkt for meticillinresistente *Staphylococcus aureus* (MRSA) i en egen undersøkelse. MRSA ble påvist i dyre- og miljøprøver fra kun én av disse flokkene. Isolatene ble typet som CC398, t011. I tillegg ble én kontaktbesetning identifisert med MRSA CC398, t011. I begge besetningene ble det gjennomført slakting av dyr, grundig rengjøring og desinfeksjon av dyrerom etc. før oppstart med MRSA-frie griser. Framgangsmåten er i tråd med tidligere strategi for MRSA i svinebesetninger.

Kliniske isolater av tarmpatogene bakterier fra mennesker

For kliniske *Salmonella* isolater fra mennesker sett under ett var forekomsten av multiresistens (MDR) på litt over 10 %, mens forekomsten av ESBL holdt seg under 2 %. Når det gjelder blodkulturisolater (n=65), var forekomsten av MDR høyest for *Salmonella* spp. (alle serovarer unntatt *S.* Typhi, Paratyphi, Typhimurium og Enteritidis). Forekomsten av resistens var høyere for flere antibiotika i *S.* Typhimurium-gruppen (inkludert *S. enterica* serovar 4,[5],12:i:-) enn hos andre *Salmonella* serovarer. Forekomsten av resistens mot tetracyklin og ampicillin er også økende i denne gruppen, både for innenlandssmittede og for pasienter smittet i utlandet.

Når det gjelder *Campylobacter* er det økende resistens mot tetracyklin og kinoloner hos isolater ervervet ved innenlandssmitte, men forekomsten er fortsatt betydelig lavere enn for utenlandssmittede isolater. De fleste tilfeller av *Shigella* infeksjoner i Norge kan knyttes til smittekilder i utlandet. Antibiotikaresistens var følgelig utbredt hos *Shigella* isolater, spesielt hos *S. flexneri,* i likhet med det som rapporteres fra andre land. MDR hos *S. flexneri* ligger på ca 70-80 % og hos *S. sonnei* ca. 40 %. ESBL hos *Shigella* er foreløpig uvanlig med en forekomst på knappe 5 % i 2013 og vel 7 % i 2014. Det er også en tendens til økende resistens mot kinoloner hos *Shigella.*

Antibiotikaresistens hos *Yersinia enterocolitica* ligger stabilt lavt, bortsett fra artens naturlige resistens mot ampicillin.

Resistens hos kliniske isolater fra mennesker

Forekomsten av antibiotikaresistente kliniske bakterie-isolater fra mennesker var meget lav i 2014. Det ble påvist ni tilfeller av meticillinresistente *Staphylococcus aureus* (MRSA) blant de 1163 blodkulturisolatene (0,8 %) som ble inkludert i NORM-protokollen. Dette samsvarer godt med tall fra laboratorienes datasystemer som rapporterte at 24 av 1720 (1,4 %) *S. aureus* fra blodkultur og spinalvæske var MRSA. Andelen er på samme nivå som i 2012 (1,0 %) og 2013 (1,0 %). Meldesystemet for infeksjonssykdommer (MSIS) registrerte 832 tilfeller av MRSA infeksjon i 2014 mot 575 i 2012 og 659 i 2013. De fleste tilfellene var pasienter med sårinfeksjoner og abscesser. MRSA utgjør fortsatt en svært liten andel av *S. aureus* isolater fra sårprøver (12 av 955, 1,3 %) hvilket er på samme nivå som i 2012 (0,7 %) og 2013 (1,2 %). MSIS registrerte videre 1035 tilfeller av MRSA kolonisering i 2014 mot 823 i 2013. Det totale antallet MRSA meldinger økte dermed fra 1482 i 2013 til 1867 i 2014 (+26 %). Overvåkingen viser at det totale antallet personer med påvist infeksjon eller kolonisering med MRSA fortsetter å øke, men at antallet med alvorlige infeksjoner foreløpig er stabilt på et lavt nivå. Forekomsten av fusidinresistens blant *S. aureus* isolater fra sårprøver holder seg stabil og utgjør 8,6 % sammenliknet med 9,0 % i 2013.

Blodkulturisolater av *E. coli* og *Klebsiella* spp. viste økt forekomst av resistens mot bredspektrede antibiotika i 2014. Forekomsten av resistens og nedsatt følsomhet for gentamicin hos *E. coli* var 8,6 % i 2014 sammenliknet med 5,8 % i 2013. Forekomsten av resistens og nedsatt følsomhet for ciprofloxacin i *E. coli* fortsatte å øke til 13,6 % i 2014 sammenliknet med 11,7 % i 2012 og 12,3 % i 2013. Det er en klar samvariasjon mellom forbruket av fluorokinoloner og nedsatt følsomhet for denne antibiotikagruppen. *Klebsiella* spp. hadde lavere forekomst av resistens mot aminoglykosider og fluoro-kinoloner enn *E. coli*.

Produksjon av bredspektrede betalaktamaser (ESBL) er blitt et utbredt problem i mange land, og forekomsten har også vært økende i Norge. Til sammen 95/1645 *E. coli* (5,8 %) og 26/754 (3,4 %) *Klebsiella* spp. fra blodkultur

ble rapportert som ESBL positive. Forekomsten er stabil fra 2012 (5,5 % i *E. coli* og 2,3 % i *Klebsiella* spp.) og 2013 (5,0 % i *E. coli* og 2,8 % i *Klebsiella* spp.). Andelen av ESBL positive isolater var fortsatt høyere blant *E. coli* fra blodkulturer (5,8 %) enn fra urinprøver (3,8 %). Karbapenemaseproduserende Enterobacteriaceae, *Pseudomonas aeruginosa* og *Acinetobacter* spp. har vært meldepliktige til MSIS siden juli 2012. Forekomsten av slike mikrober er fortsatt meget lav i Norge. Det ble i 2014 meldt totalt 33 slike funn til MSIS (6 *K. pneumoniae*, 4 *E. coli*, 2 *Enterobacter* spp., 6 *P. aeruginosa* og 15 *Acinetobacter* spp.).

Blant *Haemophilus influenzae* isolater fra systemiske infeksjoner (n=69) var 13,0 % betalaktamase positive og 13,0 % resistente mot cefuroxim som uttrykk for kromosomal betalaktamresistens. Det ble påvist tilsvarende resistens i isolater fra luftveisprøver. Alle de 13 *Neisseria meningitidis* isolatene fra systemiske infeksjoner hadde nedsatt følsomhet for penicillin G, men de var fortsatt følsomme for andre relevante antibiotika. *Neisseria gonorrhoeae* (n=255) viste nedsatt følsomhet for penicillin G (96,1 %) og azitromycin (37,2 %). Hele 75,7 % var resistente mot ciprofloxacin. Tre isolater (1,2 %) var også resistente mot ceftriaxon.

Det ble påvist tre enterokokkisolater fra blodkultur med klinisk signifikant vankomycinresistens i 2014 (1 VanA og 2 VanB *E. faecium*). Forekomsten av nedsatt følsomhet for ampicillin i *E. faecium* ligger fortsatt på 80-90 %. Høygradig gentamicinresistens ble påvist i 18,7 % av *E. faecalis* og 40,8 % av *E. faecium*, dette er en svak nedgang fra 23,6 % og 46,8 % i 2013. Nesten alle *E. faecium* isolater med høygradig gentamicinresistens (70/71) hadde samtidig nedsatt følsomhet for ampicillin. Det ble ikke påvist linezolidresistente enterokokker i 2014.

Det ble påvist nedsatt følsomhet for penicillin G hos 5,5 % av *Streptococcus pneumoniae* fra blodkultur/spinalvæske og 5,9 % fra luftveisprøver. Dette er på det samme nivået som i 2012 (6,3 %) og 2013 (3,0 %). Ett enkelt blodkultur-isolatisolat var penicillinresistent og hadde samtidig nedsatt følsomhet for cefalosporiner. Forekomsten av makrolidresistens var 4,3 % blant systemiske pneumokokkisolater og 7,6 % blant isolater fra luftveisprøver.

Streptococcus pyogenes (betahemolytiske streptokokker gruppe A) fra blodkultur hadde svakt økende erytromycinresistens (3,7 %) sammenliknet med 2013 (1,9 %). Tilsvarende har forekomsten av makrolidresistens blant *Streptococcus agalactiae* (betahemolytiske streptokokker gruppe B) økt fra 12,0 % i 2012 til 18,1 % i 2014. Alle isolatene var følsomme for penicillin G.

I alt 327 tilfeller av tuberkulose ble meldt til MSIS i 2014. Det ble utført resistensbestemmelse av 265 isolater av *Mycobacterium tuberculosis*. Ti isolater (2,5 %) fra pasienter smittet i henholdsvis Afrika (n=6), Asia (n=2) og Europa utenfor Norge (n=2) ble klassifisert som multiresistente.

Det ble utført resistensbestemmelse av 210 *Candida* blodkulturisolater av ni ulike species. De vanligste artene var *C. albicans* (n=146), *C. glabrata* (n=33), *C. tropicalis* (n=9) og *C. parapsilosis* (n=9). Kun ett gjærsoppisolat hadde nedsatt følsomhet for amfotericin B. Det ble kun påvist enkelte tilfeller av ervervet resistens mot fluconazol, men som forventet ble det påvist høy forekomst av resistens mot azoler hos *C. glabrata*. Tre *C. albicans* hadde nedsatt følsomhet for echinocandiner og ett *C. parapsilosis* isolat var resistent mot anidulafungin. Resultatene er i samsvar med tidligere studier fra Norge.

Konklusjon

Antibiotikaresistens er fortsatt et begrenset problem i Norge når det gjelder bakterier fra både mennesker og dyr. Det lave forbruket av antibiotika og det fordelaktige forbruksmønsteret må opprettholdes for å bevare den gunstige situasjonen. Resultatene som presenteres i denne rapporten, viser at norske strategier for antibiotikabruk og antibiotikaresistens hittil har vært vellykkede både i husdyrholdet og i helsevesenet. Faren for økende resistensproblemer er imidlertid til stede i form av økt antibiotikaforbruk i Norge og import av resistente bakterier fra andre land. Det er derfor nødvendig med fortsatt aktiv innsats for å sikre at vi også i fremtiden kan gi effektiv antibiotikabehandling til dem som trenger det. NORM/NORM-VET-rapporten er et viktig bidrag til arbeidet med å forebygge utvikling og spredning av antibiotikaresistens.

SUMMARY

This is the 15th joint report from the NORM surveillance programme for antimicrobial resistance in human pathogens and the NORM-VET monitoring programme for antimicrobial resistance in animal pathogens and the food production sectors. The report presents data on the occurrence of antimicrobial resistance and the usage of antimicrobial agents in humans and animals for the year 2014. The NORM and NORM-VET programmes were established as part of the Norwegian Government's Action Plan against Antimicrobial Resistance issued in 2000. NORM is coordinated by the Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø. NORM-VET is coordinated by the Norwegian Zoonosis Centre, Norwegian Veterinary Institute, Oslo. Successful collaboration has been established between NORM and NORM-VET and a joint report is issued annually.

Usage of antimicrobial agents in animals

The usage of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in food producing animals in Norway is low. In 2014, the total sales of antimicrobial VMPs for terrestrial animals were 5,927 kg. The annual sales, in kg active substance, of antimicrobial VMPs approved for use in terrestrial animals decreased by approximately 38% from 1995 to 2014. The reduction in use is solely accounted for by a reduction in the use in food producing animals (41% reduction) while for antimicrobial VMPs marketed for companion animals an increase of 19% in the sales is observed. The sales patterns of antimicrobial VMPs for terrestrial food producing animals have gradually become more favourable as the proportion of penicillin use has increased; the proportion accounted for by pure penicillin preparations rose gradually from 25% of total sales in 1995 to 53% in 2014. In this period the sales of aminoglycosides decreased from 27% to 10% of total sales; this is due to a reduction in the use of combination preparations of penicillin and dihydro-streptomycin in food producing animals. The reduced sales of antimicrobial VMPs in terrestrial animals as well as the favourable prescribing patterns are mainly explained by a campaign on prudent use of antimicrobials conducted by the Norwegian husbandry organisations and the Norwegian Medicine Authority during the second part of the 1990s. Furthermore, a target set by the Norwegian husbandry organisations to reduce the sales by 25% with 1995 as the reference year is thought to have had a major impact on this decrease.

In 2014, the total sales of antimicrobial agents for therapeutic use in farmed fish were 511 kg of active substance of which amphenicols accounted for 79%. The sales of antimicrobial VMPs in Norwegian aquaculture declined by approximately 99% from 1987 to 1996 and have thereafter remained relatively constant. This reduction is mainly attributed to the introduction of effective vaccines in salmonids.

In 2014, the total sales of ionophore coccidiostat feed additives, in kilograms of active substance, were more than four times the amounts used prior to the withdrawal of antimicrobial growth promoters in 1995. This is explained by increased production of broilers. While monensin was the most frequently used ionophore in poultry in 1995, the usage of coccidiostats has since then been dominated by narasin.

Usage of antimicrobial agents in humans

In 2014, the overall sales of antibacterials for systemic use in humans were 19.3 defined daily doses (DDD)/1,000 inhabitants/day. This was a reduction of 4% compared to 2013. The total consumption has been relatively stable over many years, although there has been a gradual increase in consumption and a shift among the various subgroups. The sales of narrow spectrum penicillin have dropped. For many years the use of the urinary antiseptic agent methenamine has increased. In 2014, the sales levelled off but still accounted for 19% of total sales, measured in DDDs.

In 2014, penicillins accounted for 42% of total antibiotic human use measured in DDDs. The use of penicillins with extended spectrum was stable while the use of beta-lactamase sensitive penicillins was reduced. Tetracyclines accounted for 18% of total consumption in 2014 while the consumption of macrolides and lincosamides accounted for 9%. Sales of cephalosporins, monobactams and carbapenems constituted 2% of total sales. Over the last two years, quinolone use has declined. This group accounted for only 3% of total consumption in 2014, but sales have more than doubled in 10 years.

Approximately 85% of all DDDs are sold through prescriptions in ambulatory care. The use of antibacterials varies according to gender, age and area of residence. In 2014, sales to hospitals amounted to 8% of total antibiotic sales. Penicillins accounted for around 48% of the sales to hospital and 41% in ambulatory care. The other main group in hospitals was cephalosporins (17%), and in ambulatory care tetracyclines (20%).

Resistance in indicator bacteria from animals

The prevalence of antimicrobial resistance among certain bacteria of the normal enteric microflora can serve as an indicator for the selective antimicrobial pressure in various populations.

In 2014, 205 *Escherichia coli* isolates from broiler caecal samples were included in the surveillance. The prevalence of resistant *E. coli* was moderate with 85.4% of the isolates being susceptible to all antimicrobial agents examined and only 2.5% of the isolates being multiresistant. Since the start of NORM-VET in 2000, the prevalence of resistance to several antimicrobial agents has decreased, and in an international perspective the occurrence of resistance among *E. coli* from Norwegian broiler is quite low. This favourable situation is probably due to the very limited use of antibiotics in Norwegian broiler production.

By use of a selective method, extended-spectrum beta-lactamase (ESBL) producing *E. coli* were detected in 35.5% and 28.9% of broiler caecal samples and broiler meat samples, respectively. All isolates had a beta-lactam resistance profile corresponding to an AmpC phenotype and carried the $bla_{\text{CMY-2}}$ gene. Quantification methods applied to the same sample material indicate that the majority of broiler flocks positive for ESBL/AmpC-producing *E. coli* have very low levels of these bacteria present among caecal *E. coli*. Correspondingly, the levels

of contamination on broiler meat samples are generally very low, though some variation may occur.

By use of a selective method, quinolone resistant *E. coli* was found in 89.5% and 70.7% of broiler caecal samples and broiler meat samples, respectively. A majority of the isolates were resistant only to the quinolones nalidixic acid and ciprofloxacin, though further resistance to one additional antimicrobial agent was observed in 12.8% and 18.6%; and to two or more in 10.7% and 9.3% of the isolates, respectively. All quinolone resistant isolates had MIC profiles indicating that the phenotype was probably mediated by mutations in the bacterial chromosome. Although the selective method detected quinolone resistant *E. coli* in a majority of the samples, only a few isolates were usually detected by the non-selective procedure. This indicates that the within-flock prevalence of quinolone resistant *E. coli* is low. The findings were to some degree surprising, as there is no selection pressure from quinolone use in Norwegian broiler production. It is unknown when, why and how this quinolone resistance has emerged in broilers and what impact this reservoir may have. Further investigations are needed in order to examine these aspects.

Enterococcus faecalis and *E. faecium* from 65 and 143 broiler flocks, respectively, were included in the programme. There was a very high occurrence of resistance among *E. faecalis* and a high occurrence of resistance among *E. faecium* isolates. In total, 32.3% of *E. faecalis* and 76.2% of *E. faecium* were susceptible to all antimicrobial agents included in the test panel. Resistance to one (mainly tetracycline) and two antimicrobial agents (mainly tetracycline and erythromycin) was detected in 60% and 6% of *E. faecalis*, respectively. In addition, 1.5% of *E. faecalis* isolates were resistant to three antimicrobial agents. Among *E. faecium,* resistance to one (mainly tetracycline or erythromycin) and two antimicrobial agents was detected in 21.7% and 2.1% of isolates, respectively.

By use of selective methods, vancomycin resistant *Enterococcus* spp. (VRE) was isolated from 6.7% of the broiler flocks. All isolates were identified as *E. faecium* carrying the *vanA* gene. This is a significant decrease compared to the prevalence in 2011 and at a similar level as the result from 2009. However, the sampling method has changed and comparison of the results should therefore be made with caution.

Resistance in zoonotic bacteria and non-zoonotic enteropathogenic bacteria

Animal isolates

In 2014, a total of 25 *Salmonella* spp. isolates from animals were susceptibility tested. 13 out of 25 isolates were fully susceptible; six isolates were resistant to one antimicrobial, one to two antimicrobials, and three to three antimicrobials. One isolate originating from a cattle herd was multiresistant to five antimicrobials. Another isolate originating from a chicken farm was multiresistant to seven of the antimicrobial substances tested for.

During spring 2014, a total of 986 swine herds were investigated for methicillin resistant *Staphylococcus aureus* (MRSA) in a separate survey. MRSA was identified in animal and environmental samples from only one of these herds. The isolates were typed as CC398, t011. In addition, one contact herd was identified with MRSA CC398, t011. In both herds all pig were slaughtered and thorough cleaning and disinfection of rooms etc. was performed before restart with MRSA free pigs in line with previous strategy for MRSA in swine herds.

Human clinical enteropathogenic isolates

The frequency of multidrug resistance (MDR) in human clinical isolates of all *Salmonella* serovars was just above 10%, and the frequency of ESBL stayed below 2%. Among the 65 *Salmonella* blood culture isolates, the highest frequency of MDR was found in *Salmonella* serovars other than *S.* Typhi, *S.* Paratyphi, *S.* Typhimurium-group. Antimicrobial resistance in general is more prevalent in the *S.* Typhimurium-group (including *S. enterica* serovar 4,[5],12:i-) than in other serovars, and resistance to ampicillin and tetracycline is still increasing in this group. This applies to domestically acquired strains as well as to strains acquired abroad.

For *Campylobacter*, domestically acquired isolates are increasingly resistant to quinolone and tetracycline. However, resistance has not yet reached the same level as seen in isolates acquired abroad.

Most cases of shigellosis are acquired abroad, and there is widespread resistance, especially in *S. flexneri*, as reported from other countries. There may be a trend of increasing resistance to quinolones. The frequency of MDR is approximately 70-80% in *S. flexneri* and 40% in *S. sonnei.* The ESBL prevalence in *Shigella* was 7.3% in 2014. Antimicrobial resistance in *Yersinia enterocolitica* remains low, except for intrinsic resistance to ampicillin.

Resistance in human clinical isolates

The prevalence of resistance in human clinical isolates was still low in Norway in 2014. Only nine methicillin resistant *Staphylococcus aureus* (MRSA) blood culture isolates were detected among the 1,163 strains included in the NORM protocol (0.8%). During 2014 the total number of systemic *S. aureus* isolates from blood cultures and cerebrospinal fluids was 1,720, including 24 MRSA strains (1.4%). This prevalence is at the same level as in 2012 (1.0%) and 2013 (1.0%). The Norwegian Surveillance System for Communicable Diseases (MSIS) registered 832 cases of MRSA infections in 2014 compared to 575 in 2012 and 659 in 2013. The majority of MRSA cases were reported to be wound infections and/or abscesses. The prevalence of MRSA among non-invasive *S. aureus* isolates is still very low at 1.3% (12/955) which is at the same level as 0.7% in 2012 and 1.2% in 2013. Furthermore, MSIS registered 1,035 cases of MRSA colonisation in 2014 compared to 823 in 2013. The total number of MRSA notifications thus increased from 1,482 in 2013 to 1,867 in 2014 (+ 26%). The results indicate an increasing number of MRSA infections and colonisations, while the prevalence of invasive disease has until now remained stable at a low level. The prevalence of fusidic acid resistant *S. aureus* wound isolates has stabilised at 8.6% compared to 9.0% in 2013.

E. coli and *Klebsiella* spp. blood culture isolates were increasingly resistant to broad-spectrum antimicrobials in 2014. The prevalence of gentamicin non-susceptibility in *E. coli* was 8.6% in 2014 compared to 5.8% in 2013. The increase in the prevalence of *E. coli* non-susceptibility to fluoroquinolones continued and reached 13.6% in 2014 compared to 11.7% in 2012 and 12.3% in 2013. There is a clear correlation between the total usage of fluoroquinolones and non-susceptibility to these agents.

The prevalence of resistance to aminoglycosides and fluoroquinolones is lower in *Klebsiella* spp. isolates than in *E. coli*.

Extended spectrum beta-lactamases (ESBL) have emerged as a significant clinical problem in many countries, including Norway. A total of 95/1,645 (5.8%) *E. coli* and 26/754 (3.4%) *Klebsiella* spp. blood culture isolates were reported with this phenotype in 2014. The prevalence is essentially unchanged from 2012 (5.5% *E. coli* and 2.3% *Klebsiella* spp.) and 2013 (5.0% *E. coli* and 2.8% *Klebsiella* spp.). The proportion of ESBL positive isolates is higher among *E. coli* from blood cultures (5.8%) than among urinary tract isolates (3.8%). Carbapenemase producing Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp. have been notifiable to MSIS since July 2012. The prevalence of these phenotypes is still very low in Norway. A total of 33 isolates were reported in 2014 (6 *K. pneumoniae*, 4 *E. coli*, 2 *Enterobacter* spp., 6 *P. aeruginosa* and 15 *Acinetobacter* spp.).

Among *Haemophilus influenzae* isolates from systemic infections (n=69), 13.0% displayed beta-lactamase production and 13.0% were resistant to cefuroxime, thus indicating chromosomal resistance to beta-lactam antibiotics. Similar rates were detected in respiratory tract isolates. All 13 *Neisseria meningitidis* isolates from systemic infections were intermediately susceptible to penicillin G, but all remained susceptible to other relevant antibiotics. *Neisseria gonorrhoeae* isolates (n=255) demonstrated non-susceptibility to penicillin G (96.1%) and azithromycin (37.2%), as well as resistance to ciprofloxacin (75.7%) and even ceftriaxone in three cases (1.2%).

Three enterococcal blood culture isolates with clinically significant vancomycin resistance were detected in 2014 (1 VanA and 2 VanB *E. faecium*). The prevalence of non-susceptibility to ampicillin in *E. faecium* has stabilised around 80-90%. High-level gentamicin resistance (HLGR) was detected in 18.7% of *E. faecalis* and 40.8% of *E. faecium*, which is a slight decline from 23.3% and 46.8% in 2013. Almost all HLGR *E. faecium* (70/71) isolates were also non-susceptible to ampicillin. Enterococcal resistance to linezolid was not detected in 2014.

Non-susceptibility to penicillin G was detected in 5.5% of *Streptococcus pneumoniae* isolates from blood cultures and cerebrospinal fluids and in 5.9% from respiratory tract specimens. This is at the same level as 6.3% in 2012 and 3.0% in 2013. A single blood culture isolate was resistant to penicillin G and at the same time showed reduced susceptibility to cephalosporins. The prevalence of macrolide resistance was 4.3% among pneumococcal blood culture isolates and 7.6% in isolates from respiratory tract samples.

Streptococcus pyogenes (group A streptococcus) isolates from blood cultures had a slightly increasing prevalence of erythromycin resistance (3.7%) compared to 2013 (1.9%). Similarly, the prevalence of macrolide resistance in *Streptococcus agalactiae* (group B streptococci) increased from 12.0% in 2012 to 18.1% in 2014. All isolates were susceptible to penicillin G.

A total of 327 cases of tuberculosis were reported to MSIS in 2014. Susceptibility testing was performed on 265 *Mycobacterium tuberculosis* isolates. Ten isolates (2.5%) originating from Africa (n=6), Asia (n=2) and Europe outside Norway (n=2), were classified as multidrug resistant (MDR).

Susceptibility testing was performed on 210 *Candida* spp. blood culture isolates of nine different species. The most common species were *C. albicans* (n=146), *C. glabrata* (n=33), *C. tropicals* (n=9) and *C. parapsilosis* (n=9). Only a single yeast isolate was non-susceptible to amphotericin B. Single isolates with acquired fluconazole resistance were detected, but as expected there was a high prevalence of resistance to azoles among *C. glabrata*. Three *C. albicans* were non-susceptible to echinocandines and one *C. parapsilosis* isolate was resistant to anidulafungin. The results are in accordance with previous studies from Norway.

Conclusion

Antimicrobial resistance is still a limited problem among clinically important microbes in Norway. The relatively low usage of antimicrobial agents as well as appropriate patterns of use must be maintained to preserve this rather favourable situation. The data presented in this report show that Norwegian antimicrobial policies in food production and health care have been successful. However, the situation may rapidly change if the use of antimicrobial agents in Norway increases or resistant clones are imported from abroad. A continued effort is needed to prevent the development and spread of antimicrobial resistance and thereby ensure the effectiveness of antimicrobials when such treatment is needed. The NORM/NORM-VET report is a vital component in the work aimed at preventing the development and spread of antimicrobial resistance in Norway.

POPULATION STATISTICS

Population statistics for human and animal populations are presented in order to facilitate comparison of Norwegian data with corresponding figures from other countries. The data are collected by Norwegian authorities as shown in the various tables below.

TABLE 1. Human population in Norway as of January 1st, 2015.
Data provided by Statistics Norway.

Age group	All	Males	Females
0 to 4 years	308,598	158,290	150,308
5 to 14 years	623,795	319,119	304,676
15 to 24 years	670,583	345,048	325,535
25 to 44 years	1,415,436	727,576	687,860
45 to 64 years	1,313,088	670,589	642,499
65 years and older	834,302	378,202	456,100
All age groups	5,165,802	2,598,824	2,566,978

TABLE 2. Livestock population in Norway in 2014.
Data provided by the Register of Production Subsidies as of 12.06.2015.

	Number* of	
Animal category	Herds	Animals
Cattle	14,500	839,000
Dairy cows only**	8,400	201,000
Suckling cow only**	4,100	67,700
Combined production (cow)**	750	30,800
Goat	1,200	64,400
Dairy goat**	300	31,600
Sheep	14,300	2,277,000
Breeding sheep > 1 year**	14,200	882,000
Swine	2,100	815,000
Breeding animal > 6 months**	1,100	51,400
Fattening pigs for slaughter**	1,900	448,000
Laying hen flocks > 250 birds	570	4,203,000
Broilers	689[1]	-
Turkey, ducks, geese for slaughter (flock > 250 birds)	47	743,000

* Numbers > 100 rounded to the nearest ten, numbers > 1000 rounded to the nearest hundred. ** Included in above total.
[1]Number tested in the surveillance for Salmonella.

TABLE 3. Production volume of the most important species in Norwegian aquaculture during the time period 1992-2014. *Data provided by the Norwegian Directorate of Fisheries updated by 12.06.2015.*

Year	Atlantic salmon (tonnes)	Rainbow trout (tonnes)	Cod (tonnes)	Arctic char (tonnes[2])	Halibut (tonnes[2])	Blue mussels (tonnes)	Scallops[1] (tonnes)	Oysters (tonnes)
1992	141,000	-	-	-	-	-	-	-
1993	170,000	-	-	-	-	-	-	-
1994	204,686	14,571	569	262	63	542	-	-
1995	261,522	14,704	284	273	134	388	-	-
1996	297,557	22,966	191	221	138	184	-	-
1997	332,581	33,295	304	350	113	502	-	-
1998	361,879	48,431	203	200	291	309	-	-
1999	425,154	48,692	147	498	451	662	67	41
2000	440,061	48,778	169	129	548	851	38	8
2001	435,119	71,764	864	318	377	920	22	3
2002	462,495	83,560	1,258	319	424	2,557	5	2
2003	509,544	68,931	2,185	272	426	1,829	1	2
2004	563,915	63,401	3,165	365	648	3,747	46	3
2005	586,512	58,875	7,409	352	1,197	4,885	3	2
2006	629,888	62,702	11,087	897	1,185	3,714	4	1
2007	744,222	77,381	11,104	394	2,308	3,165	6	4
2008	737,694	85,176	18,052	468	1,587	2,035	4	3
2009	862,908	73,990	20,924	421	1,568	1,649	7.7	3.8
2010	939,575	54,451	21,240	492	1,610	1,930	10.3	2.1
2011	1,064,868	58,472	15,273	276	2,767	1,743	13	2
2012	1,241,482	70,364	10,033	309	1,741	1,967	21	2
2013	1,168,324	71,449	3, 770	281	1, 385	2,328	23	5
2014[3]	1,272,358	68,954	1, 386	285	1,257	1,983	13	4

[1]From the wild population. [2]After 2001 in numbers of 1,000 individuals. [3] Preliminary numbers.

Import of live animals

Import of live animals (excluding fish and companion animals) to Norway in 2014 was 20 cattle, 56 camelides, 43 sheep and 24,570 day old chicks.

USAGE OF ANTIMICROBIAL AGENTS

USAGE IN ANIMALS

Kari Grave

Therapeutic usage of veterinary antimicrobial agents

In 2014, total sales of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in terrestrial food producing animals and companion animals in Norway were 5,927 kg. Annual sales of AMPs for use in these animal categories in the period 1995-2014 are shown in Figure 1. The data are based on sales from drug wholesalers to Norwegian pharmacies and from feed mills to fish farmers (see Appendix 1) of veterinary antimicrobial agents for therapeutic use and include pharmaceutical formulations approved for food animals, including horses, and companion animals. Thus, the figures represent national sales data for veterinary antimicrobial agents (see Appendix 1 for inclusion criteria).

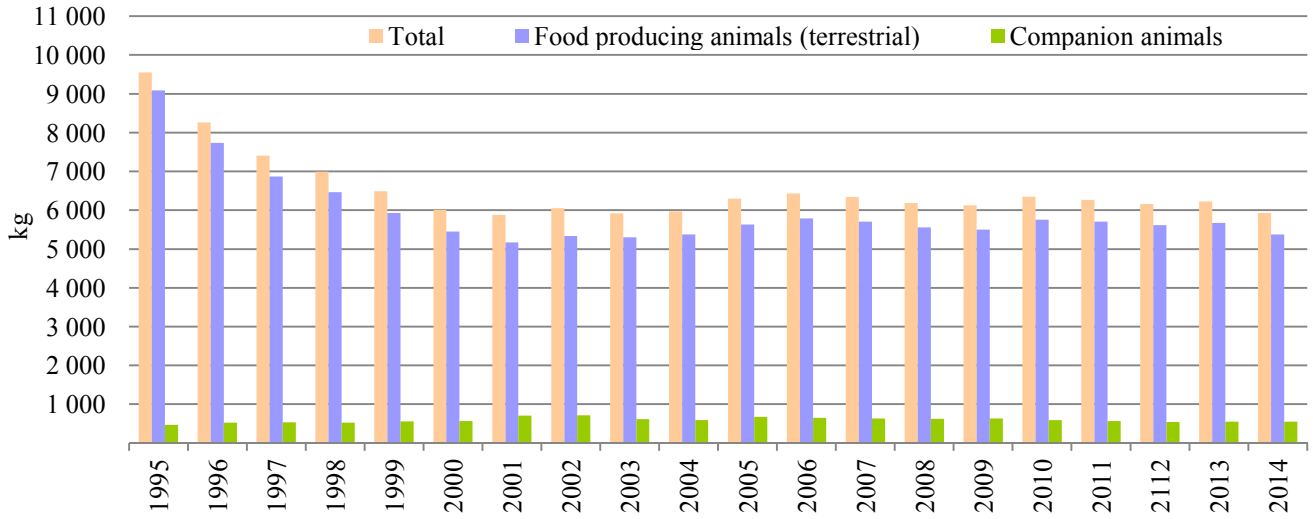

FIGURE 1. Total sales, in kilograms active substance, and estimated sales for food producing animals (terrestrial animals) and companion animals of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in Norway for the years 1995-2014.

In the period 1995-2014 the total sales of antimicrobial VMPs for use in terrestrial animals decreased by 38%. Of antimicrobial VMPs used almost solely for food production animals the reduction was 41%, while for products used in companion animal only an increase of 19% was observed (Figure 1).

An increase in the sales of pure penicillin VMPs for food producing terrestrial animals is observed for the period 1995-2014 - from 25% to 53% of total sales - and this is accounted for by products used in food producing and companion animals (Figures 2-3). In this period the sales of aminoglycosides decreased from 27% to 10% of the total sales; this is due to a reduction in the use of combination preparations of penicillin and dihydro-streptomycin in food producing animals (Figure 2). The observed peak in the sales of sulfonamides in companion animals in 2001-2002 is probably due to use in sheep of a trimethoprim-sulfonamide VMP marketed for companion animals because of a withdrawal in 2001 of a product used for mastitis in sheep (Figure 3).

The sales of the antimicrobial VMPs defined by the World Health Organization (WHO) with highest priority for human medicine i.e. fluoroquinolones and macrolides (Figures 2-3) are negligible. Note that there is no cephalosporin VMPs marketed in Norway for food producing animals.

The reduced sales of antimicrobial VMPs in terrestrial food producing animals as well as the favourable prescribing patterns are mainly explained by a campaign on prudent use of antimicrobials conducted by the Norwegian husbandry organisations and Norwegian Medicine Authority during the second part of the 1990s. Furthermore, a target set by the Norwegian husbandry organisations to reduce the sales by 25% with 1995 as the reference year is thought to have had a major impact on this decrease.

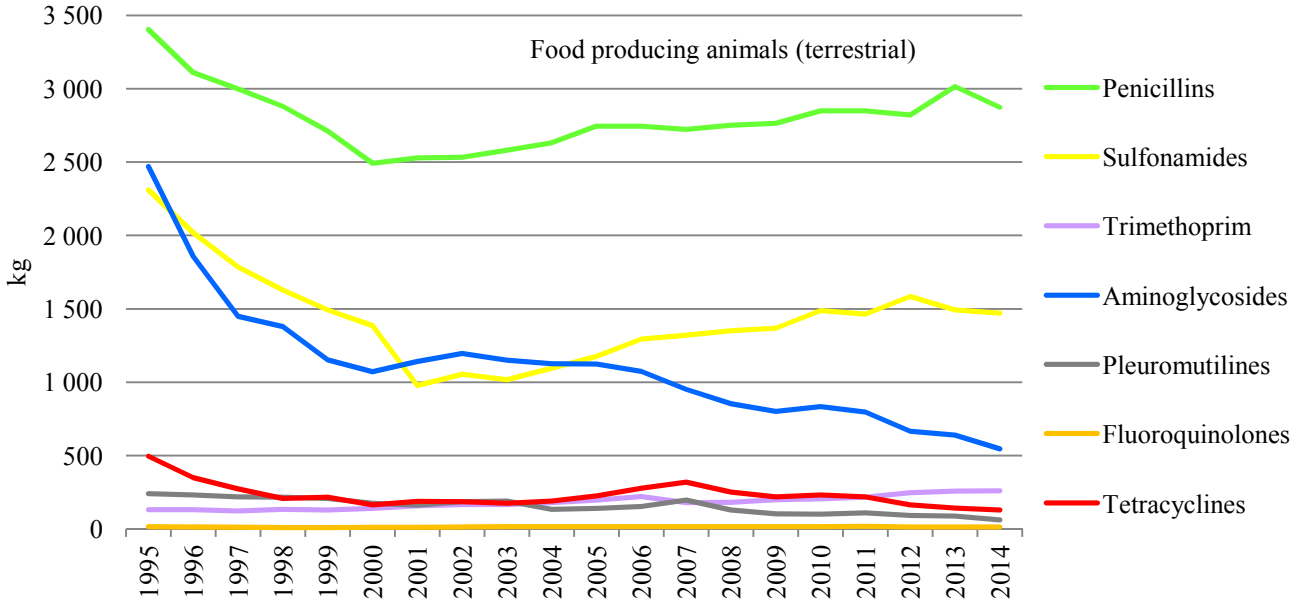

FIGURE 2. Sales in Norway (kilograms active substance) of antimicrobial veterinary medicinal products (VMPs) mainly for therapeutic use in food producing animals for the years 1995-2014 (farmed fish not included). In addition, minor amounts of amphenicols (range 17-27 kg) were sold in 2008-2014 and of macrolides (range 0.2-18 kg) during 1995-2014.

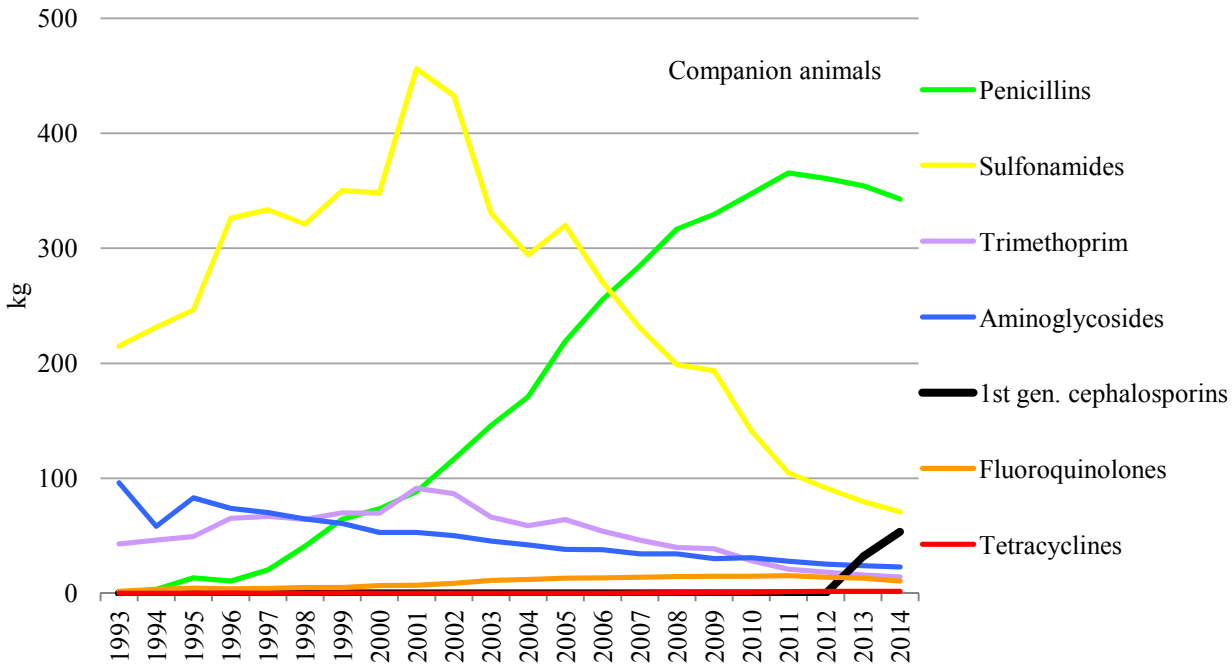

FIGURE 3. Sales in Norway, in kilograms active substance, of antimicrobial veterinary medicinal products (VMPs) marketed for therapeutic use in companion animals for the years 1995-2014. In addition, minor amounts of a 3rd generation cephalosporin (0.7-1.1 kg) were sold annually during 2008-2014 and of macrolides (0.4-5kg) from 1998-2005.

From 1995-2014 an increase of 19% (from 465 to 548 kg) in the sales of antimicrobial VMPs marketed for companion animals is observed (Figure 3). This increase is mainly accounted for by penicillins, and in 2014 approximately 85% (242 kg) of the penicillins products sold for companion animals (approved for) was as combination of amoxicillin and clavulanic acid (Figure 4).

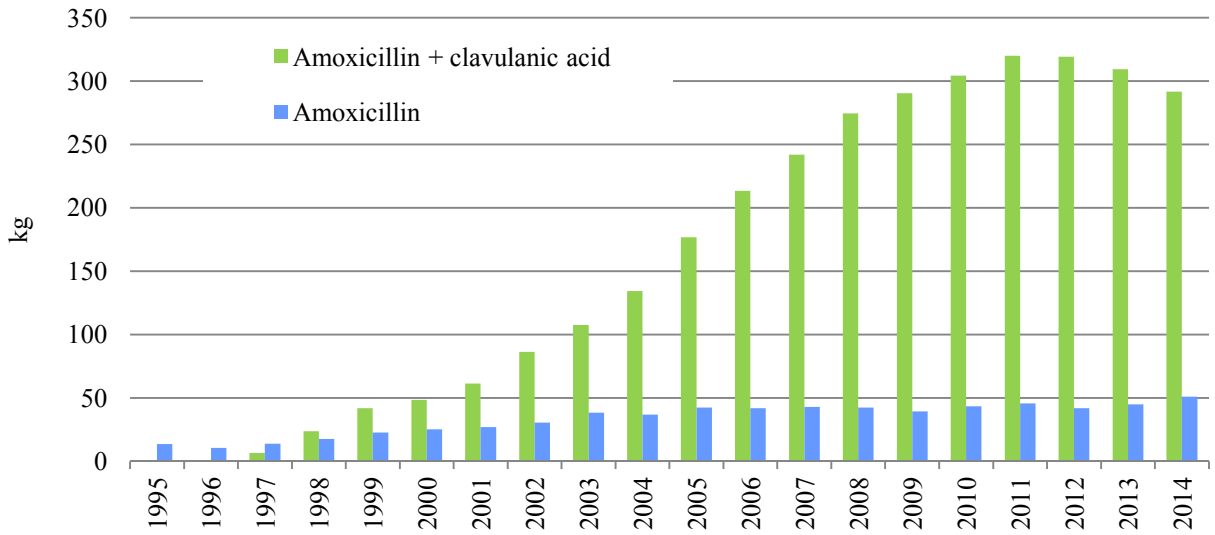

FIGURE 4. Sales, in kg active substance, of penicillin veterinary medicinal products for companion animals 1995-2014.

The annual sales of antimicrobial VMPs for use in farmed fish peaked in 1987 when the sales amounted to 48 tonnes (Figure 5). In 2014, the sales of antimicrobial VMPs for use in farmed fish were 511 kg active substance, of which 79% were amphenicols (Table 4); this implies that the sales have declined by approximately 99% from 1987. Note that in 2013, the sales of antimicrobial VMPs for use in farmed fish were dominated by quinolones (76%). The significant decrease in the usage of antimicrobial agents in Norwegian aquaculture from 1987 is mainly attributed to the introduction of effective vaccines against bacterial diseases in Atlantic salmon and rainbow trout and to some extent also to improved health management.

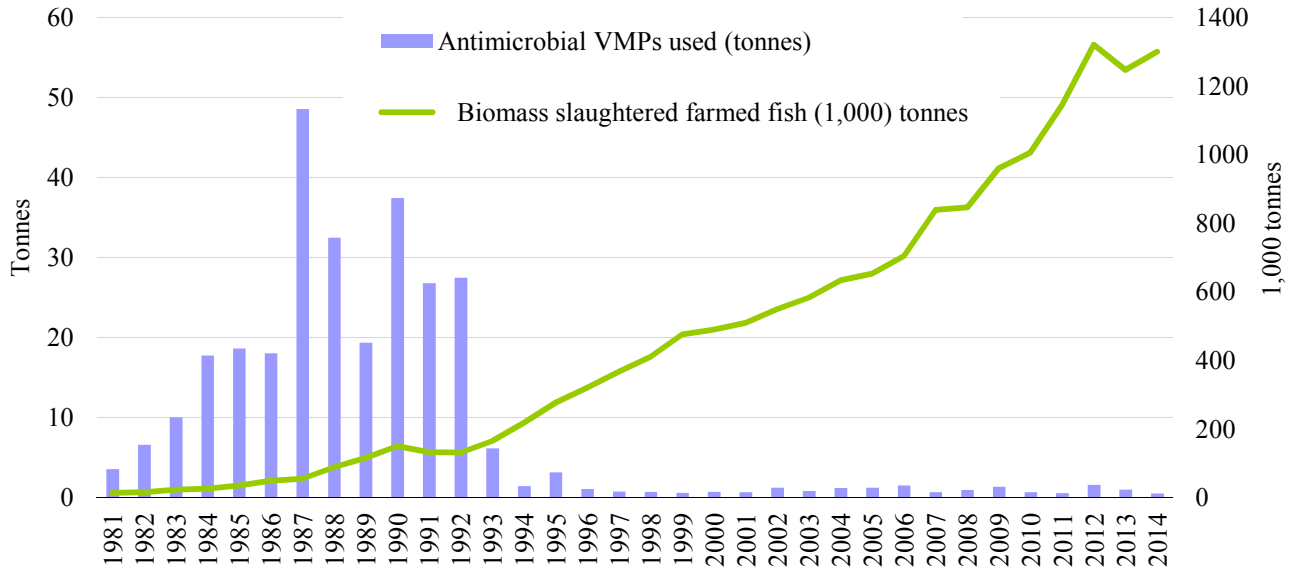

FIGURE 5. Total sales, in tonnes of active substance, of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in farmed fish in Norway in the period 1981-2014 versus produced biomass (slaughtered) farmed fish.

TABLE 4. Total sales, in kilograms of active substance, of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in farmed fish in Norway in the period 2004-2014.

Group of substances/active substance		2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Tetracyclines:	Oxytetracycline	9	8	0	19	23	40	10	1	1	0	0
Amphenicols:	Florfenicol	111	202	302	139	166	303	275	336	191	300	403
Quinolones:	Flumequine	4	28	7	18	1	1	0	0	0	0	0
	Oxolinic acid	1,035	977	1,119	406	681	926	308	212	1,399	672	108
Combinations:	Spectinomycin + lincomycin (2+1)	0	0	50	66	70	43	57	0	0	0	0
Total		**1,159**	**1,215**	**1,478**	**648**	**941**	**1,313**	**649**	**549**	**1,591**	**972**	**511**

Antimicrobial and coccidiostatic feed additives

Data on the sales of various substances and categories of feed additives (Table 5) were obtained through annual reports from the the Norwegian Food Safety Authority.
The glycopeptide avoparcin was licensed in Norway as growth promoter in broilers and turkeys in 1986. In 1995 the food animal production industry voluntarily abandoned the use of all antimicrobial growth promoters (AGPs), including avoparcin. These measures resulted in an immediate decline in the use of AGPs (Figur 6). No antimicrobial growth promoters have been used in animals in Norway since 1997.

TABLE 5. Total sales, in kilograms of active substance, of coccidiostats as feed additives in Norway 2004-2014. Data were obtained through annual reports from the Norwegian Food Safety Authority.

Active substance	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Lasalocid	173	37	13	17	16	63	0	0	0	0	0
Monensin	817	852	889	919	897	885	805	1,060	1,080	1,174	1,313
Salinomycin	0	0	0	0	0	0	0	0	0	0	0
Narasin	5,270	5,318	5,615	7,065	9,212	8,621	9,080	9,394	10,378	12,345	12,409
Total ionophore coccidiostats	**6,260**	**6,207**	**6,517**	**8,001**	**10,125**	**9,569**	**9,885**	**10,454**	**11,458**	**13,519**	**13,722**
Amprolium/etopabat	0.8	0	0	0	0	0	0	0	0	0	0
Total others	**0.8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

The total sales of ionophore coccidiostats (kilograms of active substance) have been doubled since the withdrawal of antimicrobial growth promoters in 1995 and have since then almost totally been dominated by narasin (Table 5, Figur 6). The sales of ionophore coccidiostats are correlated to the number of slaughtered chicken produced in this period.

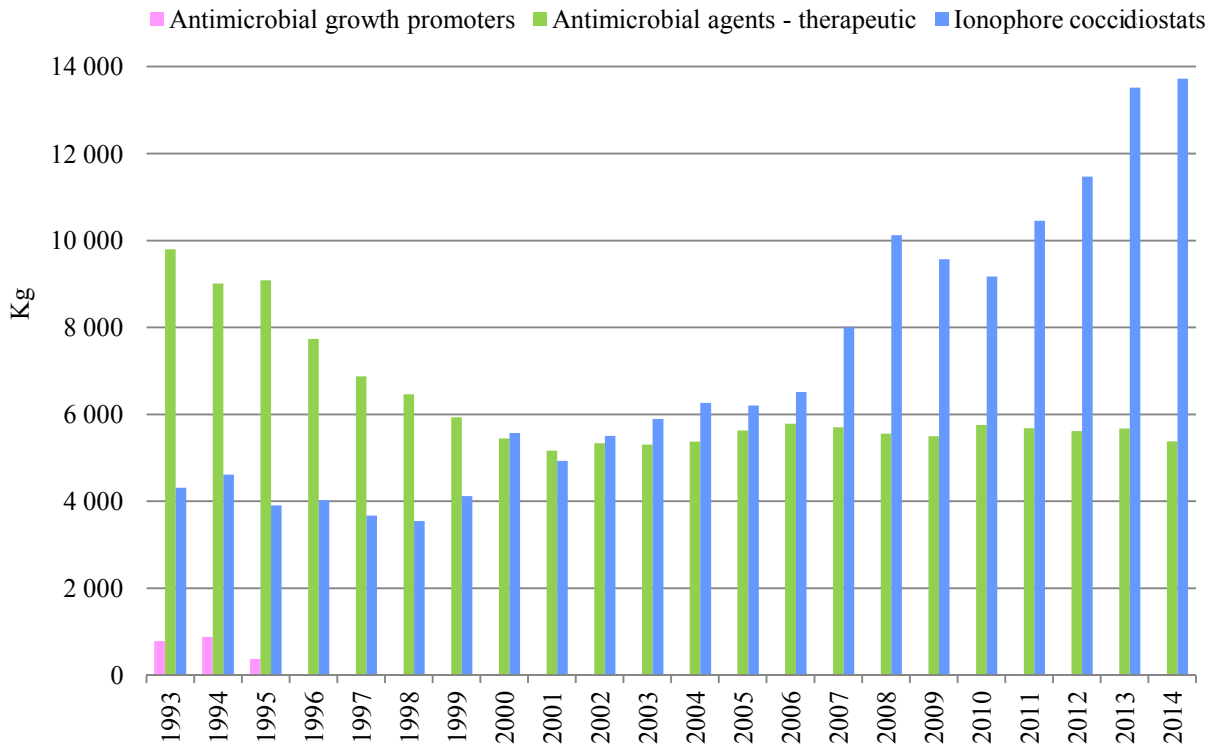

FIGURE 6. Sales, in kg active substance, of antimicrobial veterinary medicinal products (VMPs) for food producing animals (terrestrial), antimicrobial growth promoters, and ionophore coccidiostats in Norway during 1993-2014.

USAGE IN HUMANS

Hege Salvesen Blix

In 2014, the overall sales of antibacterials for systemic use in humans were 19.3 defined daily doses (DDD)/1,000 inhabitants/day. The use of antibiotics has decreased since 2011 and 2012, when a *Mycoplasma pneumonia* epidemic caused higher prescriptions of antibiotics. Increased sales of ATC group J01-Antibacterials in the first decade of this century are mainly caused by increased use of the penicillins and the urinary antiseptic methenamine. When methenamine is excluded, the level of antibiotic use in 2014 was 15.7 DDD/1,000 inhabitants/day (Table 6, Figure 7).

TABLE 6. Human usage of antibacterial agents in Norway 2007-2014 by ATC groups. The usage is presented as DDD/1,000 inhabitants/day and in % change 2013-2014. Collection methodology of data on human usage of antimicrobial agents is presented in Appendix 2.

ATC	Groups of substances	2007	2008	2009	2010	2011	2012	2013	2014	Change (%) 2013-2014
J01A	Tetracyclines	3.32	3.22	3.09	3.12	3.47	3.87	3.53	3.44	- 3
J01B	Amphenicols	0.001	0.001	0.002	0.001	<0.001	<0.001	<0.001	<0.001	-
J01CA	Penicillins with extended spectrum	2.93	3.09	3.15	3.19	3.21	3.34	3.33	3.34	-
J01CE	Beta-lactamase sensitive penicillins	4.70	4.71	4.47	4.44	4.47	4.3	4.1	3.8	- 7
J01CF	Beta-lactamase resistant penicillins	0.72	0.77	0.80	0.82	0.88	0.90	0.78	0.89	+ 14
J01CR	Combination of penicillins	0.02	0.02	0.02	0.03	0.03	0.04	0.05	0.08	-
J01D	Cephalosporins, monobactams, carbapenems	0.60	0.60	0.58	0.55	0.56	0.55	0.52	0.47	- 10
J01E	Sulfonamides and trimethoprim	1.02	0.98	0.94	0.87	0.87	0.87	0.85	0.84	- 1
J01F	Macrolides, lincosamides and streptogramins	2.30	2.13	1.89	2.01	2.31	2.26	1.93	1.66	- 14
J01G	Aminoglycosides	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.08	-
J01M	Quinolones	0.67	0.70	0.71	0.73	0.75	0.75	0.71	0.65	- 8
J01X*	Other antibacterials	3.30	3.48	3.65	3.84	3.93	4.04	4.12	4.07	- 1
	Total exclusive of methenamine	**16.9**	**16.8**	**16.2**	**16.3**	**17.2**	**17.4**	**16.3**	**15.6**	**- 4**
	Total all antimicrobial agents	**19.7**	**19.8**	**19.4**	**19.7**	**20.6**	**21.0**	**20.0**	**19.3**	**- 4**

*J01X includes glycopeptides, colistin, fusidic acid, metronidazol (i.v.), nitrofurantoin, linezolid and methenamine. Of total J01X, methenamine constitutes 3.7 DDD/1,000 inhabitants/day in 2014.

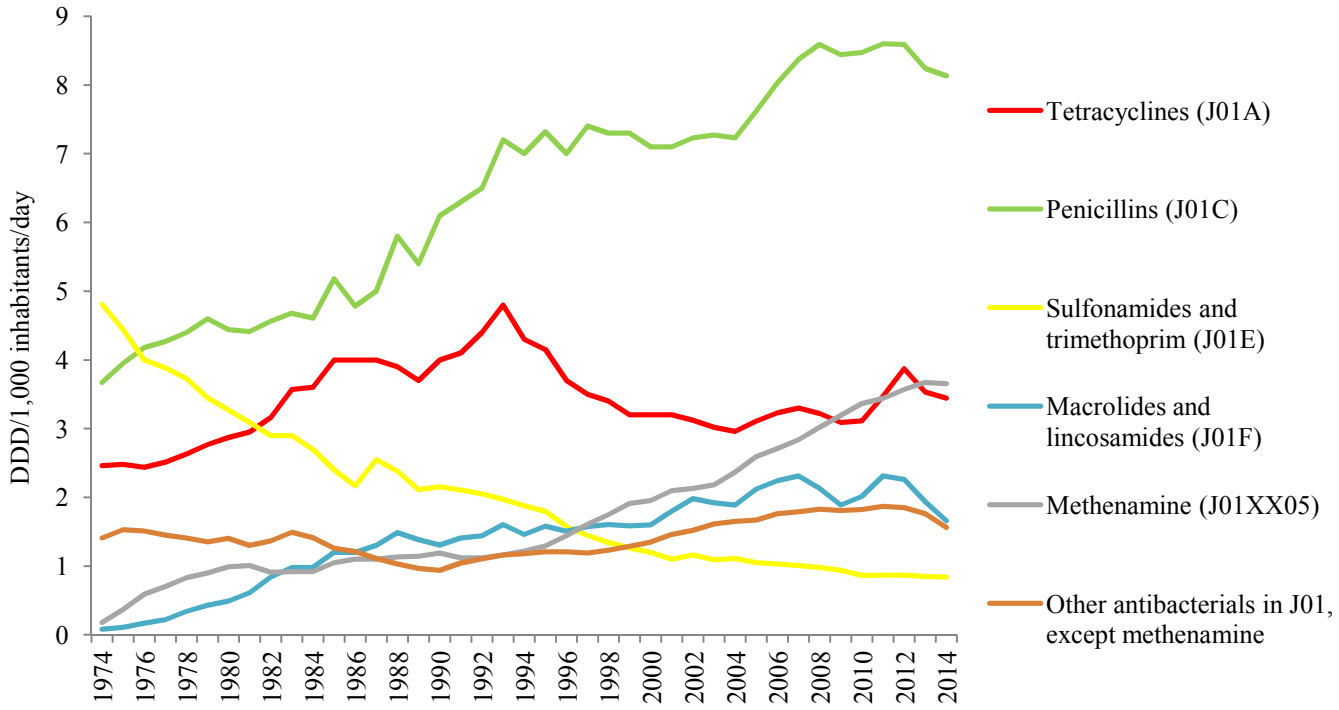

FIGURE 7. Sales of penicillins (J01C), tetracyclines (J01A), macrolides, lincosamides and streptogramines (J01F), sulfonamides and trimethoprim (J01E), methenamine and other antibacterials in Norway 1974-2014. Other types of antibacterials include all other antibacterials in ATC group J01, except methenamine (J01XX05).

Antimicrobial total usage in humans and animals, measured in weight of active substance

In 2014, the overall sales in Norway of antibacterials for use in humans, terrestrial animals and farmed fish measured in weight of active substance were 55.2 tonnes (Figure 8). Humans accounted for 88% of the total use, terrestrial animals for 11% of the total use, and the use in aquaculture only for 1% of the total use. The increase of 11% (in tonnes) from 2005 is caused by increased use in humans. When excluding methenamine, the increase was 1% (from 41.0 tonnes in 2005 to 41.6 tonnes in 2014). During these years the use in terrestrial animals has been stable.

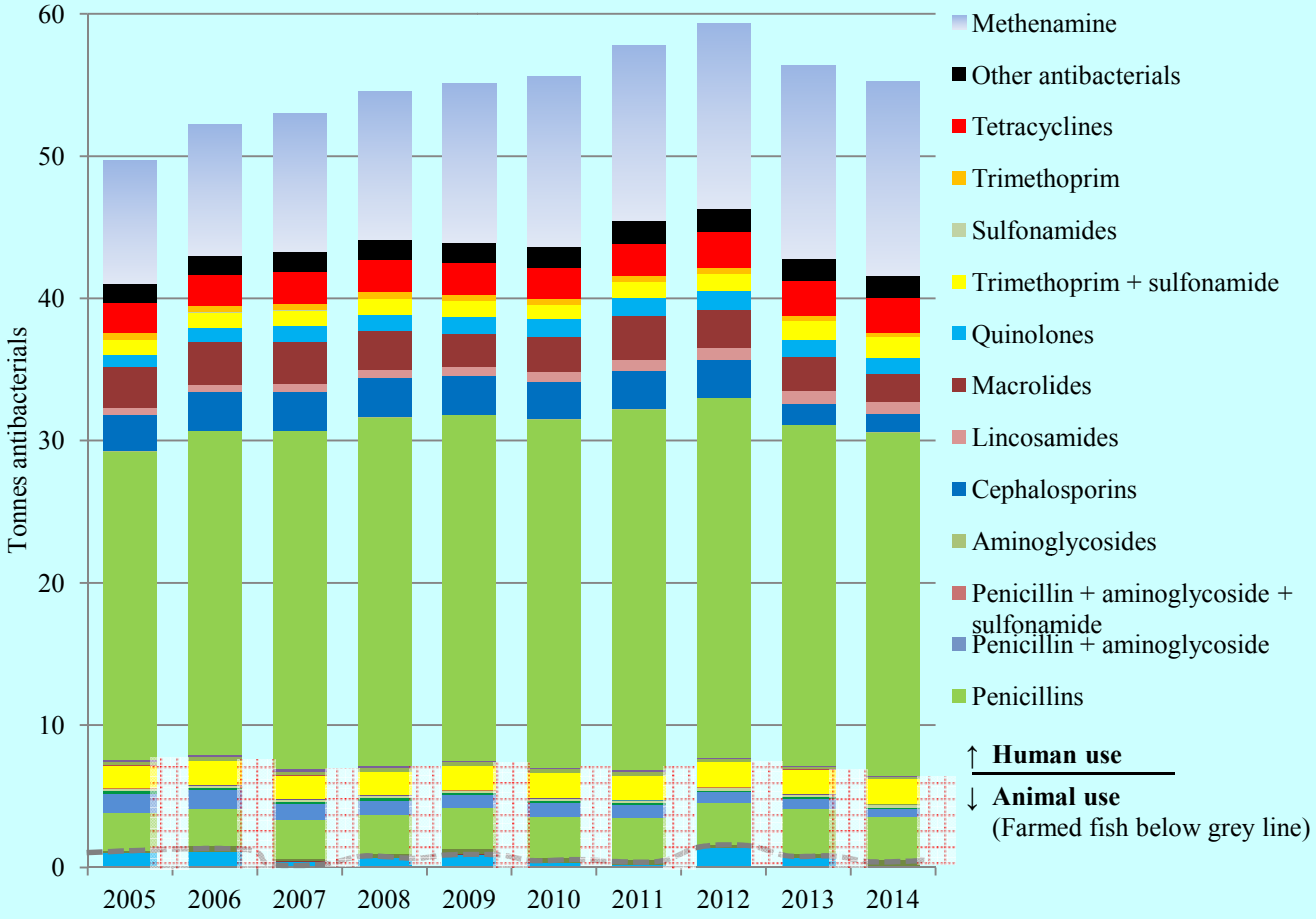

FIGURE 8. Sales, in tonnes of active substance, of human and veterinary antibacterials, for the years 2005-2014. Use in farmed fish is included and appears below the grey line.

According to Table 7, oral formulations are dominating in human medicine while for veterinary medicine the dominating formulations are the parenteral ones. The oral formulations represent 83% of the total weight followed by parenteral formulations with 16% of the total weight. Use of other formulations e.g. for eye, ear and skin is very limited.

TABLE 7. Sales, in kg of active substance, of human and veterinary antibacterials according to formulation in 2014.

Formulation	Humans	Terrestrial animals	Aquaculture
Dermal	105	11	
Oral	42,785	2,250	511
Parenteral	5,783	3,249	
Eye / ear	34	12	
Intramammary		322	
Others	54	105	
Total	48,761	5,949	511

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In 2014, the penicillins (ATC group J01C) accounted for 42% of the total antibacterial use in Norway (Figure 9). Over the years there has been a shift towards use of more broadspectered penicillins. The beta-lactamase sensitive penicillin group (J01CE) is the largest of the penicillin subgroups. Penicillins with extended spectrum (J01CA) now represent 41% of the penicillin group compared to around 30% a decade ago (2003) (Figure 9 and Figure 10). This is mainly due to increasing use of amoxicillin and pivmecillinam. Pivmecillinam is used for urinary tract infections, at the expense of the subgroup of sulfonamides and trimethoprim, which has decreased over the years (Figure 7).

The use of the group J01F macrolides, lincosamides and streptogramins has followed a wavy pattern over the years; the internal pattern within the group has remained relatively unchanged (Figure 7 and Figure 11). The shifts in use could be explained to some degree by the recurrent epidemics of *M. pneumoniae* in Norway, occurring with four- to six-years intervals.

In recent years, the sales of cephalosporins, monobactams and carbapenems have been stable and this group represents 2% of the total sales of antibacterials (Figure 9). The internal subgroup pattern has changed over time (Figure 12). Today, 1st and 3rd generation cephalosporins hold 47% and 31% of ATC group J01D.

The use of quinolones has decreased over the last 2 years. The quinolones represent only a small fraction (3%) of total antibacterial sales (Figure 9). Ciprofloxacin is the main substance accounting for 97% of the quinolone group in 2014. The use of ATC group J01X is mainly due to the urinary prophylactic agent methenamine, accounting for 90% of subgroup J01X and 19% of total antibacterial use (Figure 9).

Geographical variation

The usage of antibacterials varies among the 19 Norwegian counties, with the county using the least using around 70% (in DDDs) of the county using the most (Figure 13). There is a trend of the same high-use and low-use counties over the years. The same pattern is seen when using number of prescriptions/1000 inhabitants as measurement.

Antibiotic use in primary care

Antibacterials are prescription-only drugs in Norway. Around 85% of the total human sales of antibacterials are used in primary care, i.e. outside institutions (hospitals and nursing homes).

For ambulatory care, the most important antibiotic groups in 2014 were penicillins (J01C, 41% of DDDs), tetracyclines (J01A, 20%) and macrolides and lincosamides (J01F, 9%). The three most used antibiotics for outpatients in 2014 were phenoxymethylpenicillin, pivmecillinam and doxycycline. These three represented 47% of all prescriptions and 50% of all DDDs prescribed when methenamine is excluded.

Females use more antibiotics than males; 27% of the females purchased at least one antibiotic course in 2014 compared to 19% of the males. The gender pattern is similar in all regions of the country (Figure 14). The highest use is found among young children, young women and the elderly (Figure 15). Among those who use antibacterials, the elderly use more, both with regard to the amount (measured in DDDs) and to the number of prescriptions. For those above 70 years, 2-3 prescriptions are dispensed every year compared to 1-2 for younger persons. Since the dosages for young children are much lower than in adults, the number of DDDs per user will be less than in adults (Figure 16).

Antibiotics prescribed by dentists

Physicians are the main prescribers to humans, but dentists prescribe 5% (measured in DDDs) of antibiotics (J01) to humans in ambulatory care. The prescription of antibiotics by dentists has increased by 49% (measured in DDD/1,000 inhabitants/day) from 2004-2014. In 2014, dentists most often prescribed phenoxymethylpenicillin (72% of all antibiotic DDDs prescribed by dentists) followed by amoxicillin (11%) and clindamycin (6%).

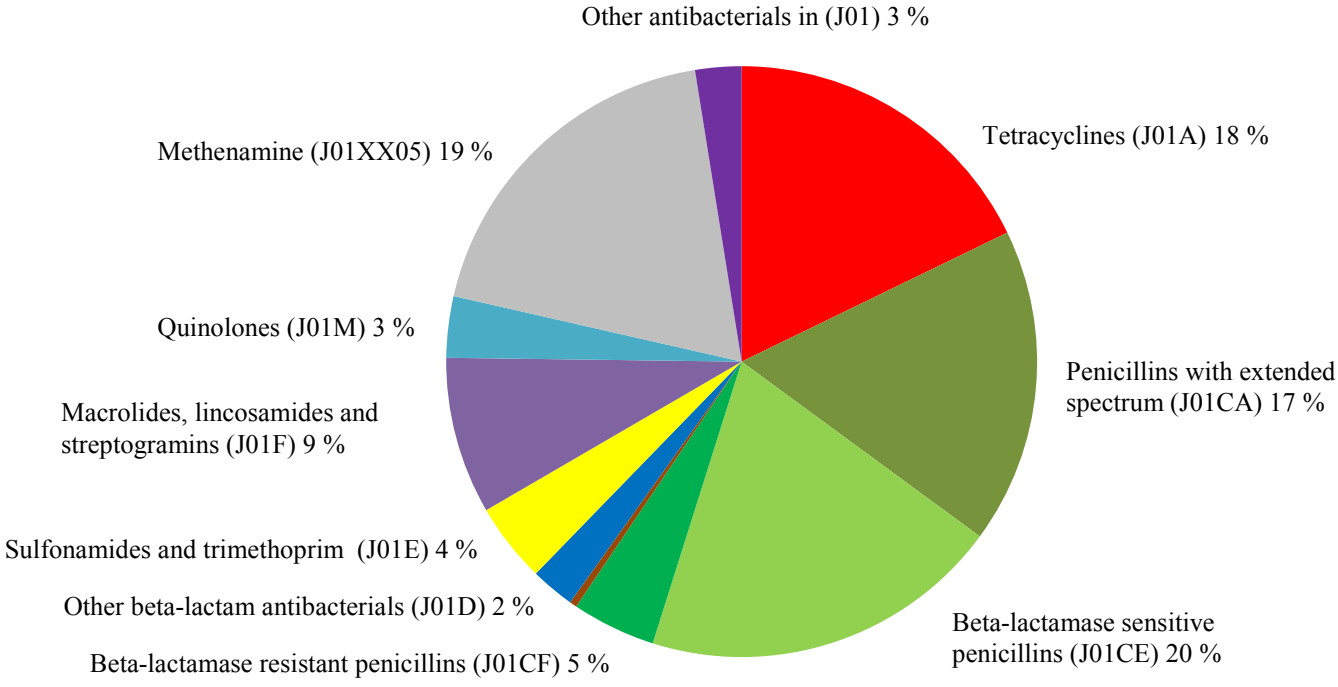

FIGURE 9. Relative amount of antibacterial agents for systemic use in 2014 in Defined Daily Doses (DDDs) (total sales).

TABLE 8. Human usage of single antibacterial agents for systemic use in Norway 2009-2014. Sales are given in DDD/1,000 inhabitants/day. The methodology for collection of data on human usage of antibacterial agents is presented in Appendix 2.

ATC group	ATC code	Substance	2009	2010	2011	2012	2013	2014
J01A - Tetracyclines	J01A A02	Doxycycline	1.78	1.83	2.09	2.36	2.02	1.97
	J01A A04	Lymecycline	0.54	0.59	0.76	0.90	1.00	0.96
	J01A A06*	Oxytetracycline	0.16	0.15	0.03	-	<0.001	<0.001
	J01A A07	Tetracycline	0.60	0.54	0.58	0.62	0.54	0.50
	J01A A08*	Minocycline	0.0003	0.001	0.002	0.006	0.009	0.003
	J01A A12	Tigecycline	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
J01B - Amphenicols	J01B A01	Chloramphenicol	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
J01CA - Penicillins with extended spectrum	J01C A01	Ampicillin	0.11	0.09	0.09	0.09	0.10	0.12
	J01C A04	Amoxicillin	1.31	1.34	1.39	1.45	1.40	1.41
	J01C A08	Pivmecillinam	1.72	1.75	1.73	1.78	1.83	1.80
	J01C A11	Mecillinam	0.008	0.008	0.008	0.008	0.008	0.007
J01CE - Beta-lactamase sensitive penicillins	J01C E01	Benzylpenicillin	0.28	0.22	0.24	0.24	0.22	0.23
	J01C E02	Phenoxymethyl-penicillin	4.19	4.22	4.23	4.07	3.85	3.60
	J01C E08*	Benzathine benzylpenicillin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
J01CF - Beta-lactamase resistant penicillins	J01C F01	Dicloxacillin	0.67	0.70	0.74	0.76	0.57	0.71
	J01C F02	Cloxacillin	0.13	0.12	0.14	0.14	0.21	0.18
	J01C F05*	Flucloxacillin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
J01CR - Combination of penicillins, incl. beta-lactamase inhibitors	J01C R02*	Amoxicillin and enzyme inhibitor	0.003	0.003	0.002	0.004	0.007	0.012
	J01C R05	Piperacillin and enzyme inhibitor	0.02	0.02	0.03	0.03	0.04	0.06
J01DB - First gen. cephalosporins	J01D B01	Cefalexin	0.21	0.20	0.19	0.18	0.17	0.14
	J01D B03	Cefalotin	0.07	0.07	0.08	0.08	0.08	0.09
J01DC - Second gen. cephalosporins	J01D C02	Cefuroxime	0.10	0.09	0.09	0.08	0.07	0.05
J01DD - Third gen. cephalosporins	J01D D01	Cefotaxime	0.11	0.11	0.12	0.12	0.12	0.11
	J01D D02	Ceftazidime	0.01	0.01	0.01	0.01	0.01	0.01
	J01D D04	Ceftriaxone	0.02	0.02	0.03	0.03	0.03	0.02
J01DF - Monobactams	J01D F01	Aztreonam	<0.001	<0.001	<0.001	<0.001	0.001	0.001
J01DH - Carbapenems	J01D H02	Meropenem	0.04	0.04	0.04	0.05	0.05	0.05
	J01D H03	Ertapenem	0.002	0.002	0.002	0.002	0.002	0.002
	J01D H51	Imipenem and enzyme inhibitor	0.002	0.002	0.002	0.002	0.002	0.002
J01DI – Other cephalo-sporins and penems	J01DI02	Ceftaroline fosamil						<0.001
J01E - Sulfonamides and trimethoprim	J01E A01	Trimethoprim	0.60	0.56	0.55	0.51	0.48	0.44
	J01E E01	Sulfamethoxazole and trimethoprim	0.33	0.31	0.32	0.36	0.37	0.40
J01F - Macrolides, lincosamides and streptogramins	J01F A01	Erythromycin	0.92	0.94	1.18	1.06	0.85	0.74
	J01F A02	Spiramycin	0.01	0.01	0.01	0.01	0.01	0.01
	J01F A06	Roxithromycin					<0.001	<0.001
	J01F A09	Clarithromycin	0.31	0.34	0.37	0.39	0.30	0.23
	J01F A10	Azithromycin	0.37	0.41	0.44	0.48	0.41	0.35
	J01FS15	Telithromycin				<0.001	<0.001	<0.001
	J01F F01	Clindamycin	0.28	0.31	0.32	0.33	0.37	0.33

ATC group	ATC code	Substance	2009	2010	2011	2012	2013	2014
J01G - Aminoglycosides	J01GA01*	Streptomycin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	J01G B01	Tobramycin	0.03	0.03	0.03	0.03	0.03	0.03
	J01G B03	Gentamicin	0.04	0.04	0.05	0.05	0.05	0.05
	J01G B06*	Amikacin	<0.001	0.001	0.001	0.001	0.001	0.001
J01M - Quinolones	J01M A01	Ofloxacin	0.03	0.03	0.03	0.02	0.02	0.01
	J01M A02	Ciprofloxacin	0.67	0.70	0.71	0.72	0.69	0.69
	J01MA12*	Levofloxacin	0.004	0.003	0.002	0.002	0.001	0.001
	J01MA14*	Moxifloxacin	0.001	0.004	0.006	0.004	0.005	0.007
J01X - Other antibacterials	J01X A01	Vancomycin	0.01	0.01	0.01	0.01	0.01	0.02
	J01X A02	Teicoplanin	0.001	0.001	0.001	0.001	0.001	<0.001
	J01X B01	Colistin	0.005	0.004	0.004	0.004	0.005	0.006
	J01X C01	Fusidic acid	0.005	0.004	0.005	0.005	0.004	0.004
	J01X D01	Metronidazole	0.07	0.07	0.07	0.07	0.06	0.05
	J01X E01	Nitrofurantoin	0.36	0.37	0.39	0.37	0.36	0.33
	J01XX01	Fosfomycin				<0.001	<0.001	<0.001
	J01X X05	Methenamine	3.19	3.37	3.44	3.57	3.67	3.65
	J01XX08	Linezolid	0.008	0.009	0.01	0.01	0.007	0.007
	J01XX09	Daptomycin	<0.001	<0.001	<0.001	0.001	0.001	<0.001
Antibiotics in other ATC groups	J04AB02	Rifampicin	0.004	0.004	0.004	0.005	0.004	0.005
	J04A	Rifampicin**	0.087	0.086	0.082	0.086	0.082	0.079
	A07AA09	Vancomycin	0.001	0.001	0.001	0.002	0.002	0.002
	A07AA11	Rifaximin	0.001	0.001	0.002	0.004	0.007	0.01
	A07AA12	Fidaxomicin				<0.001	<0.001	<0.001
	P01AB01	Metronidazole	0.22	0.23	0.24	0.23	0.24	0.24
	D06AX09/ R01AX06*	Mupirocin in kg cream/oint. (2%)	5.1	4.5	4.6	7.3	8.6	8.5

*Drugs not licensed in the Norwegian market in 2014. ** Given as the amount DDD/1,000 inhabitants/day of rifampicin in plain and combination products.

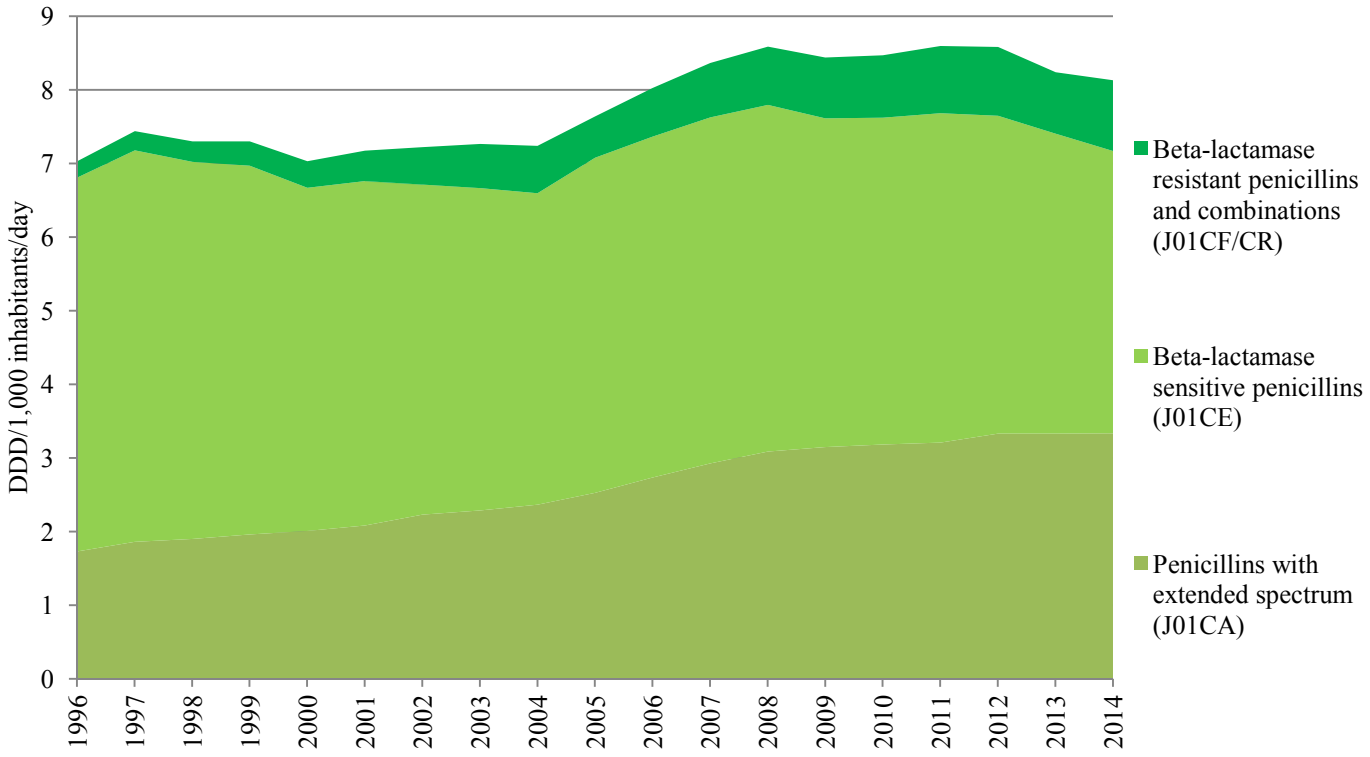

FIGURE 10. Sales of penicillins (J01C) in Norway 1996-2014 and changes within groups of penicillins.

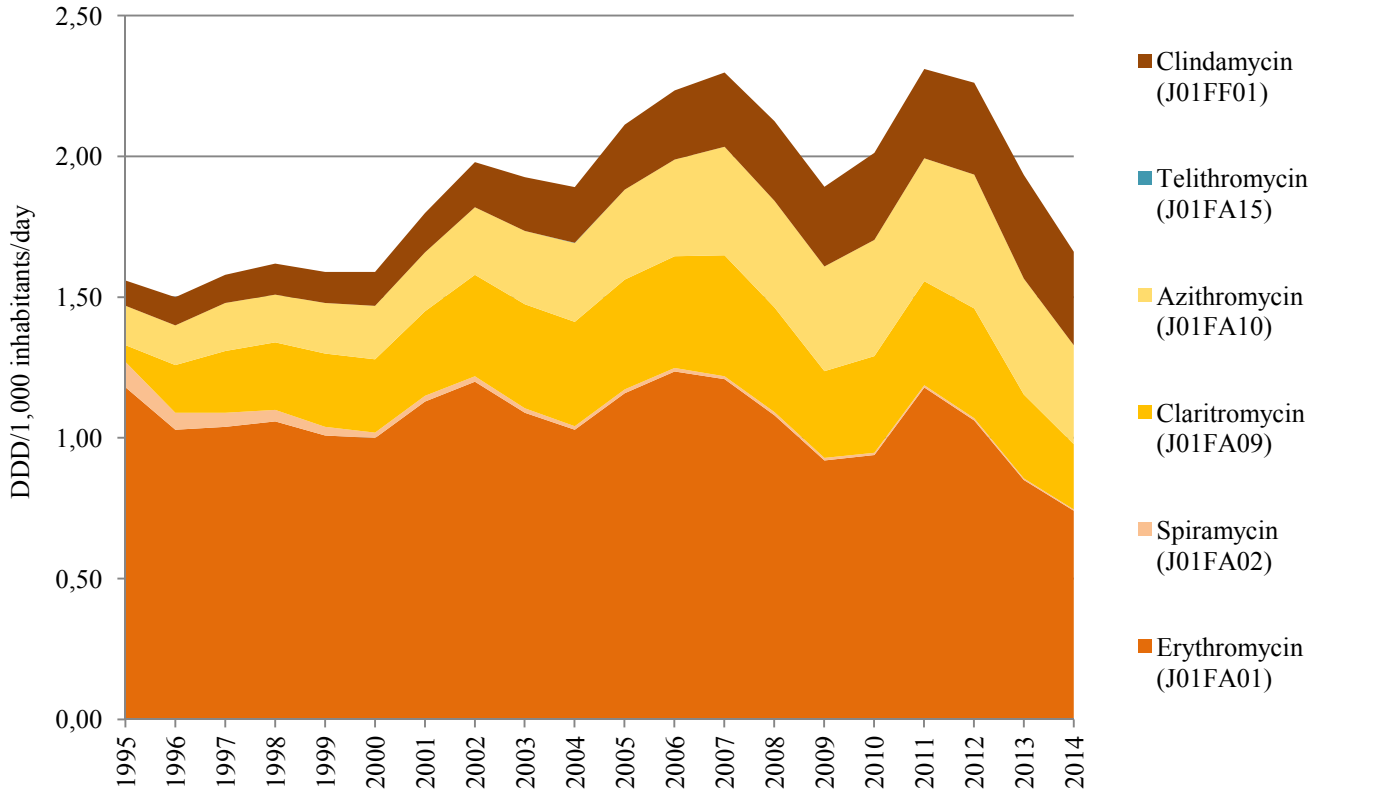

FIGURE 11. Sales of macrolides, lincosamides and streptogramins (J01F) in Norway 1995-2014.

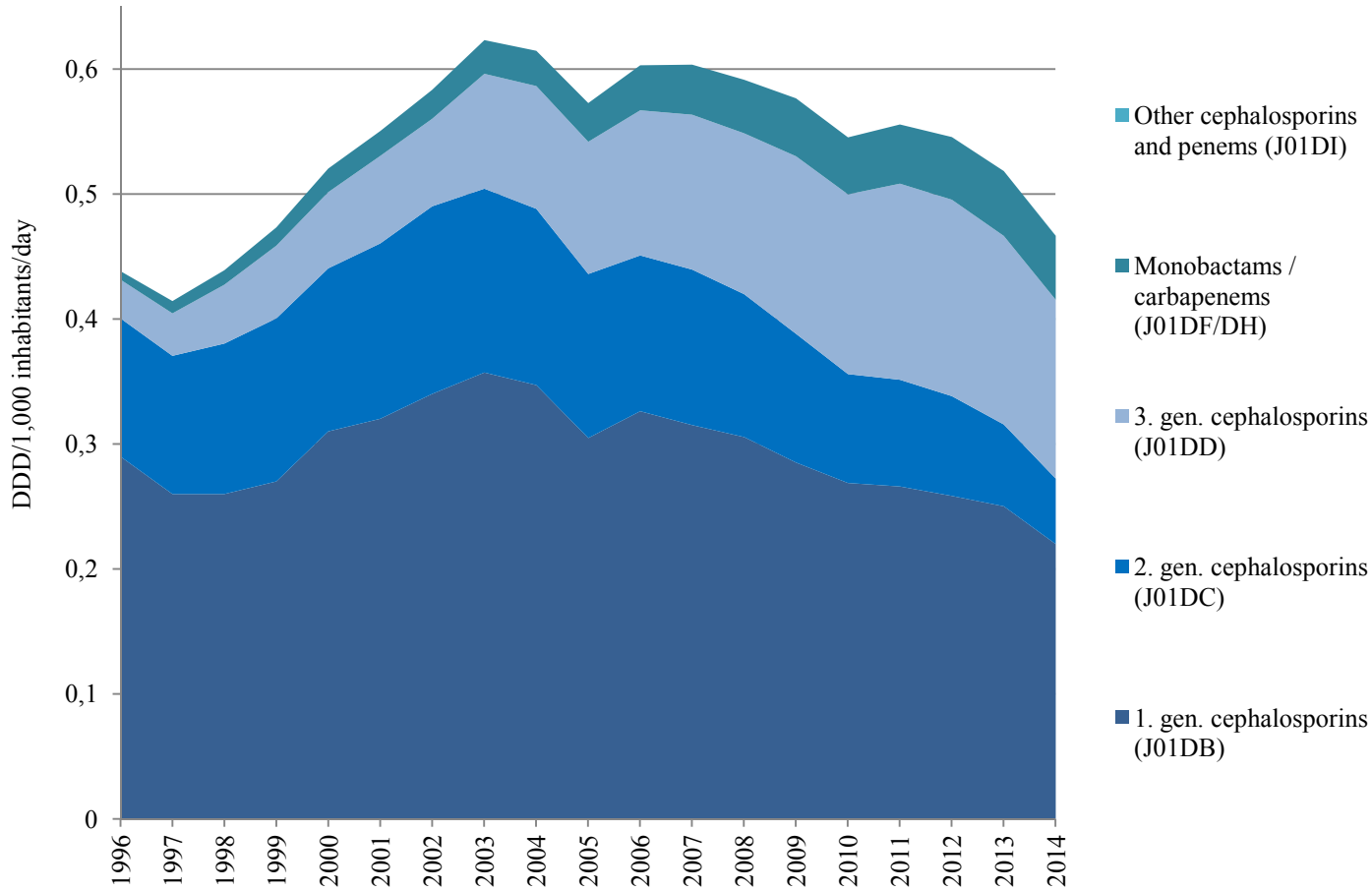

FIGURE 12. Sales of cephalosporins, monobactams and carbapenems (J01D) in Norway 1996-2014 and changes within generations of cephalosporins and monobactams/carbapenems.

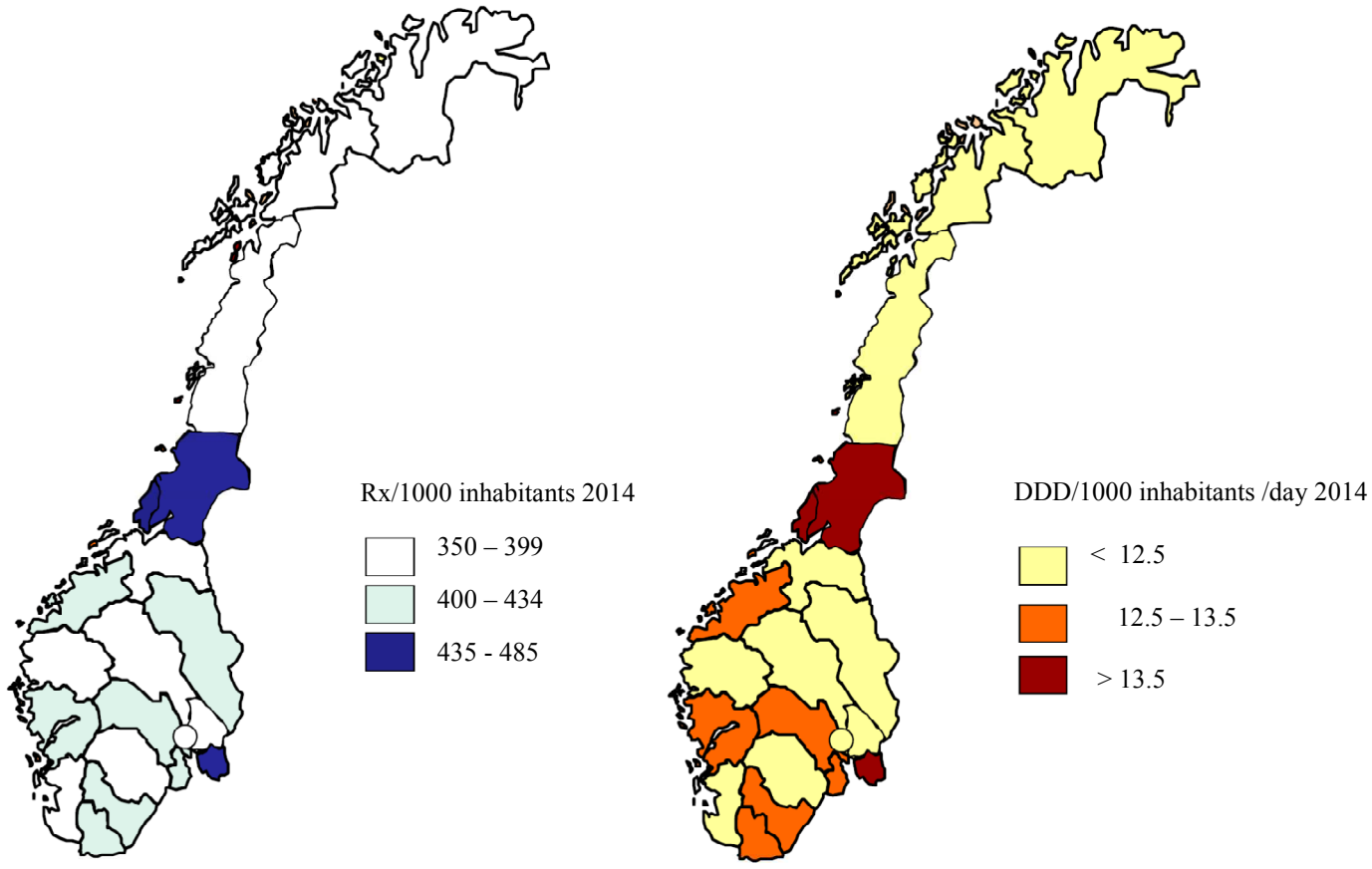

FIGURE 13. Sales of antibacterial agents for systemic use (ATC group J01, excl. methenamine) in outpatients in the different counties of Norway in 2014, measured as number of prescriptions (Rx)/1,000 inhabitants and number of DDD/1,000 inhabitants/day. Data from NorPD (excl. health institutions).

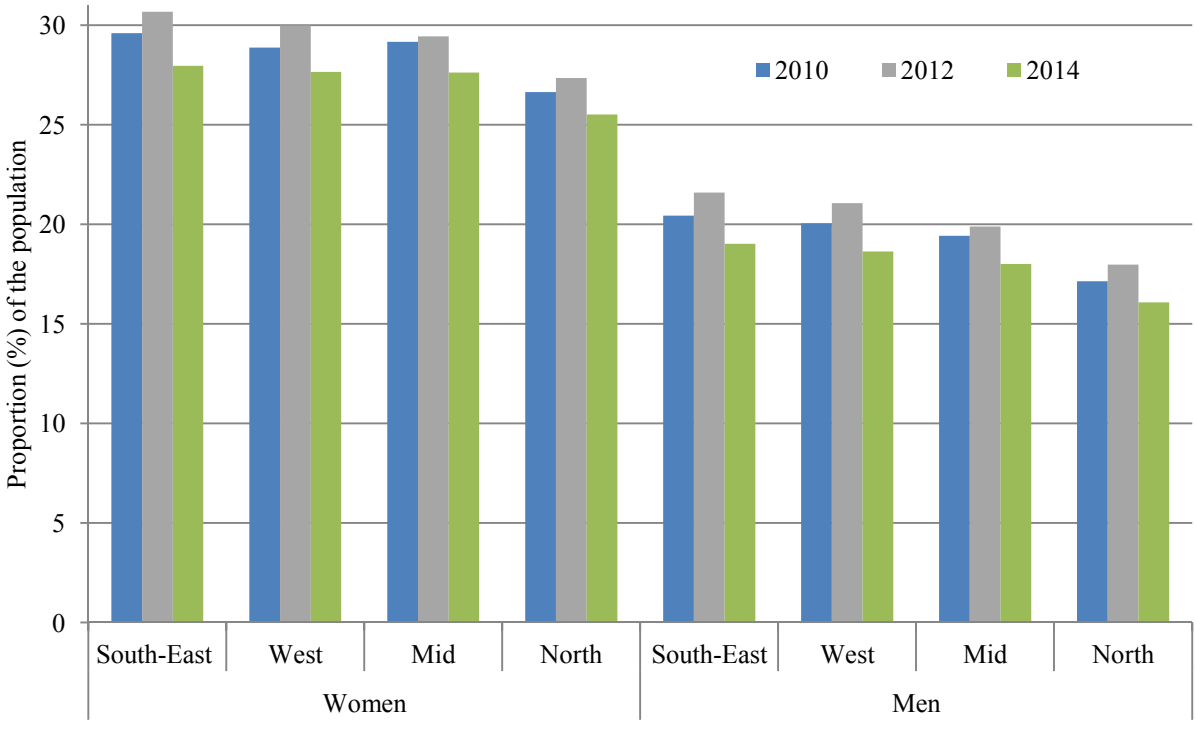

FIGURE 14. One year prevalence of systemic antibacterial use in ambulatory care by gender and health region in Norway for the years 2010, 2012 and 2014. Antibacterials for systemic use include ATC group J01, vancomycin (A07AA09), rifaximin (A07AA11), fidaxomicin (A07AA12) and metronidazole (P01AB01).

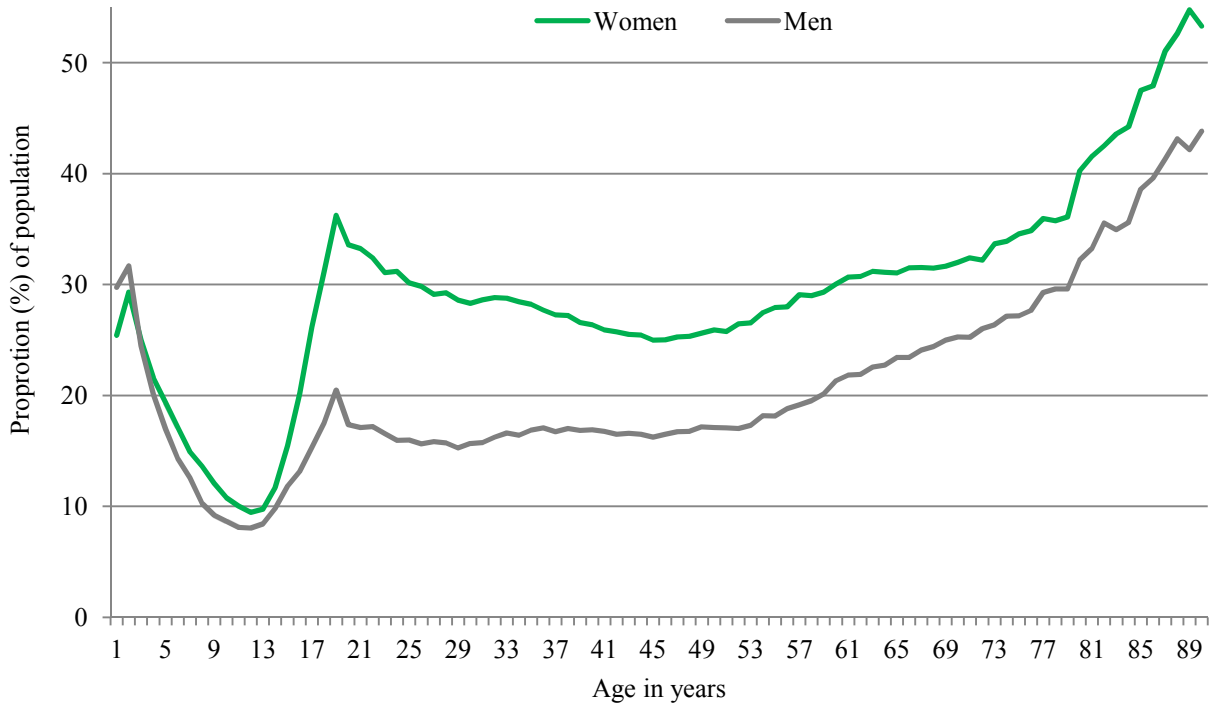

FIGURE 15. Proportion (%) of the population having dispensed at least one prescription of antibacterials (one year prevalence) in ambulatory care by gender and age in Norway in 2014. Antibacterials included are antibacterials for systemic use (ATC group J01, excl. methenamine). Prevalence in age groups above 65+ is adjusted according to persons from these age groups living outside institutions.

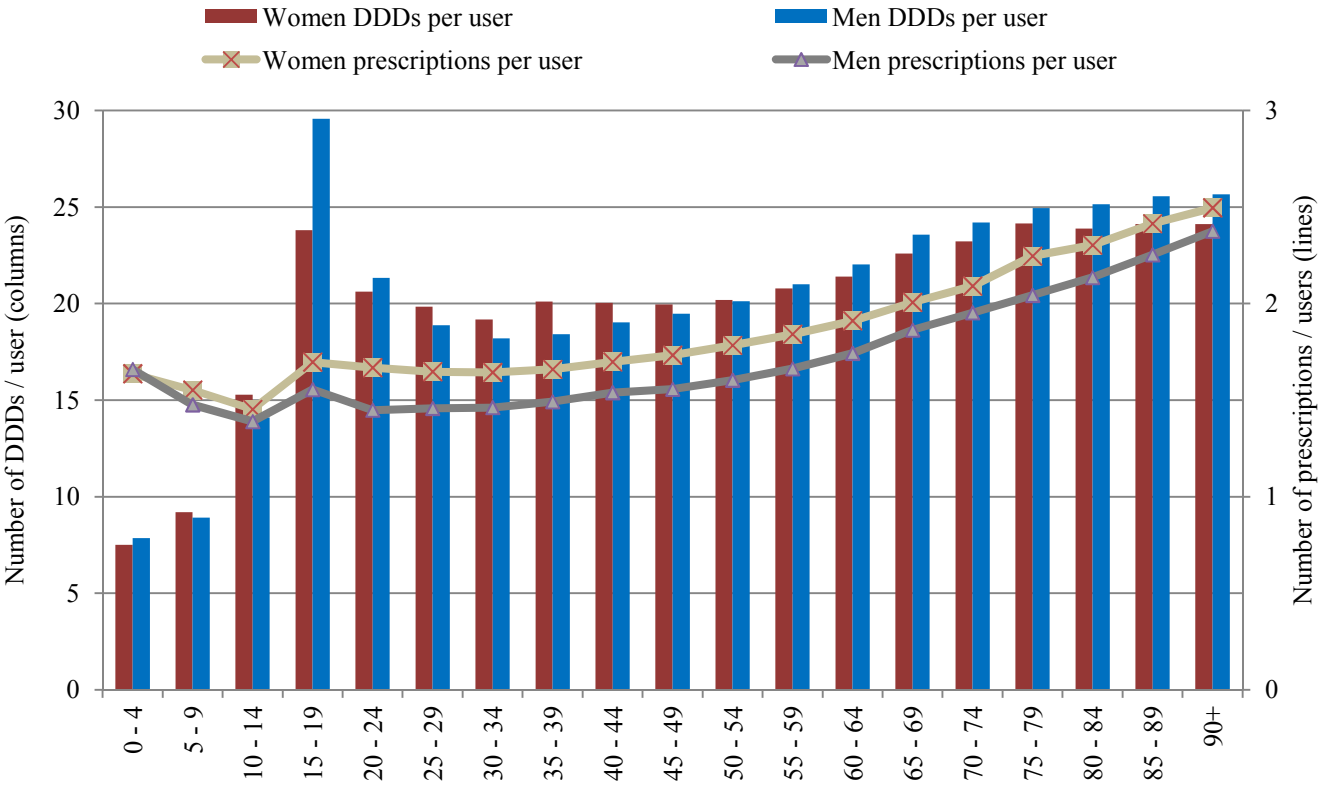

FIGURE 16. Mean number of prescriptions per person and mean number of DDDs per person among users of antibacterials in ambulatory care by gender and age in Norway in 2014. Antibacterials included are antibacterials for systemic use (ATC group J01, excl. methenamine).

Hospital use

In 2014, the antibacterial sales (in DDDs) to hospitals represented around 8% of total sales of antibacterials for human use in the country. The therapy pattern of antibacterials in hospitals does not change much from one year to another (Figure 17).

Penicillins (J01C) represent 48% of the use measured in DDDs in hospitals (J01CE 17%, J01CA 16% and J01CF 11%, J01CR 4%). The second largest group is the cephalosporins; 17% of all DDDs, the dominant subgroup being 3rd generation cephalosporins (J01DD). In 2014, seven substances accounted for 51% of DDDs used. These were benzylpenicillin, cloxacillin, cefotaxime, ampicillin, cefalotin, ciprofloxacin and pivmecillinam. Three single substances accounted for 30% of all antibacterial use in hospitals; benzylpenicillin (14%), cloxacillin (9%) and cefotaxime (7%).

Six antibiotic groups mainly used in hospitals are shown in Figure 18. Since 2006, there has been a stable increase in the use of carbapenems and piperacillin with the beta-lactamase inhibitor tazobactam. The use of 3rd generation cephalosporins decreased in 2014 and the use of 2nd generation cephalosporins has been decreasing over many years.

Figure 19 shows national trends in antibiotic use by hospital activity data (bed days and admissions) instead of population statistics. The two measurements together show the interplay between shorter hospital stays and intensity of antibiotic treatment. Although the health system is similar all over the country, there are large variations in volume of antibiotics used, measured in DDD/100 bed days, and in therapy profile (Figure 20). This is probably caused by differences in therapy traditions.

Antimycotics

The use of antimycotics for systemic use has been increasing in Norway (Figure 21). Hospital use of antimycotics represent 24% of total antifungal use measured in DDDs. Fluconazole is the most used agent. In July 2013, a warning regarding the use of oral ketoconazole was issued due to increased risk of liver damage. This resulted in decreased use of ketoconazole in ambulatory care in 2013 and no use in 2014. Of total DDDs, 24% was used in hospital. In ambulatory care, 2% of the DDDs were parenteral use and in hospitals, 62 % was parenteral use.

National Guidelines

The national guidelines for antibiotic use in ambulatory care and nursing homes were updated in 2013 and new national guidelines for hospital use was published in 2013. The Antibiotics Center for Primary Health Care (ASP) was established in 2006 and the Norwegian Advisory Unit for Antibiotic Use in Hospitals (KAS) was established in 2011. These centres are responsible for the continuous updating of national treatment guidelines. The updated guidelines and the commitment of the national centres will hopefully have a positive impact on therapy traditions and antibacterial prescribing in Norway.

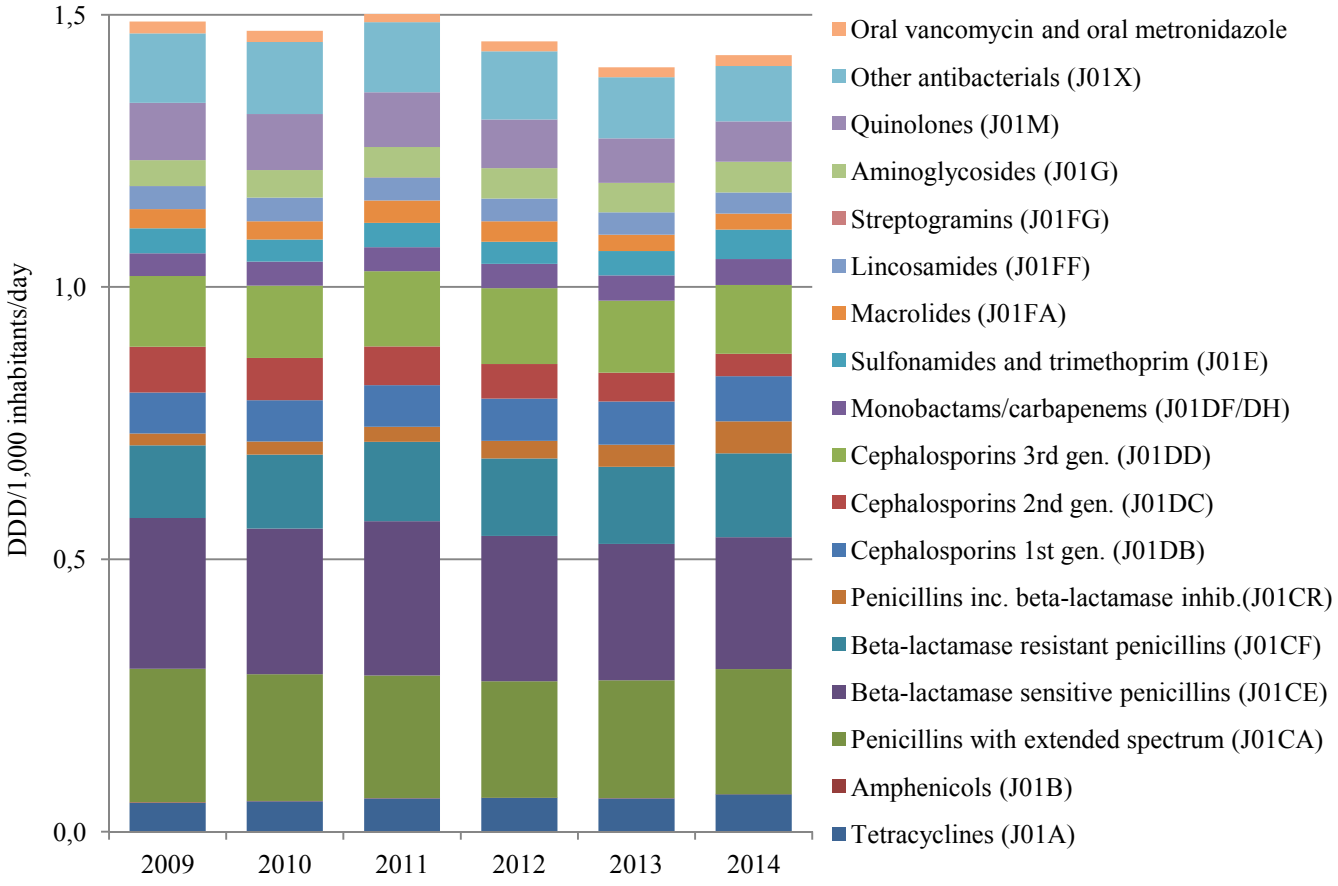

FIGURE 17. Proportions of antibacterial agents for systemic use in Norwegian hospitals 2009-2014, measured in DDD/1,000 inhabitants/day.

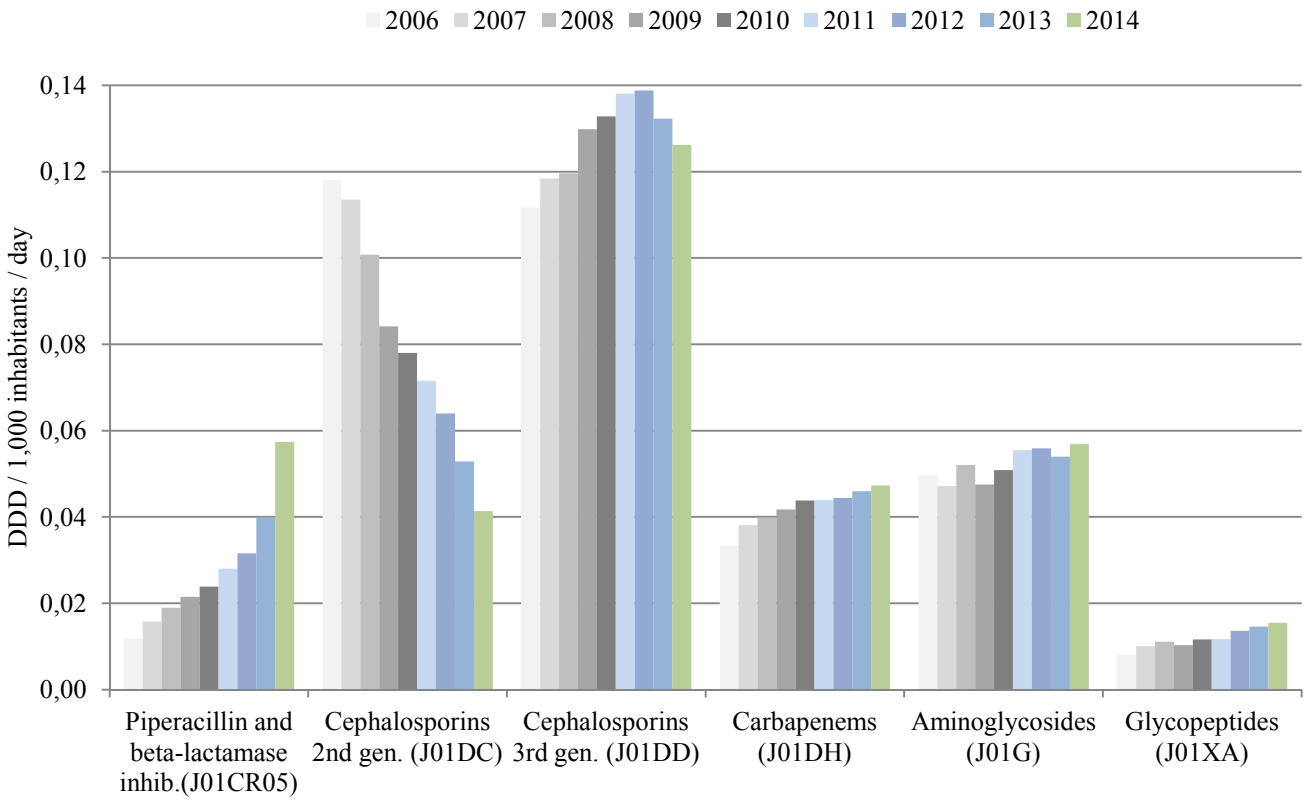

FIGURE 18. Proportions of selected antibacterial agents for systemic use in Norwegian hospitals 2006-2014, measured in DDD/1,000 inhabitants/day.

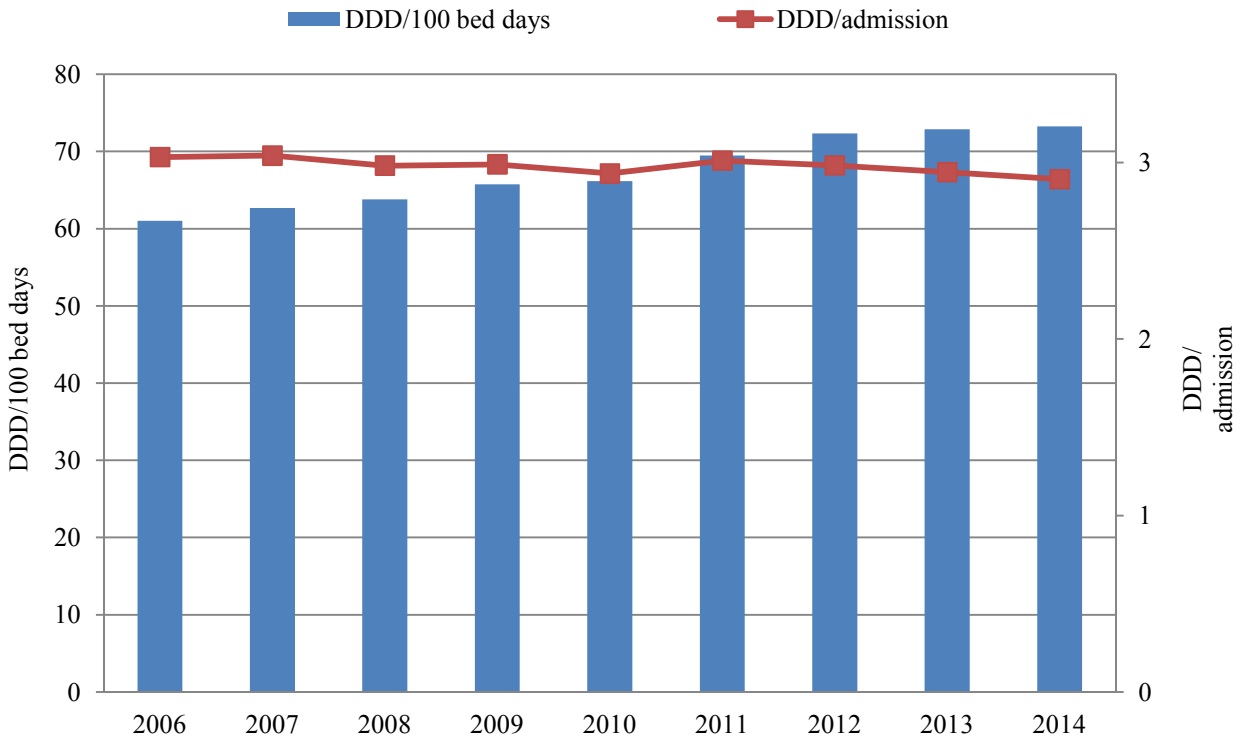

FIGURE 19. Total use of antibiotics in Norwegian hospital (somatic) 2006-2014, measured in DDD/100 bed days (blue bars) and DDD/admission (red line). Antibiotics are defined as J01 antibacterials for systemic use, A07AA09 vancomycin (oral), A07AA12 fidaxomycin and P01AB01 metronidazole (oral and rectal).

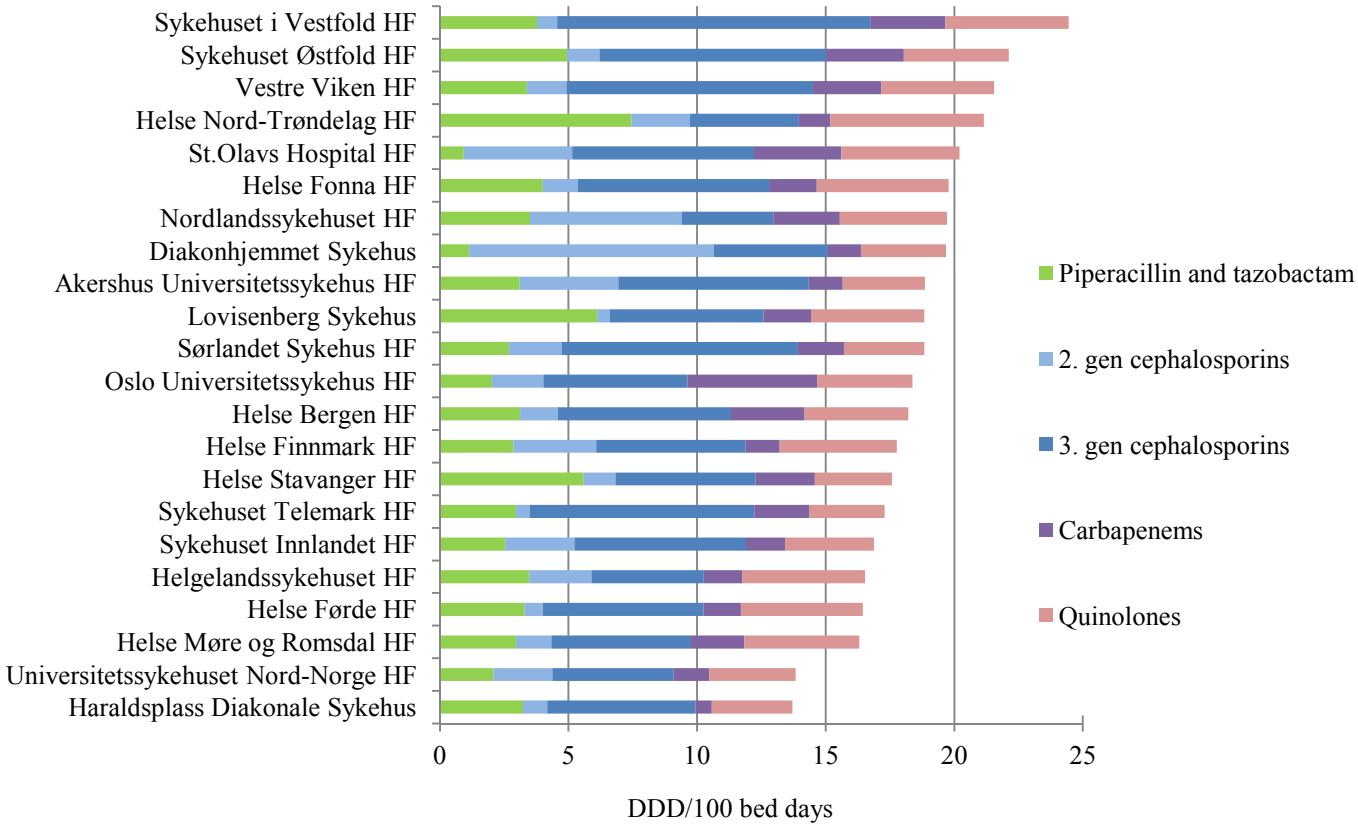

FIGURE 20. Proportions of selected antibacterial agents for systemic use (ATC J01CR05, ATC group J01DC, J01DD and J01M) in Norwegian hospitals/health trusts in 2014, measured in DDD/100 bed days.

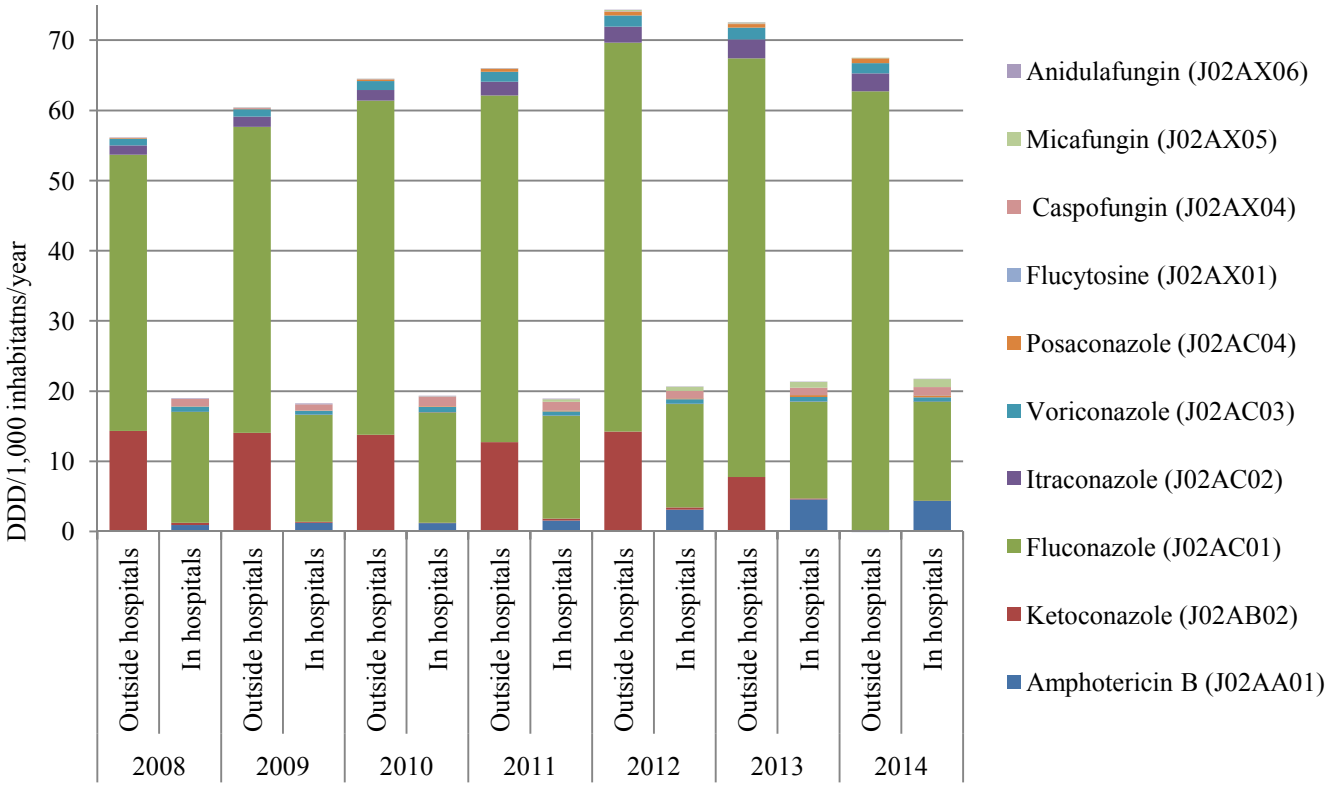

FIGURE 21. Proportions of antimycotics for systemic use in Norway for ambulatory care and hospitals 2008-2014, measured in DDD/1,000 inhabitants/year.

OCCURRENCE OF ANTIMICROBIAL RESISTANCE

INDICATOR BACTERIA FROM ANIMALS AND FOOD

Madelaine Norström, Jannice Schau Slettemeås, Anne Margrete Urdahl

The prevalence of acquired antimicrobial resistance among certain bacteria of the normal enteric microflora can be used as an indicator of the selective pressure from use of antimicrobial agents in various populations. These bacteria may form a reservoir of transferable resistance genes from which antimicrobial resistance can be spread to other bacteria, including those responsible for infections in animals or humans. Thus, monitoring of resistance among indicator bacteria of the normal enteric microflora from healthy animals, as well as from feed and food, is important to get an overview of the prevalence of antibiotic resistance, detect trends and evaluate effects of interventions.

In NORM-VET, *Escherichia coli* and *Enterococcus* spp. are used as indicator bacteria. In 2014, *E. coli* and *Enterococcus* spp. from caecal samples from broiler flocks, as well as *E. coli* from broiler meat, were included. The substances included in the test panels might not always be those used in veterinary medicine, but are included because of their importance for human health. Some of the cut-off values defining resistance applied in NORM-VET have been changed over the years. To facilitate comparisons in this report, data on prevalence of resistance presented in earlier reports have been recalculated using the cut-off values applied in 2014. Sampling, laboratory methods and data processing are described in Appendix 3.

Escherichia coli from broiler

Caecal samples from a total of 210 broiler flocks were examined and *E. coli* isolates were obtained from 205 samples (97.6%). One isolate per positive sample was susceptibility tested. The results are presented in Table 9 and Figures 22-23, and in the text.

TABLE 9. Antimicrobial resistance in isolates of *Escherichia coli* from caecal samples from broiler flocks (n=205) in 2014.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*															
			0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	1.5	[0.3-4.2]								98.5					0.5	1.0		
Tigecycline	0	[0.0-1.8]					91.2	5.9	2.9									
Chloramphenicol	0	[0.0-1.8]										99.5	0.5					
Ampicillin	6.3	[3.5-10.6]							5.4	46.3	41.5	0.5				6.3		
Cefotaxime	1.5	[0.3-4.2]					98.5		0.5	1.0								
Ceftazidime	1.5	[0.3-4.2]						98.5		1.0	0.5							
Meropenem	0	[0.0-1.8]		99.5		0.5												
Sulfamethoxazole	3.4	[1.4-6.9]										74.6	19.0	2.9				3.4
Trimethoprim	3.4	[1.4-6.9]					86.8	9.3	0.5						3.4			
Azithromycin	ND	ND								23.9	58.0	18.0						
Gentamicin	0	[0.0-1.8]						52.7	43.4	3.9								
Ciprofloxacin	3.4	[0.0-1.8]	87.3	9.3		1.0	2.4											
Nalidixic acid	3.4	[1.4-6.9]									96.6			1.0	1.5	1.0		
Colistin	0	[0.0-1.8]							96.6	3.4								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. CI=confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC-value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

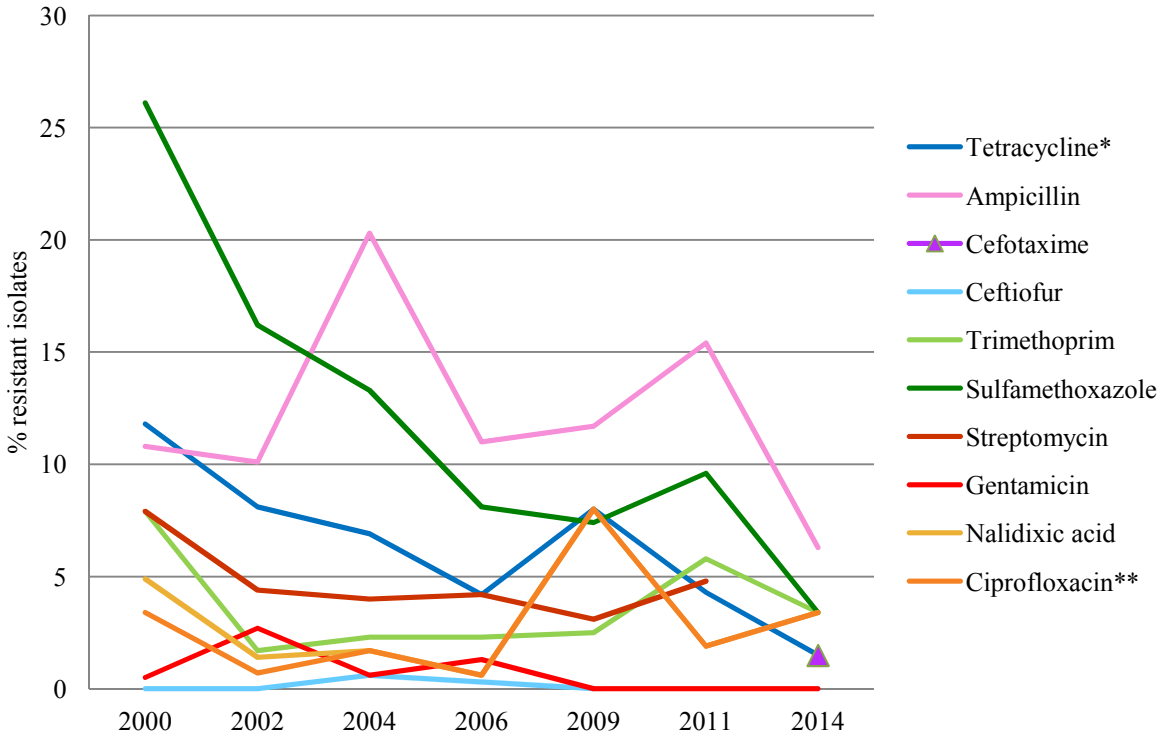

FIGURE 22. Prevalence of resistance to various antimicrobials in *Escherichia coli* from broiler isolates in 2000-2014. The cut-off values used in NORM-VET 2014 were applied. *Oxytetracycline in 2002 and 2004. **Enrofloxacin before 2006.

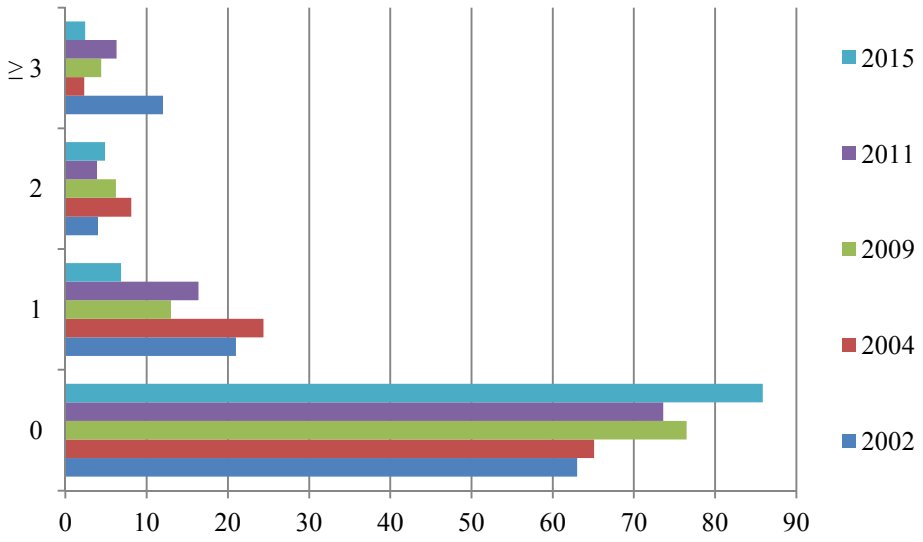

FIGURE 23. Antimicrobial resistance profile for *Escherichia coli* faecal isolates from broiler in 2002-2014. Proportions of isolates susceptible to all or resistant to one, two, and three or more antimicrobial agents are illustrated. Antimicrobial agents tested for varies between the years as also shown in the figure above and this probably has an effect on the results.

RESULTS AND COMMENTS

The 2014 data indicate a moderate occurrence of resistance among *E. coli* from broiler caecal samples. In total, 85.4% of the isolates were susceptible to all antimicrobial agents included. Altogether, 6.8% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin and sulfamethoxazole), 4.9% to two (nalidixic acid and ciprofloxacin), 2.0% to three and 0.5% to four antimicrobial agents (Figure 23). Resistance to ampicillin was the most frequently identified resistance determinant, followed by resistance to sulfamethoxazole, trimethoprim, ciprofloxacin and nalidixic acid. Compared to previous data, the proportion of isolates being fully susceptible has increased and the proportion being resistant to one or more than three antimicrobial agents has decreased. There has, however, been a change in the panel of antimicrobial agents tested for compared to previous years, and this may have had an effect on the results. Nevertheless, since the start of NORM-VET in 2000, the prevalence of resistance to some antimicrobial agents in *E. coli* from broilers has indeed decreased as illustrated in Figure 22, especially resistance to sulfamethoxazole and tetracycline (highly significant decrease from 2000 to 2014 with $p=1.1*10^{-10}$ and $p=2.7*10^{-5}$, respectively). Trimethoprim resistance has also decreased although not significantly ($p=0.052$), as well as ampicillin resistance from 2011 to 2014 ($p=0.003$). Resistance to the fluoroquinolone ciprofloxacin and to the quinolone nalidixic acid was low and identified in only 3.4% of the isolates in 2014 compared to 2% in 2011 and 8% in 2009 (Figure 22). With the exception of the 8% finding in 2009, the level of resistance to quinolones among *E. coli* from broiler seems to be low and relatively stable. However, due to the peak in 2009 and the finding of possible plasmid-mediated quinolone resistance among *E. coli* from broiler faecal and meat samples in 2009 and 2012, respectively, a selective method was used in the same sample material to investigate these aspects further (see below).

By using the non-selective *E. coli* method, three of the *E. coli* isolates from broilers were resistant to third generation cephalosporines, with the bla_{CMY-2} gene identified in all three. In addition, a selective method was used to further investigate the reservoir of ESBL/AmpC-producing *E. coli* in the same sample material (see below). Quantitative methods were also applied in order to estimate the burden of the AmpC producing *E. coli* among the total amount of *E. coli* isolates present in caecal contents or as contaminants of meat samples (see separate presentation on page 35).

In an international perspective, the occurrence of resistance among *E. coli* from Norwegian broiler is quite low, though the occurrence varies markedly between countries reporting to EFSA with the Nordic countries having the lowest resistance levels (EFSA and ECDC Summary report 2013). This favourable situation is probably due to the very limited use of antibiotics in the Norwegian broiler production.

Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from broiler and broiler meat

Acquired resistance to third generation cephalosporins among Gram-negative bacteria has received special attention in recent years. Production of extended-spectrum beta-lactamases (ESBLs) or plasmid-mediated AmpC are major mechanisms behind such resistance. Internationally, poultry seem to be the species among production animals associated with the highest prevalences of *Escherichia coli* and *Salmonella* producing ESBL/AmpC. In Norway, a selective method for the detection of ESBLs/AmpC producing *E. coli* was included in NORM-VET from 2011. Like many other countries, this selective method showed that Norwegian broiler production had a high prevalence of *E. coli* resistant to third generation cephalosporins encoded by the plasmid-borne bla_{CMY-2} gene with 43% [95% CI: 36.7-49.2] positive broiler flocks (NORM-VET 2011) and 32.2% [95% CI: 25.9-39.1%] positive broiler meat samples (NORM-VET 2012). Among the poultry breeder flocks, 7.3% [95% CI: 3.8-12.4%] were found positive for ESBL/AmpC producing *E. coli* (NORM-VET 2012). There is no selection pressure from cephalosporin usage in Norway, but the poultry production is dependent on import of breeding animals and these animals have been shown to be the source of introduction. Since the finding of this ESBL/AmpC producing *E. coli* reservoir in Norwegian broiler production, the industry has taken measures to limit the number of imported breeding animal flocks carrying ESBLs/AmpC producing *E. coli.*

In 2014, selective screening for ESBL/AmpC producing *E. coli* was performed on samples from broiler and broiler meat. A total of 210 caecal samples from broiler flocks and 201 broiler meat samples were screened for the presence of ESBL producing *E. coli*.

Sampling, laboratory methods and data processing are described in Appendix 3. The data are presented in Table 10 and in the text.

TABLE 10. Antimicrobial resistance in ESBL/AmpC-producing *Escherichia coli* isolates from caecal samples from broiler flocks (n=75) and broiler meat samples (n=58) in 2014.

Substance	Sample	Resistance (%)	[95% CI]	Distribution (%) of MIC values (mg/L)*														
				0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	Caecal	0	[0.0-4.8]								98.7	1.3							
	Meat	3.4	[0.4-11.9]								93.1	3.4					3.4		
Tigecycline	Caecal	0	[0.0-4.8]					100											
	Meat	0	[0.0-6.2]					100											
Chloramphenicol	Caecal	0	[0.0-4.8]										100						
	Meat	0	[0.0-6.2]										100						
Ampicillin	Caecal	100	[95.2-100]														100		
	Meat	100	[93.8-100]													6.9	93.1		
Cefotaxime	Caecal	100	[95.2-100]								1.2	10.7	88.0						
	Meat	98.3	[90.8-100]					1.7			1.7	8.6	87.9						
Ceftazidime	Caecal	100	[95.2-100]									1.3	72.0	26.7					
	Meat	98.3	[90.8-100]						1.7		6.9	50.0	41.4						
Meropenem	Caecal	0	[0.0-4.8]		98.7	1.3													
	Meat	0	[0.0-6.2]		100														
Sulfamethoxazole	Caecal	40	[28.9-52.0										50.7	9.3					40.0
	Meat	15.5	[7.3-27.4]											56.9	27.6				15.5
Trimethoprim	Caecal	1.3	[0.03-7.2]					97.3	1.3						1.3				
	Meat	0	[0.0-6.2]					98.2	1.8										
Azithromycin	Caecal	ND	ND								61.3	38.7							
	Meat	ND	ND								65.5	34.5							
Gentamicin	Caecal	0	[0.0-4.8]						22.7	73.3	4.0								
	Meat	0	[0.0-6.2]					1.7	29.3	63.8	5.2								
Ciprofloxacin	Caecal	2.7	[0.3-9.3]	92.0	5.3			2.7											
	Meat	10.3	[3.9-21.2]	79.3	10.3			10.3											
Nalidixic acid	Caecal	2.7	[0.3-9.3]									97.3					2.7		
	Meat	10.3	[3.9-21.2]									89.7				1.7	6.9	1.7	
Colistin	Caecal	0	[0.0-4.8]							98.7	1.3								
	Meat	0	[0.0-6.2]							100									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. CI=confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC-value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

By use of the selective method, *E. coli* resistant to third generation cephalosporins were found in 35.7% (95% CI: 29.2-42.6) and 28.9% (95% CI: 22.7-35.6) of caecal and meat samples, respectively. All isolates had a beta-lactam resistance profile corresponding to an AmpC phenotype, and real-time PCR showed that all isolates contained the bla_{CMY-2} gene. As described above, only three cephalosporin resistant isolates, all with the bla_{CMY-2} gene, were found by using the non-selective procedure indicating that the within-flock prevalence probably is low. These aspects are further investigated by quantititaive methods (see separate presentation on page 35).

The majority (63.2%) of the ESBL/AmpC producing *E. coli* from broiler caecal and meat samples was only resistant to beta-lactams, i.e. ampicillin and the third-generation cefalosporins cefotaxime and ceftazidime. In addition, there was some resistance observed for sulfamethoxazole, tetracycline, trimethoprim and the quinolones ciprofloxacin and nalidixic acid (Table 10). Among the isolates, 10.6% and 24.1% of the caecal and meat isolates, respectively, showed decreased susceptibility to the carbapenem ertapenem (data not shown), with MIC values above the EUCAST cut-off value at 0.06 mg/L, i.e. MIC 0.12-0.25 mg/L. Ertapenem is known to have lower specificity to detect carbapenemase producing Enterobacteriaceae than the two other carbapenems imipenem or meropenem (Cohen *et al.* 2010). None of the isolates were resistant to imipenem or meropenem (data not shown).

The current findings by the selective method indicate that there has been a small reduction with regard to prevalence of ESBL/AmpC producing *E. coli* in broiler flocks and broiler meat since the investigations in 2011 (43%) and 2012 (32.2%), respectively, though the reduction observed is not statistically significant. Also, a change in sampling procedure has been made from boot samples to pooled caecal samples per flock, and this may have had an impact on the result. Boot swab sampling mirrors the prevalence in the broiler house, while the caecal samples mirror presence in the animals.

The results from broiler flocks and broiler meat are in contrast to the results reported by the industry that only a small proportion of imported breeding animal flocks were positive in 2014 (http://www.animalia.no/Listesider/Aktuelt-og-fagstoff/Tiltak-mot-antibiotikaresistens-virker-/). However, the imported breeding flocks were tested at hatching and were too young to be the parenting flocks to the majority of the broiler flocks tested in the NORM-VET programme in 2014. Therefore, it is probably too early to observe any effect of the measures implemented by the industry on the results in this report.

The presence of bacteria resistant to cephalosporins in food production animals is of great concern. Resistant bacteria in the food chain may have an impact on resistance development in human bacterial populations and it should be an overall goal to keep the level of resistant bacteria in production animals and through the meat processing chain at the lowest possible level. The situation regarding ESBL/AmpC producing *E. coli* in broilers in Norway is similar to the situation in Sweden. A Swedish report from 2014 (Egervärn *et al.* 2014), concludes that food on the Swedish market, including the ESBL/AmpC producing *E. coli* in broilers, is a limited contributor to the prevalence of ESBL-producing *E. coli* within the human healthcare sector for the time being. However, it points out that antibiotic resistance is dynamic and that national rates can change rapidly and need to be followed further. Preliminary results from a Norwegian study indicate that although highly similar ESBL/AmpC producing *E. coli* and AmpC resistance plasmids can be found in both human patients and Norwegian poultry meat, this is a very limited proportion of the total cases of human ESBL producing *E. coli* (Berg *et al.* 2015). However, this is a small study and further studies are needed in order to fully understand the role of resistant bacteria in food and their impact on the resistance epidemiology in humans.

Quantification of extended-spectrum beta-lactamase (ESBL)/AmpC producing *Escherichia coli* in caecal and meat samples from broilers

Acquired resistance to third-generation cephalosporins among Gram-negative bacteria is of concern as third-generation cephalosporins are among the antimicrobial agents defined as critically important for human health by WHO. Production of extended-spectrum beta-lactamases (ESBLs) or plasmid-mediated AmpC are major mechanisms behind such resistance. Internationally, poultry seems to be the production animals associated with the highest prevalence of ESBL/AmpC-producing *Escherichia coli*. In 2006, third-generation cephalosporin resistance was identified in an *E. coli* isolate originating from Norwegian broilers for the first time (2, 6). The introduction of a selective method for the detection of ESBLs/AmpC producing *E. coli* in NORM-VET from 2011 indicated that the Norwegian broiler production had a high prevalence of *E. coli* resistant to third-generation cephalosporins encoded by the plasmid-borne bla_{CMY-2} gene with 43% [95% CI: 36.7-49.2] positive broiler flocks (3) and 32.2% [95% CI: 25.9-39.1%] positive broiler meat samples (4). However, among indicator *E. coli* isolated by a non-selective method from the same samples, only very few displayed cephalosporin resistance, suggesting that AmpC producing *E. coli* was present at a low level. In order to estimate the burden of the AmpC producing *E. coli* among the total amount of *E. coli* isolates present in caecal contents or as contaminants of meat samples, quantitative methods were applied.

Material and Methods

In NORM-VET 2014, selective screening for ESBL/AmpC producing *E. coli* was performed on caecal samples from 210 broiler flocks and on 201 broiler meat samples. Of these, quantification was conducted on caecal samples and broiler meat samples from 75 broiler flocks and 58 broiler meat samples, respectively, all identified as positive for ESBL/AmpC producing *E. coli* in the selective screening.

Caecal samples

In short, 1 g of caecal content was mixed with 9 mL (0.1 g) 1% peptone glycerol (Peptone: Becton, Dickinson and Company, Glycerol: Merck). Subsequently, a tenfold dilution was made by mixing 20 µL of caecal content in peptone glycerol with 180 µL physiological saline, in total six dilutions including undiluted sample. From each dilution 10 µL were plated by running drop method (1) onto a square MacConkey agar plate (Difco); one agar plate with 1 mg/L cefotaxime and one without supplements. The agar plates were incubated for 24±3 hours at 41±0.5°C. The plate count was determined as the highest dilution with visible growth. A presumptive positive colony from the least diluted sample and from the highest dilution with growth were streaked onto MC agar, MC agar containing 1 mg/L cefotaxime, and blood agar (Heart infusion agar, Difco) containing 5% bovine blood. The colonies were verified as *E. coli* using MALDI-TOF MS (Bruker Daltronik GmbH, Bremen, Germany) and the bla_{CMY-2} gene was identified using real-time PCR (5). The fraction of ESBL/AmpC-producing *E. coli* due to presence of the bla_{CMY-2} gene in relation to the total number of the *E. coli* was determined.

Meat samples

The semi-quantification of ESBL/AmpC-producing *E. coli* in meat samples was performed using a protocol developed by the Technical University of Denmark (personal communication, Yvonne Agersø). In short, 15 g of meat were mixed with 120 mL MacConkey (MC) broth containing 1 mg/L cefotaxime. Subsequently, 45 mL (5 g) and 10 mL (1 g) of the broth were transferred to new containers. Further, 1 mL from the 10 mL container was transferred to a tube with 9 mL MC broth containing 1 mg/L cefotaxime. This step was repeated until a concentration of 10^{-4} (0.0001 g sample) was reached. From the last tube, 1 mL of the broth was discarded. After incubation of the broth for 16-18 hours at 41±0.5°C, 10 µL from each dilution were plated by running drop method (Herigstad et al. 2001) onto a square MC agar containing 1 mg/L cefotaxime. The plates were further incubated for 24±3 hours at 41±0.5°C. One presumptive positive colony from the least diluted sample and from the highest dilution with growth were streaked onto MC agar containing 1 mg/L cefotaxime, and blood agar (Heart infusion agar, Difco) containing 5% bovine blood. The colonies were verified as *E. coli* using MALDI-TOF MS (Bruker Daltronik GmbH, Bremen, Germany) and the bla_{CMY-2} gene identified using real-time PCR (5).

Results

The results from the quantification of ESBL/AmpC-producing *E. coli* in caecal samples of broilers is shown in Table 11, and the results from the semi-quantification of ESBL/AmpC-producing *E. coli* in broiler meat samples are shown in Table 12.

TABLE 11. Number of isolates identified as ESBL/AmpC-producing *Escherichia coli* due to presence of the bla_{CMY-2} gene in relation to the total number of the *E. coli* of the gut flora (caecum) of broilers sampled in 2014.

ESBL/AmpC-producing ***E. coli*** **(%)**	**No of samples**	**Proportion of samples (%)**
0.001	10	13.5
0.01	25	33.8
0.1	23	31.1
1.0	12	16.2
10.0	4	5.4

TABLE 12. Number of isolates identified as ESBL/AmpC-producing *Escherichia coli* due to presence of the bla_{CMY-2} per gram broiler meat sample.

ESBL/AmpC-producing ***E. coli*** **(cfu/g)**	**No of Samples**	**Proportion of samples (%)**
0.2	51	87.9
1	4	6.9
10	2	3.4
100	1	1.7

The occurrence of ESBL/AmpC-producing *E. coli* among the total caecal *E. coli* is less than 0.1 % in more than 78.3% of the samples. A 10% occurrence of ESBL/AmpC-producing *E. coli* was detected in only 5.4% of the samples (Table 11). These low levels of ESBL/AmpC-producing *E. coli* among the total caecal *E. coli* correspond well with the results from the quantification of ESBL/AmpC-producing *E. coli* in broiler meat samples showing that a majority (87.9%) of the samples had very low levels of ESBL/AmpC-producing *E. coli* present (≤ 0.2cfu/g), as shown in Table 12. Though, in one of the broiler meat samples, the levels of ESBL/AmpC-producing *E. coli* was 100 cfu/g showing that there might be large variations in the levels of ESBL/AmpC-producing *E. coli* contamination.

To conclude, the results indicate that the majority of broiler flocks positive for ESBL/AmpC-producing *E. coli* have very low levels of the bacteria present among the caecal *E. coli*. Further, the levels of contamination on broiler meat samples are generally very low, though some variation may occur. This was the first time quantification of ESBL/AmpC-producing *E. coli* was performed. The methods used for caecal and meat samples are different, and comparison of the results therefore has to be performed with caution. Moreover, the numbers of investigated samples were low, and more quantitative data on occurrence of ESBL/AmpC-producing *E. coli* are therefore needed to elaborate these aspects more thoroughly.

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Quinolone resistant *Escherichia coli* from broiler and broiler meat

Fluoroquinolones are defined by the WHO as critically important for treatment of human infections. Monitoring the resistance to these substances in the bacterial population is therefore of special interest. Through the years from 2000, the prevalence of resistance to quinolones among indicator *E. coli* from broiler (caecal or meat samples) seems to be low and relatively stable. However, due to a peak in 2009 and the finding of possible plasmid-mediated quinolone resistance among *E. coli* from broiler caecal and meat samples in 2009 and 2012, respectively, a selective method was implemented in NORM-VET to investigate these aspects further.

In 2014, selective screening for quinolone resistant *E. coli* was performed on caecal samples from broiler and broiler meat. A total of 210 caecal samples from broiler flocks and 198 broiler meat samples were screened for the presence of quinolone resistant *E. coli*.

Sampling, laboratory methods and data processing are described in Appendix 3. The data are presented in Table 13 and in the text.

TABLE 13. Antimicrobial resistance in quinolone resistant isolates of *Escherichia coli* from caecal samples from broiler flocks (n=188) and from broiler meat (n=140) in 2014.

Substance	Sample	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*														
				0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	Caecal	9.6	[5.8-14.7]]								90.4					3.7	5.9		
	Meat	7.2	[3.5-12.7]								92.9					2.9	4.3		
Tigecycline	Caecal	0	[0.0-1.9					100											
	Meat	0	[0.0-2.6]					99.3	0.7										
Chloramphenicol	Caecal	2.6	0.9-6.1										97.3				1.6	1.1	
	Meat	0.7	[0.02-3.9]										99.3				0.7		
Ampicillin	Caecal	14.9	[10.1-20.8]							1.1	17.0	63.3	3.7	0.5			14.4		
	Meat	13.6	[8.4-20.4]							5.0	11.4	60.7	9.3			0.7	12.9		
Cefotaxime	Caecal	2.1	[0.6-5.4]					97.9	0.5			0.5	1.1						
	Meat	2.8	[0.8-7.2]					97.1					2.1				0.7		
Ceftazidime	Caecal	1.6	[0.3-4.6]						98.4				1.6						
	Meat	2.8	[0.8-7.2]						97.1				1.4	1.4					
Meropenem	Caecal	0	[0.0-1.9]		100														
	Meat	0	[0.0-2.6]		100														
Sulfamethoxazole	Caecal	10.6	[6.6-16.0]										43.6	36.2	9.0	0.5			10.6
	Meat	15.0	[9.1-22.0]										61.4	14.3	9.3				15.0
Trimethoprim	Caecal	6.4	[3.3-10.9]					68.6	22.9	2.1						6.4			
	Meat	5.0	[2.0-10.0]					72.1	17.1	5.7						5.0			
Azithromycin	Caecal	ND	ND								45.2	52.1	2.7						
	Meat	ND	ND								53.6	45.0	1.4						
Gentamicin	Caecal	0	[0.0-1.9]						71.3	26.1	2.7								
	Meat	0	[0.0-2.6]						60.0	35.7	4.3								
Ciprofloxacin	Caecal	100	[98.1-100]				9.0	81.4	2.1	1.6	0.5	0.5	4.8						
	Meat	97.3	[96.1-100]			0.7	10.7	72.1	7.9	0.7	0.7	2.1	4.3	0.7					
Nalidixic acid	Caecal	100	[98.1-100]												2.1	17.0	57.4	23.4	
	Meat	100	[97.4-100]												16.4	37.1	46.4		
Colistin	Caecal	0	[0.0-1.9]							100									
	Meat	0	[0.0-2.6]							96.4	3.6								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = cut-off not defined by EUCAST. CI=confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC-value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

By use of a selective method *E. coli* resistant to quinolones were found in 188 of the 210 caecal samples and in 140 of the 198 broiler meat samples investigated (89.5%, 95% CI: 84.6-93.3 and 70.7%, 95% CI: 63.8-76.9, respectively). A majority of the isolates were resistant only to the quinolones nalidixic acid and ciprofloxacin, 76.6% and 72.1%, respectively. Further resistance to one additional antimicrobial agent was observed in 12.8% and 18.6%; and to two and more in 10.7% and 9.3% of the isolates, respectively. Out of these multi-resistant isolates, three and four caecal isolates, and one each of the broiler meat isolates, were resistant to four (ampicillin, trimethoprim, sulfamethoxazole, tetracycline) and five (ampicillin, trimethoprim, sulfamethoxazole, tetracycline, chloramphenicol) additional antimicrobial agents, respectively.

All, but one of the isolates had decreased susceptibility to both nalidixic acid and the fluoroquinolone ciprofloxacin with MIC values above the EUCAST cut-off at 16 mg/L and 0.064 mg/L, respectively. Resistance to quinolones in bacteria is usually caused by mutations in the quinolone resistance determining region (QRDR) involving the

genes *gyrA*, *gyrB*, *ParC,* and *ParE*. In addition, plasmid-mediated quinolone resistance (PMQR) genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, *aac(6')-1b-cr, oqxAB*) are responsible for low-level resistance to quinolones. Among the current isolates, none had MIC profiles corresponding to possible presence of plasmid-mediated quinolone resistance genes (PMQR), indicating that the resistance probably is mediated by mutations in the QRDR. This is supported by preliminary results from a pilot study performed by the industry that found the resistance to be caused by mutations in the *gyrA* gene. However, in cases of concomitant mutations and plasmid-mediated resistance, the presence of PMQR cannot be discovered by MIC profiles and further characterisation of the isolates is necessary.

Although the selective method detects quinolone resistant *E. coli* in the majority of the samples, only a few isolates are usually detected by the non-selective procedure, indicating that the within-flock prevalence of quinolone resistant *E. coli* in general is low. Nevertheless, the findings were to some degree surprising, as there is no selection pressure from use of quinolones in the Norwegian broiler production. It is unknown when, why and how this quinolone resistance has emerged in broilers and what impact this reservoir may have. Further investigations are needed in order to examine these aspects further.

Enterococcus spp. from broiler

Caecal samples from a total of 210 broiler flocks were collected. *E. faecium* or *E. faecalis* was identified in 208 of the samples (99%). One isolate per positive sample was susceptibility tested. Sampling, laboratory methods and data processing are described in Appendix 3. The results are presented in Tables 14-15, Figure 24, and in the text.

TABLE 14. Antimicrobial resistance in *Enterococcus faecalis* spp. (n=65) from caecal samples from broiler flocks in 2014.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*																
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥ 2048
Tetracycline	52.3	[39.5-64.9]						47.7					12.3	30.8	9.2				
Tigecycline	0	[0.0-5.5]	3.1	41.5	46.2	9.2													
Chloramphenicol	3.1	[0.4-10.7]								63.1	33.8			3.1					
Ampicillin	0	[0.0-5.5]					20.0	67.7	10.8	1.5									
Erythromycin	20.0	[11.1-31.8]						49.2	26.2	4.6	9.2	3.1				7.7			
Quinupristin - dalfopristin	ND	ND							4.6	6.2	70.8	18.5							
Gentamicin	0	[0.0-5.5]									27.7	72.3							
Ciprofloxacin	0	[0.0-5.5]					15.4	78.5	6.2										
Vancomycin	0	[0.0-5.5]						36.9	41.5	21.5									
Teicoplanin	0	[0.0-5.5]					100												
Linezolid	0	[0.0-5.5]					1.5	47.7	50.8										
Daptomycin	1.5	[0.04-8.3]					4.6	26.2	61.5	6.2	1.5								

*Bold vertical lines denote microbiological cut-off values for resistance. ND = cut-off not defined by EUCAST. CI=confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC-value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

TABLE 15. Antimicrobial resistance in *Enterococcus faecium* (n=143) from caecal samples from broiler flocks in 2014.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*																
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥ 2048
Tetracycline	11.2	[6.5.1-17.5]						88.1	0.7		1.4		0.7	6.3	2.1	0.7			
Tigecycline	0	[0.0-2.5]	26.6	57.3	14.7	1.4													
Chloramphenicol	0	[0.0-2.5]								88.1	10.5	0.7	0.7						
Ampicillin	0	[0.0-2.5]					35.0	36.4	18.9	9.8									
Erythromycin	8.4	[4.4-14.2]						54.5	30.8	6.3	4.9	1.4				2.1			
Quinupristin - dalfopristin	ND	ND					14.7	28.7	23.8	31.5	0.7	0.7							
Gentamicin	0	[0.0-2.5]									83.2	15.4	1.4						
Ciprofloxacin	5.6	[2.4-11.7]					3.5	38.5	16.8	35.7	5.6								
Vancomycin	0	[0.0-2.5]						74.1	23.1	2.8									
Teicoplanin	0	[0.0-2.5]					98.6	1.4											
Linezolid	0	[0.0-2.5]						36.4	63.6										
Daptomycin	0.7	[1.8-3.8]				0.7		7.0	39.9	51.7	0.7								

*Bold vertical lines denote microbiological cut-off values for resistance. ND = cut-off not defined by EUCAST. CI=confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC-value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

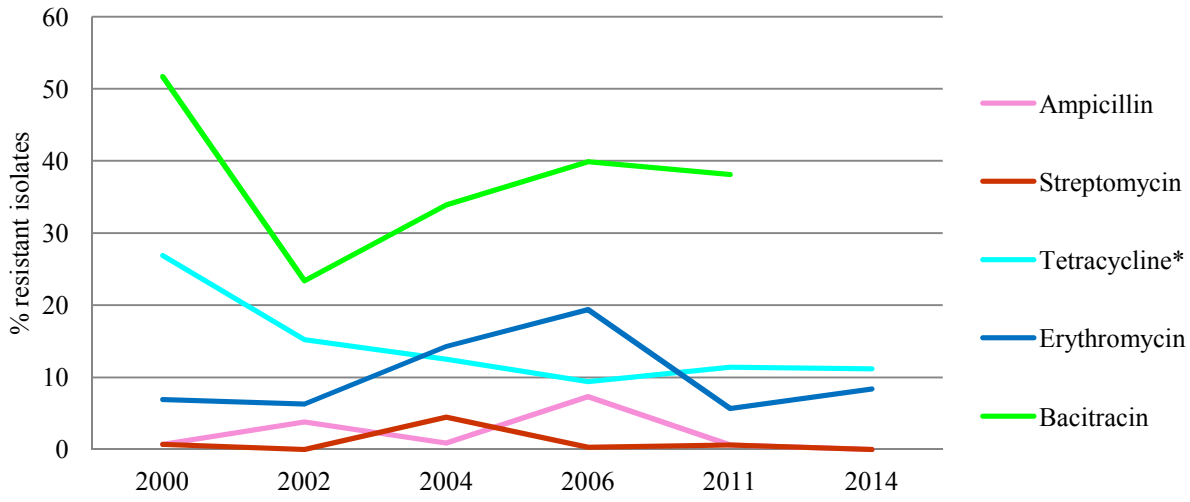

FIGURE 24. Prevalence of resistance to various antimicrobials in *Enterococcus faecium* from broiler (meat and caecal samples) 2000-2014. The breakpoints used in NORM-VET 2014 were applied. * Oxytetracycline in 2002 and 2004.

RESULTS AND COMMENTS

There was a very high occurrence of resistance among *E. faecalis* and a high occurrence among *E. faecium* isolates.

E. faecalis: In total 32.3% of the isolates were susceptible to all antimicrobial agents included in the test panel. Resistance to one (mainly tetracycline) and two antimicrobial agents (mainly tetracycline and erythromycin) was detected in 60% and 6%, respectively. In addition, 1.5% of the isolates were resistant to three antimicrobial agents.

E. faecium: In total 76.2% of the isolates were susceptible to all antimicrobial agents included in the test panel. Resistance to one (mainly tetracycline and erythromycin) and two antimicrobial agents was detected in 21.7% and 2.1%, respectively.

Surprisingly, there is a very high prevalence of tetracycline resistance among *E. faecalis* (52.3%) despite insignificant use of oxytetracycline for clinical purposes in Norwegian broiler production. Resistance to erythromycin has decreased compared to the survey in 2006, but is still fairly common in particular for *E. faecalis*. Erythromycin has never been used in broilers in Norway. However, resistance may have been acquired by former use of spiramycin as cross-resistance between erythromycin and spiramycin is common. Spiramycin was licensed for use in poultry until 1998 when it was withdrawn due to limited sales. No vancomycin resistant isolates of *E. faecium* or *E. faecalis* were detected by random selection. Avoparcin, which induces cross-resistance to vancomycin, was routinely used as a growth promoter in Norwegian broiler and turkey production from 1986 until it was banned in 1995. Studies have shown that this use has selected for an extensive reservoir of vancomycin resistant enterococci (VRE) in Norwegian broiler production. The reservoir has persisted after the ban was implemented (see below).

Vancomycin resistant *Enterococcus* spp. (VRE) from broiler

A total of 210 caecal samples from broiler flocks were screened for the presence of VRE. The results are presented in Table 16, and in the text. Laboratory methods and data processing are described in Appendix 3.

TABLE 16. Antimicrobial resistance in vancomycin resistant *Enterococcus faecium* (n=14) from caecal samples from broiler flocks in 2014.

Substance	Resistance (n)	Distribution (n) of MIC values (mg/L)*															
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥ 2048
Tetracycline	2						12					1	1					
Tigecycline		10	4															
Chloramphenicol	0								14									
Ampicillin	9					5				9								
Erythromycin	0						14											
Quinupristin - dalfopristin	ND					1		9	4									
Gentamicin	0									14								
Ciprofloxacin	0				12	1		1										
Vancomycin	14														14			
Teicoplanin	14											4	10					
Linezolid	0						10	4										
Daptomycin	0						3	10	1									

*Bold vertical lines denote microbiological cut-off values for resistance. ND = cut-off not defined by EUCAST. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

VRE was isolated from a total of 14 caecal samples (6.7%, 95% CI: 3.7-10.9). This is a significant decrease compared to the prevalence in 2011 (15.9%) and at a similar level as the result from 2009 (7.5%). However, the sampling method has changed from boot swabs in 2009 and 2011 to caecal samples from 10 animals in 2014. Boot swab sampling mirrors the prevalence in the broiler house, while caecal samples mirror the prevalence in the animals, and comparison of the results should therefore be made with caution. Further follow up using the same sampling method needs to be performed to investigate if there is a changing trend in prevalence. All isolates were identified as *E. faecium* carrying the *vanA* gene, and all showed cross-resistance to teicoplanin. Nine isolates showed additional resistance to ampicillin and two were resistant to tetracycline.

Methicillin resistant *Staphylococcus aureus* in pigs in Norway 2014

MRSA CC398, t034 was detected for the first time in Norway in the 2011 monitoring in specimens from pigs sampled at one of the investigated slaughterhouses (1). In the 2012 monitoring, an anonymous study, MRSA CC398, t034 was detected in samples from only one farm (2). However, in 2013, two separate clusters of infection with MRSA CC398, t034 in Norwegian pig herds were discovered, one in the south-eastern part of the country and one in the south-western county of Rogaland. The Norwegian Food Safety Authorities decided to try to combat the bacteria in infected herds by slaughter of animals, thorough cleaning and disinfection of rooms, and restart with MRSA-free pigs. To the authors' knowledge such a strategy has not been conducted in any other countries. MRSA is not a health problem in pigs, and the measures are implemented to prevent a putative risk for humans.

To provide a basis for sound administrative decisions for combating MRSA in Norwegian pig herds, a survey of MRSA in sow herds was performed in the spring of 2014 to obtain knowledge about the prevalence in Norwegian swine herds (3).

Materials and methods

All Norwegian pig herds with more than 10 sows were sampled by the authorities during spring 2014. Pigs were sampled by using sterile SodiBox cloths moistened with sterile water. A point on the cloth was rubbed firmly against the skin behind both ears of the pig (about 5x5 cm on each side). Each cloth was used for 20 pigs, and a total of three cloths, representing 60 pigs distributed on all rooms and all age groups except suckling piglets, were taken per herd. The three cloths were analysed as one pooled sample. In addition, in each herd two cloths were used for environmental samples taken in all rooms with pigs. Each cloth was used on about 15 touch points (about 10x10 cm per location) representing furnishings, feeders, water nipples, window sills, door handles, tools, boots, ventilation system etc. These two cloths were analysed as one pooled sample.

The samples were submitted to the Norwegian Veterinary Institute's laboratory in Oslo and analysed for MRSA by a method described by the EU reference laboratory on antimicrobial resistance (DTU Food, National Food Institute, Copenhagen, Denmark): Pre-enrichment in 300 mL Mueller Hinton broth with 6.5% NaCl at 37°C for 16-20 h. Then 1 mL was transferred into 9 mL tryptone soya broth with cefoxitin (3.5 mg/L) and aztreonam (75 mg/L). After incubation at 37°C for 16-20 h, 10 µL were inoculated on Brilliance^{TM} MRSA Agar (Oxoid) and incubated at 37 °C for 24-48 h. Suspect colonies were isolated on 5% blood agar and submitted to the Norwegian human reference laboratory for MRSA at St. Olavs Hospital in Trondheim for verification and typing. 95% confidence interval (CI) was calculated based on a binomial distribution.

Results

A total of 986 herds were included in the survey. Nine herds had already been sampled in the investigation of two ongoing "outbreaks" in the same time period, and these were not sampled again. None of these had MRSA positive pigs at the time of reporting. MRSA was identified in only one herd; situated in the county of Oppland (0.1%; 95% CI: 0.003-0.6). Both the animal and the environmental samples were positive. The isolates were typed as CC398, t011. In addition, one contact herd was identified with MRSA CC398, t011. Both herds went through slaughter of animals, thorough cleaning and disinfection of rooms, and restart with MRSA-free pigs in line with the previous strategy for MRSA in swine herds.

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Narasin in the broiler production – usage and resistance

Coccidiostats are routinely used in Norwegian broiler production and since 1996 such use has been almost totally dominated by the ionophore narasin. The annual usage of narasin has been increasing since 2007 compared to the number of broiler produced as illustrated in Figure 25.

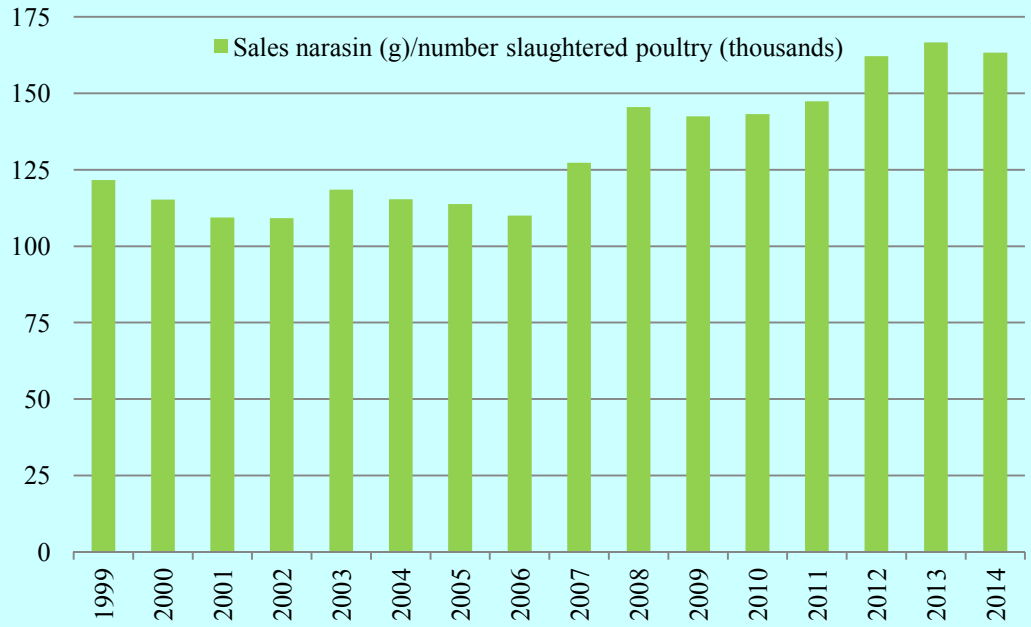

FIGURE 25. Annual sales of narasin (g active substance) per thousand slaughtered poultry in Norway during the years 1999-2014.

In addition to being a coccidiostat, narasin also has an antibacterial effect, and has therefore been included in the test panel for *Enteroccocci* spp. in the NORM-VET programme for the years 2001 to 2013. The selection pressure exerted by narasin use in broiler production is probably the reason why narasin resistance is frequently observed among enterococci from broilers, in particular for *E. faecium*. Lately it has been an increased concern that the use of narasin might have an influence on the development of resistance towards other antimicrobial agents (cross-resistance). A previous study has reported cross-resistance between narasin and salinomycin *(Butaye, Devriese et al. 2000)*. A preliminary analysis was therefore conducted to identify the possibilities of development of cross-resistance between narasin and other antimicrobial agents.

Material and methods

Resistance data of all *E. faecium* isolates originating from all animal species included in NORM-VET during 2003-2013 were included in the analysis (n=988). These isolates originated from cattle (n=49), dogs (n=12), broilers (n=577), layers (n=82), swine (n=165), sheep (n=1) and turkeys (n=102). Note that these data have been collected for different years for each species. The data were analysed using three separate models categorising the isolates into three groups; all species except broilers (non-broilers), only broilers, and all species.

The associations between narasin resistance and resistance to other antimicrobial agents that were included in the test panels during the same period (2003-2013) were analysed by logistic regression with the resistance to tetracycline, ampicillin, erythromycin, gentamicin, bacitracin and streptomycin as the outcome variables and resistance to narasin as explanatory variable. The isolates were categorised as resistant or susceptible based on the previous epidemiological cut-off value for narasin (MIC > 2mg/L) applied in NORM-VET 2012 and for the other antimicrobial agents the cut-off values applied in the NORM-VET report from 2013 were used.

TABLE 17. Percentage of *Enterococcus facium* isolates classified as resistant to the substances tested for during NORM-VET 2003-2013. Resistance to the substances marked with * were included as outcome variables in the logistic regression models.

Substances	% Resistance		
	All species except for broilers (n=411)	Only broilers (n=577)	All species (n=988)
Tetracycline*	15.8	10.6	12.8
Chloramphenicol	0	0	0
Ampicillin*	4.9	4.0	4.4
Erythromycin*	22.6	14.2	17.7
Gentamicin*	23.1	19.4	21
Streptomycin*	28.0	19.8	23.2
Vancomycin	0	0.7	0.4
Kanamycin	1.5	1.0	1.2
Bacitracin*	12.4	38.1	27.2
Linezolid	0	0	0
Narasin	16.8	72.4	49.3
Virginiamycin	3.4	3.3	3.3

Results

For the majority of substances, the percentage of resistant isolates was higher among non-broilers than broilers. However, broiler isolates were more resistant to narasin and bacitracin than isolates from non-broilers. The logistic regression model found a significant positive association ($p<0.001$) between narasin and bacitracin resistance regardless of whether the included isolates were from broilers or not. Furthermore, a significant positive association between narasin and gentamicin resistance ($p<0.001$) and between narasin and streptomycin resistance ($p<0.05$) was observed for broiler isolates. However, a significant negative association was observed between narasin and streptomycin resistance for non-broilers ($p<0.001$) as well as for all isolates ($p<0.01$).

Discussion and preliminary conclusions

Bacitracin resistance is more common among broiler isolates than from other species. Bacitracin was formerly used as an antimicrobial growth promoter (AGP), but the usage was negligible and declining during the 1990s and since 1997 bacitracin has not been used as AGP in food animal production in Norway. Therefore the relatively high occurrence of bacitracin resistance might be a result of persisting resistances. Furthermore, since narasin is used in broiler production only, and the positive associations between narasin and bacitracin resistance is observed also in non-broilers, the resistance to bacitracin is probably not due to narasin use. However, whether narasin resistance leads to cross-resistance to bacitracin or vice versa, or both resistances can be explained by some underlying cause(s) remains unclear.

The occurrence of streptomycin and gentamicin resistance is higher for non-broilers, whereas the occurrence of narasin resistance is more than four-fold higher for the broiler isolates. The results from the logistic regression show that the probability of streptomycin resistance decreases if narasin resistance is present for non-broilers, whereas for broilers the probabilities of streptomycin resistance or gentamicin resistance increases if narasin resistance is present, indicating that cross-resistance does not occur between these substances.

In order to investigate if there is a possible causal relationship between the usage of narasin and resistance to other antimicrobials further studies are needed.

References:

1. Butaye, P., L. A. Devriese and F. Haesebrouck (2000). "Incomplete cross resistance against ionophores in *Enterococcus faecium* and *Enterococcus faecalis* strains from pigs and poultry." Microb Drug Resist 6(1): 59-61

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ZOONOTIC AND NON-ZOONOTIC ENTEROPATHOGENIC BACTERIA

Madelaine Norström, Jannice Schau Slettemeås, Anne Margrete Urdahl, Astrid Louise Wester

Zoonotic and non-zoonotic enteropathogenic bacteria represent a considerable public health problem. Furthermore, the occurrence of acquired antimicrobial resistance in such bacteria represents a major public health concern. Therefore, it is of great importance to monitor the occurrence of antimicrobial resistance in zoonotic and other enteropathogenic bacteria at relevant stages in the farm-to-fork continuum. In Norway, *Salmonella* isolates from control programmes concerning feed samples, animals and food products, as well as diagnostic samples from animals are monitored for antimicrobial resistance.
Regarding human isolates, all *Salmonella*, *Shigella* and *Yersinia enterocolitica* isolates are included, as well as a representative number of *Campylobacter* isolates. Sampling, laboratory methods and data processing are described in Appendix 4.

The NORM results on human isolates of enteropathogenic *Enterobacteriaceae* are interpreted according to the clinical breakpoints given by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In case of missing clinical breakpoints, epidemiological cut-off values (ECOFFs) were used based on zone-distribution judgments or, as for *Campylobacter*, on ECOFFs given by EUCAST. Multi-drug resistance (MDR) was defined as resistance to three or more antimicrobial categories according to the 2011 ECDC/CDC joint definitions.

SALMONELLA SPP.

Salmonella from animals

The situation regarding occurrence of *Salmonella* spp. in food production animals in Norway is very favourable as such animal populations are considered virtually free from *Salmonella* spp. To document and maintain this favourable situation, Norway runs an extensive surveillance programme that covers both live animals (cattle, pigs and poultry) and meat samples. The *Salmonella* isolates examined in NORM-VET include those that are detected in this programme, as well as those detected by clinical submissions to the Norwegian Veterinary Institute. The data are presented in Table 18 and in the text.

TABLE 18. Antimicrobial resistance in *Salmonella* spp. (n=24) from animals (cattle=1, pig=4, dog=7, cat=6, poultry=4, alpaca=1; quail=1); *S.* Typhimurium (n=16) and other *Salmonella* spp. (n=8) in 2014.

		Distribution (n) of MIC values (mg/L)*																		
Substance	n resistant	0.003	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	6									18						6				
Tigecycline	0						21	2	1											
Chloramphenicol	1											23					1			
Ampicillin	5								16	2		1				5				
Cefotaxime	1						23		1											
Ceftazidime	0							23		1										
Meropenem	0			23		1														
Sulfamethoxazole	6											6	6	6						6
Trimethoprim	1						22	1							1					
Azithromycin	ND									15	9									
Gentamicin	2							19	3					1	1					
Ciprofloxacin	1	21			2			1												
Nalidixic acid	1										21	2					1			
Colistin	5								5	14	5									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = cut-off not defined by EUCAST. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested for are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

In 2015, a total of 24 *Salmonella* spp. isolates from animals were susceptibility tested. The 16 isolates of *S.* Typhimurium included one each from an alpaca, three swine herds (two monophasic), a cattle herd and a chicken flock, and four and six from dogs (one monophasic) and cats, respectively. The remaining eight isolates belonged to six different serovars; *S.* Kedougou from dogs (n=2), *S.* Infantis from one dog and one poultry flock, *S.* Taset from one swine herd, *S.* Braenderup from one quail flock, and *S.* Heidelberg and *S.* Mbandaka from two different poultry flocks.
Of the 24 isolates, 13 were fully susceptible, six isolates were resistant to one antimicrobial, one to two antimicrobials, three to three antimicrobials and one isolate was multi-resistant to five antimicrobials originating from a cattle herd. One isolate originating from a chicken farm was multi-resistant to seven of the antimicrobial substances tested for.

Salmonella from human clinical specimens

In 2014, the National Reference Laboratory (NRL) for Enteropathogenic Bacteria at the Norwegian Institute of Public Health (NIPH) performed antimicrobial susceptibility testing on a total of 1,093 unique *Salmonella* isolates from human infections. As indicated in Table 19, 23.3% was reported as acquired in Norway, 69.6% was acquired abroad, whereas the place of acquisition was unknown for 7.0%. Travel abroad is considered a risk factor for obtaining bacteria carrying antimicrobial resistance. To enable evaluations with this in mind, figures of the proportions of place of acquisition from 2010 to 2014 is shown for all *Salmonella* as a whole (Figure 24), and for some of the different serovars.

All isolates were tested for resistance against four different antibiotic groups: beta-lactams (ampicillin, cefotaxim, ceftazidime and meropenem), ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole. In addition, one third of the isolates were tested for nalidixic acid, azithromycin, tetracycline, and chloramphenicol.

TABLE 19. Distribution of human isolates of *Salmonella* serovars (n=1,093) in 2014 according to place of acquisition.

	Place of acquistion		
	Norway	Abroad	Unknown
S. Typhimurium including *S. enterica* serovar 4,[5],12:i- (n=242)	101	113	28
S. Enteritidis (n=398)	54	325	19
S. Typhi (n=7)	0	5	2
S. Paratyphi (n=8)	0	8	0
Other *Salmonella* (n=438)	100	310	28
Total (n=1,093)	**255 (23.3%)**	**761 (69.6%)**	**77 (7.0%)**

The dominating serovars were *S.* Typhimurium (n=113) and its monophasic variant (n=129), with 242 isolates (22.1%) of all *Salmonella* isolates, and *S.* Enteritidis with 398 (36.4%) of the isolates. The numbers of *S.* Typhi and *S.* Paratyphi isolates remain low. For 2014 the total numbers of isolates were seven and eight, respectively.

The results of the antimicrobial susceptibility testing for 2014 isolates are presented in Tables 20-23, in Figures 26-33, and in the text.

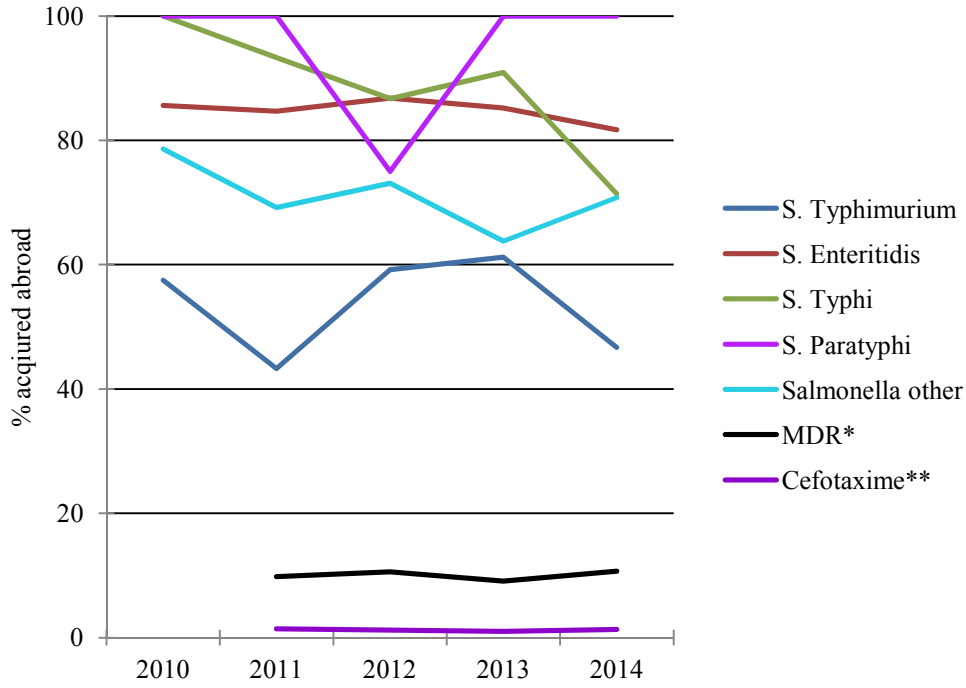


FIGURE 26. Proportion of unique isolates of *Salmonella* acquired abroad, and tested for antimicrobial resistance 2010- 2014 by serovar group, and total results for MDR and cefotaxim. *MDR testing 2011-2013 was calculated for five groups of antibiotics, whereas from 2014 onwards MDR results shown are for seven groups. **Before 2014 cefpodoxime was tested.

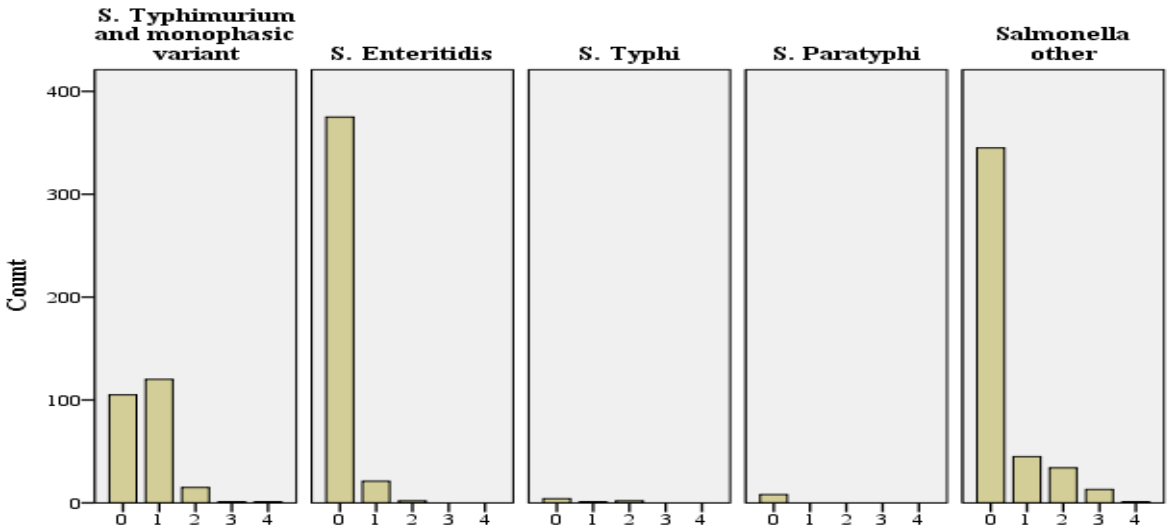


FIGURE 27. Distribution of number of antimicrobials that *Salmonella* isolates from 2014 (n=1,093) were resistant to; by serovar groups. The four antibiotic groups tested were beta-lactams, aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole.

ANITMICROBIAL RESISTANCE IN BLOOD CULTURE ISOLATES OF SALMONELLA

A total of 65 strains were isolated from blood culture, representing 0.5% of all blood culture isolates when skin contaminants are excluded. There were four *S.* Typhimurium and its monophasic variant, 19 *S.* Enteritidis (29.2%), six *S.* Typhi, six *S.* Paratyphi, and 30 (46.2%) other *Salmonella* isolates (Figure 28) representing fifteen different serovars. Most isolates from blood culture were tested against seven groups of antibiotics. The number that each group of *Salmonella* was resistant to is shown in Figure 29. Although the numbers are small, it seems that the most frequent serovar in blood culture, *S.* Enteritidis, is fairly sensitive to all groups of antimicrobials, in spite of having been acquired abroad in more than half of the cases. It should be emphasised that low-level ciprofloxacin resistance is underestimated. When calculating MDR, low-level resistance against nalidixic acid and/or high-level resistance against ciprofloxacin counted as quinolone resistance.

In addition to blood culture isolates, *Salmonella* was isolated from sample materials indicating serious infection in two patients: *S.* Paratyphi A from ascites and *S.* Enteritids from a biopsy material.

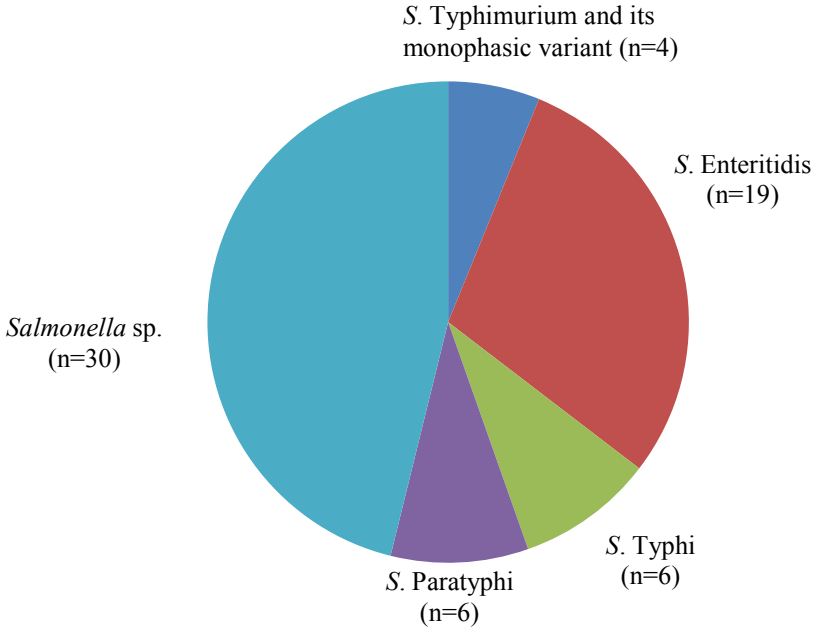


FIGURE 28. Distribution of blood culture isolates of different *Salmonella* serovars (n=65) in 2014.

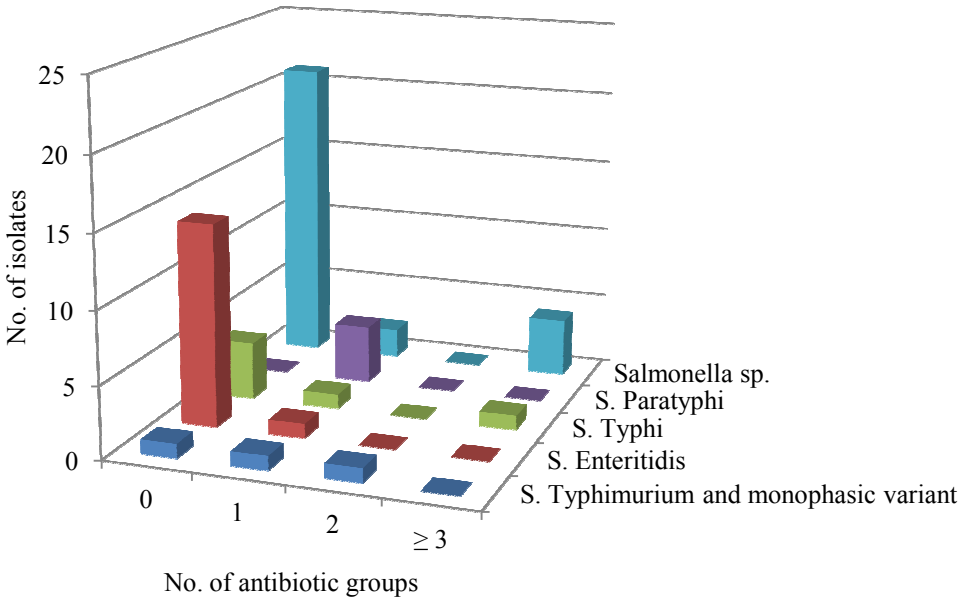


FIGURE 29. Antimicrobial resistance in *Salmonella* isolated from blood culture in 2014 tested against seven antibiotic groups (n=55 out of the total n=65) with the number of isolates resistant to none, one, two, or three or more antimicrobial groups, respectively. The seven antibiotic groups tested were beta-lactams, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, macrolides, tetracyclines, and chloramphenicol.

RESISTANCE IN SALMONELLA IRRESPECTIVE OF SAMPLE MATERIAL

TABLE 20. Human isolates of domestically acquired *Salmonella* Typhimurium-group (n=101) during 2014, including domestically acquired *S. enterica* serovar 4,[5],12:i:- (n=54). Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	46.5	-	53.5
Cefotaxime	≤ 1	> 2	100.0	0.0	0.0
Ceftazidime	≤ 1	> 4	100.0	0.0	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin [1]	≤ 0.5	> 1	100.0	0.0	0.0
Nalidixic acid [2] *	≥ 19	< 19	74.6	-	25.4
Gentamicin	≤ 2	> 4	99.0	0.0	1.0
Azithromycin [2] *	≤ 11	> 11	100.0	-	0.0
Tetracycline [2] *	≥ 13	< 13	23.8	-	76.2
Chloramphenicol *	≤ 8	> 8	90.5	-	9.5
Trimethoprim-sulfamethoxazole	≤ 2	> 4	96.0	0.0	4.0

[1] Low-level resistance against ciprofloxacin is underestimated by using these breakpoints; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. [2] Epidemiological cut-off values based on zone distribution evaluations. * Only tested in 63/101 isolates.

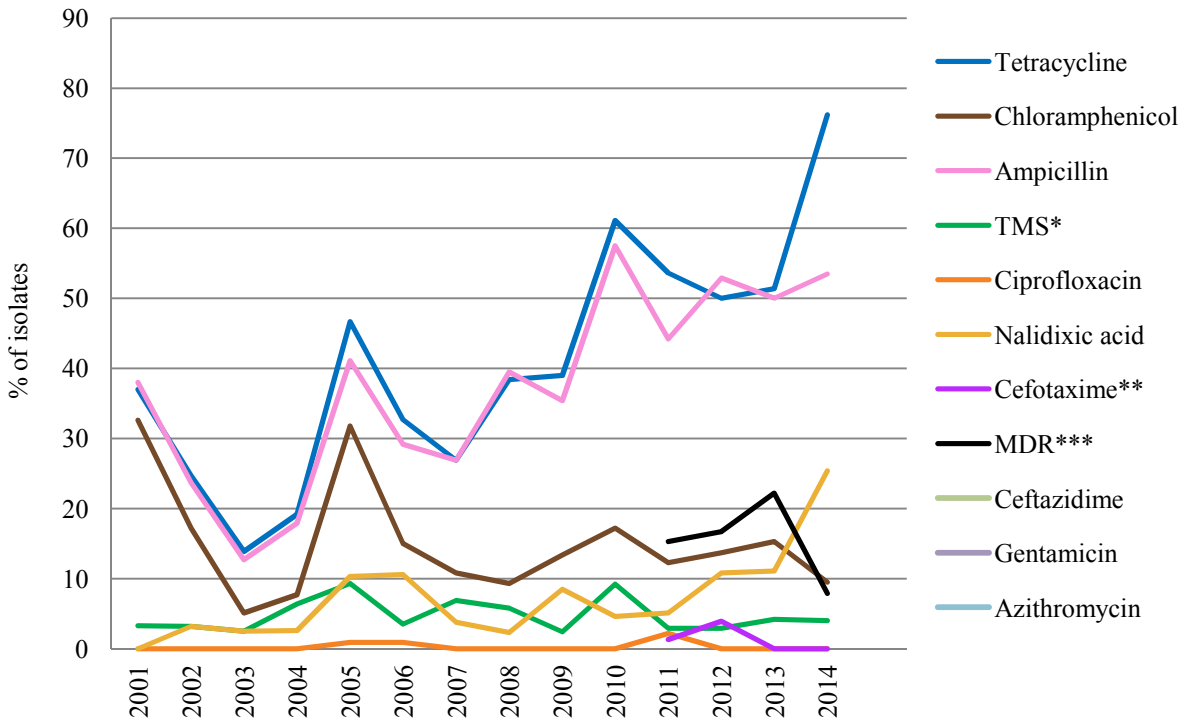


FIGURE 30. Percentage of resistance to various antimicrobial agents in *Salmonella* Typhimurium-group including *S. enterica* serovar 4,[5],12:i:- from humans infected in Norway 2001-2014. *TMS=Trimethoprim-sulfamethoxazole. **Before 2014 cefpodoxime was tested. ***MDR testing 2011-2013 was calculated for five groups of antibiotics, whereas from 2014 onwards MDR results shown are for seven groups.

TABLE 21. Human isolates of *Salmonella* Typhimurium-group acquired abroad during 2014 (n=113), including *S. enterica* serovar 4,[5],12:i:- (n=59). Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	41.6	-	58.4
Cefotaxime	≤ 1	> 2	96.5	0.0	3.5
Ceftazidime	≤ 1	> 4	95.5	1.8	2.7
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin [1]	≤ 0.5	> 1	97.3	1.8	0.9
Nalidixic acid [2]*	≥ 19	< 19	88.6	-	11.4
Gentamicin	≤ 2	> 4	93.8	0.0	6.2
Azithromycin [2] *	≤ 11	> 11	100.0	-	0.0
Tetracycline [2] *	≥ 13	< 13	16.5	-	83.5
Chloramphenicol *	≤ 8	> 8	82.3	-	17.7
Trimethoprim-sulfamethoxazole	≤ 2	> 4	93.8	0.0	6.2

[1] Low-level resistance against ciprofloxacin is underestimated by using these breakpoints; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. [2] Epidemiological cut-off values based on zone distribution evaluations. * Only tested in 79/113 isolates.

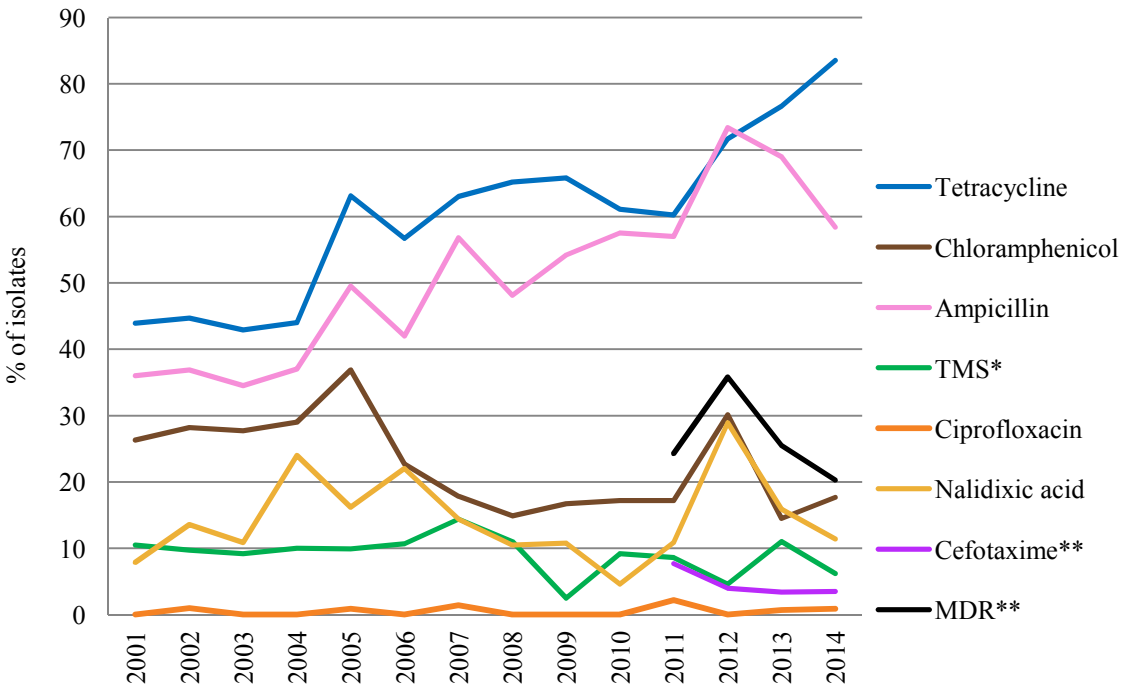


FIGURE 31. Percentage of resistance to various antimicrobial agents in *Salmonella* Typhimurium-group including *S. enterica* serovar 4,[5],12:i:- from humans infected outside Norway 2001-2014. *TMS=Trimethoprim-sulfamethoxazole. **Before 2014 cefpodoxime was tested. ***MDR testing 2011-2013 was calculated for five groups of antibiotics, whereas from 2014 onwards MDR results shown are for seven groups.

TABLE 22. Human isolates of *Salmonella* Enteritidis (n=398[#]), acquired during 2014, irrespective of place of acquisition. Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	94.5	-	5.5
Cefotaxime	≤ 1	> 2	98.3	0.0	1.7
Ceftazidime	≤ 1	> 4	98.5	1.5	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin [1]	≤ 0.5	> 1	99.4	0.3	0.3
Nalidixic acid [2]*	≥ 19	< 19	68.0	-	32.0
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Azithromycin [2] *	≤ 11	> 11	100.0	-	0.0
Tetracycline [2] *	≥ 13	< 13	93.3	-	6.7
Chloramphenicol *	≤ 8	> 8	100.0	-	0.0
Trimethoprim-sulfamethoxazole	≤ 2	> 4	99.5	0.5	0.0

[1] Low-level resistance against ciprofloxacin is underestimated by using these breakpoints; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. [2] Epidemiological cut-off values based on zone distribution evaluations. *Only tested in 75/398 isolates. #Place of acquisition; Norway (n=54), abroad (n=325), unknown (n=19).

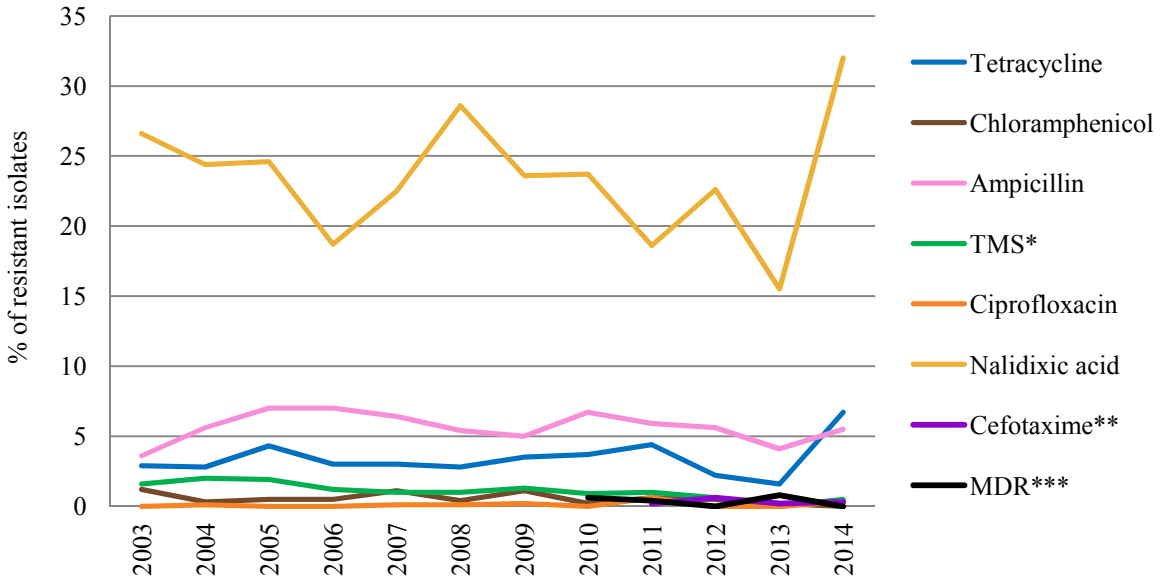


FIGURE 32. Percentage of resistance to various antimicrobial agents in *Salmonella* Enteritidis from humans in 2003-2014. *TMS=Trimethoprim-sulfamethoxazole. **Before 2014 cefpodoxime was tested. ***MDR testing 2011-2013 was calculated for five groups of antibiotics, whereas from 2014 onwards MDR results shown are for seven groups.

TABLE 23. Human isolates of *Salmonella* spp. including *S.* Paratyphi B variant Java, but excluding *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi (n=438[#]), acquired during 2014, irrespective of place of acquisition. Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	83.8	-	16.2
Cefotaxime	≤ 1	> 2	97.4	0.5	2.1
Ceftazidime	≤ 1	> 4	95.9	3.4	0.7
Meropenem	≤ 2	> 8	99.8	0.2	0.0
Ciprofloxacin [1]	≤ 0.5	> 1	94.1	0.9	5.0
Nalidixic acid [2]*	≥ 19	< 19	85.8	-	14.2
Gentamicin	≤ 2	> 4	94.7	1.6	3.7
Azithromycin [2] *	≤ 11	> 11	100.0	-	0.0
Tetracycline [2] *	≥ 13	< 13	76.4	-	23.6
Chloramphenicol *	≤ 8	> 8	90.6	-	9.4
Trimethoprim-sulfamethoxazole	≤ 2	> 4	89.5	0.0	10.5

[1] Low-level resistance against ciprofloxacin is underestimated by using these breakpoints; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. [2] Epidemiological cut-off values based on zone distribution evaluations. *Only tested in 127/438 isolates. #Place of infection; Norway (n=100), abroad (n=310), unknown (n=28).

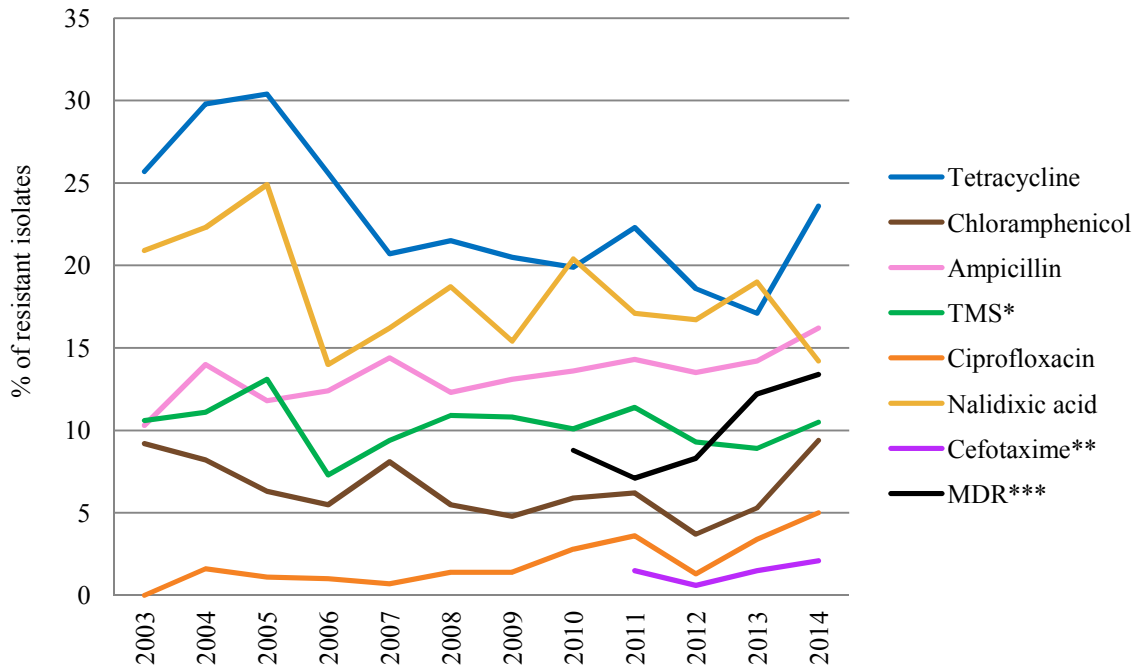


FIGURE 33. Percentage of resistance to various antimicrobial agents in *Salmonella* spp. including *S.* Paratyphi B variant Java; but excluding *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi, from humans in 2003-2014. *TMS=Trimethoprim-sulfamethoxazole. **Before 2014 cefpodoxime was tested.***MDR testing 2011-2013 was calculated for five groups of antibiotics, whereas from 2014 onwards MDR results shown are for seven groups.

RESULTS AND COMMENTS

As found earlier, the overall impression is that the "*S.* Typhimurium and its monophasic variant" group and the "*Salmonella* other" group are the two most resistant (Figure 27).

Another tendency is that the proportion of strains resistant to ampicillin and tetracycline within the *S.* Typhimurium-group continues to increase. As demonstrated in Figures 30 and 31, this trend is apparent for both domestically acquired strains and strains acquired abroad. The rates of isolates resistant to ampicillin and tetracycline have practically doubled over the last decade and are now around 50% for domestically acquired isolates and 65-70% in those acquired abroad. Several countries report an increase in MDR *S. enterica* serovar 4,[5],12:i-. In Norway, the number of isolates assigned to this serovar has increased steadily from 59 strains in 2008 to 129 in 2014. The corresponding proportions of this serovar within the *Salmonella* Typhimurium-group were around 20% in 2008 and 2009, and more than 50% in 2014. Somewhat surprisingly, MDR when seven groups of antibiotics were tested, was less frequent among *S. enterica* serovar 4,[5],12:i- than in *S.* Typhimurium (7.5% and 31.7%, respectively).

Antimicrobial resistance in *S.* Enteritidis isolates seems fairy stable (Figure 32), possibly due, in part, to a stable proportion of infections acquired abroad (Figure 26). There is still a very low level of resistance to ciprofloxacin. However, the breakpoints used underestimate low-level ciprofloxacin resistance, probably relevant in systemic *Salmonella* infections. There is no clear tendency towards an increase in resistance against nalidixic acid.

With regard to *Salmonella* spp. including *S.* Paratyphi B variant Java, but excluding *S.* Typhimurium-group, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi, most infections were acquired abroad and antimicrobial resistance was moderate and fairly stable, but for a possible increase in MDR (Table 23 and Figure 33). Also in this group, resistance to ciprofloxacin was still well below 5%, although the same reservations have to be made concerning low-level ciprofloxacin resistant strains in systemic infections.

A total of twelve strains carried extended spectrum beta-lactamases (ESBL). $ESBL_A$ was carried by three strains of *S.* Typhimurium or its monophasic variant, by four *S.* Infantis, and by one each of *S.* Enteritidis, *S.* Muenchen and *S.* Saintpaul, respectively. $ESBL_M$ was carried by two strains of *S.* Anatum.

Among isolates acquired in Norway, 2.0% were resistant to $\geq$ 3 groups and thus defined as multi-drug resistant (MDR), whereas the corresponding percentage was 1.4 among those acquired abroad ($p>0.05$). Of the 374 isolates tested for seven antimicrobial groups, the proportion of MDR was 5.8% and 12.8% among those acquired in Norway and abroad, respectively ($p>0.05$).

CAMPYLOBACTER SPP.

Campylobacter spp. from human clinical cases

Of the 3,386 human campylobacteriosis cases registered in Norway in 2014, 46.0% were reported as acquired abroad. Based on epidemiological data from patients, the vast majority of cases were judged as sporadic. However, only a fraction of the isolates are forwarded to the NRL. Consequently, quality-assured species diagnoses, complete AMR data and molecular epidemiology data on *Campylobacter* isolates are lacking due to resource limitations. Outbreaks with less clear epidemiological links may very well have been overlooked, and the antimicrobial susceptibility testing results presented may therefore be underestimated or overestimated.

Susceptibility testing was performed on a total of 266 *C. jejuni* isolates from 69 patients infected in Norway, 121 infected abroad and 76 where the place of acquisition of infection was unknown, as well as 21 *C. coli* isolates. EUCAST clinical breakpoints and epidemiological cut-off values have been used. The results for *C. jejuni* are presented in Tables 24-25, Figures 34-36, and in the text.

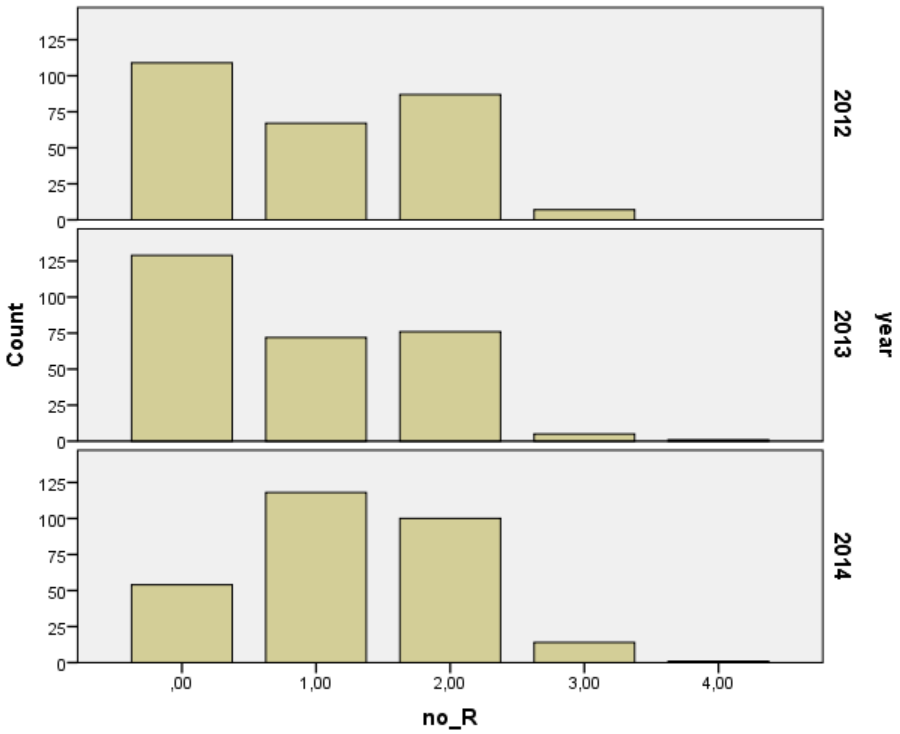


FIGURE 34. Distribution of number of antimicrobials that *Campylobacter jejuni* isolates were resistant to; by year.

TABLE 24. *Campylobacter jejuni* isolates from patients infected in Norway in 2014 (n=69). Distribution (%) of antimicrobial susceptibility categories.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline	≤ 2	> 2	85.5	-	14.5
Erythromycin	≤ 4	> 4	97.1	-	2.9
Gentamicin [1]	≤ 2	> 2	94.2	-	5.8
Nalidixic acid [1]	≤ 16	> 16	73.9	-	26.1
Ciprofloxacin	≤ 0.5	> 0.5	72.5	-	27.5

[1] Epidemiological cut-off values according to EUCAST web-pages by July 2014.

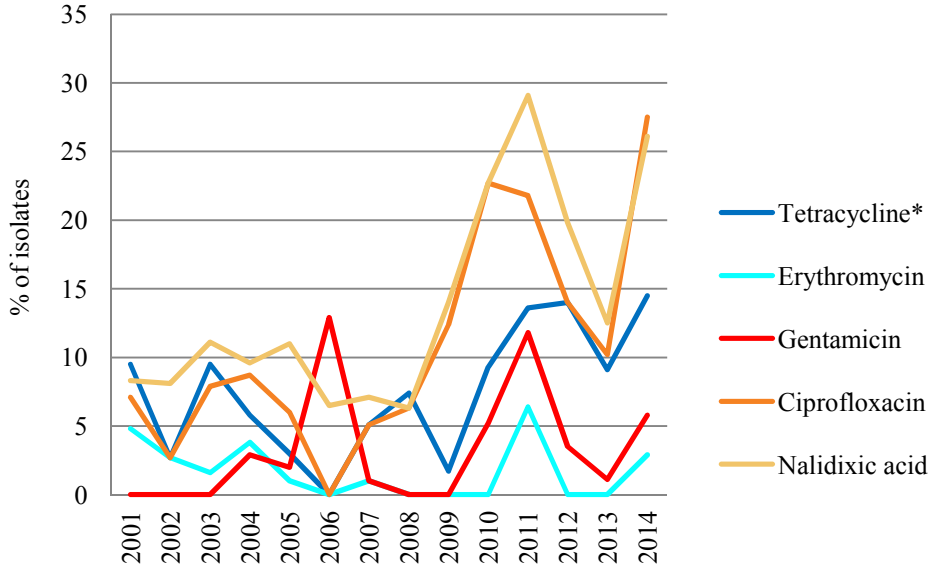


FIGURE 35. Prevalence of resistance in *Campylobacter jejuni* isolated from humans infected in Norway 2001-2014 to various antimicrobials. *Doxycycline before 2006.

TABLE 25. *Campylobacter jejuni* isolates from patients infected outside Norway in 2014 (n=121). Distribution (%) of antimicrobial susceptibility categories.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline	≤ 2	> 2	37.2	-	62.8
Erythromycin	≤ 4	> 4	95.9	-	4.1
Gentamicin [1]	≤ 2	> 2	96.7	-	3.3
Nalidixic acid [1]	≤ 16	> 16	22.3	-	77.7
Ciprofloxacin	≤ 0.5	> 0.5	22.3	-	77.7

[1] Epidemiological cut-off values according to EUCAST web-pages by July 2014.

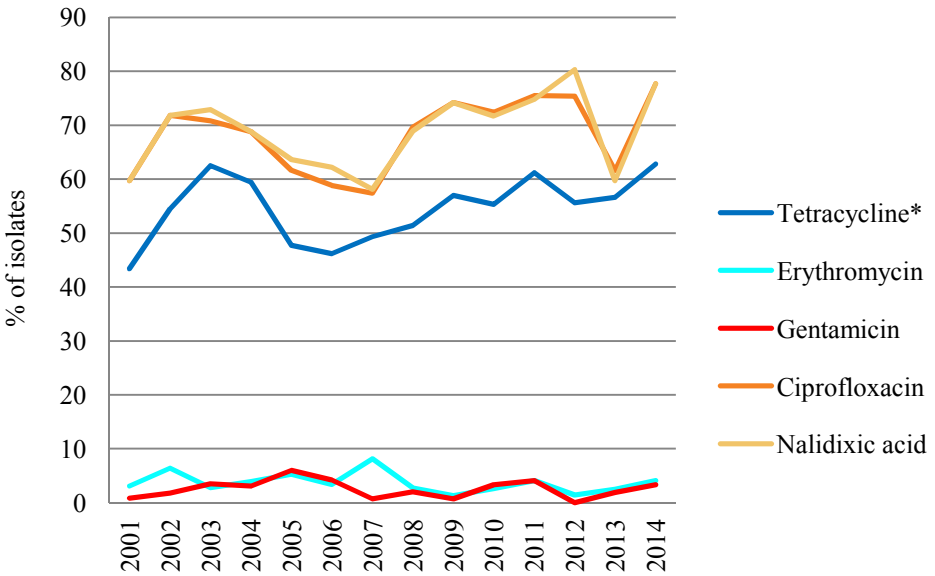


FIGURE 36. Prevalence of resistance to various antimicrobial agents in *Campylobacter jejuni* from humans infected outside Norway 2001-2014. *Doxycycline before 2006.

RESULTS AND COMMENTS

The data clearly show that resistance was more widespread among *C. jejuni* isolates recovered from patients infected abroad than in patients infected in Norway. Only 9.6% of isolates from infections acquired abroad were susceptible to all antimicrobial agents tested compared to 33.8% of the isolates from patients infected in Norway ($p<0.001$). The main differences between the two groups were seen for ciprofloxacin, nalidixic acid and tetracycline. There was a statistically significant difference in levels of resistance between those isolates acquired abroad compared to those acquired in Norway ($p<0.001$ for both antimicrobial groups).

For strains acquired abroad, resistance against all antimicrobials seems relatively stable, and for strains acquired in Norway the level of resistance seems to increase, however with broad variations probably due to a low number in strains tested. Furthermore, there might be a tendency towards more resistance against quinolones and tetracycline also in isolates acquired in Norway, possibly approaching the level seen in isolates acquired abroad.

Thirteen of the 16 *C. coli* isolates were acquired abroad, and ten of these thirteen isolates were resistant to at least one of the antimicrobial agents tested.

Yersinia enterocolitica from human clinical cases

A total of 65 unique strains of pathogenic *Yersinia enterocolitica* were analysed in 2014. Fifty-three belonged to serogroup 3 including 30 acquired in Norway, 17 acquired abroad and six with unknown place of acquisition. Eight strains belonged to serogroup 9, of which four were acquired in Norway, three acquired abroad and one strain was acquired from unknown location. One single *Y. enterocolitica* biotype 2 strain was domestically acquired, whereas two *Y. pseudotuberculosis* strains were acquired in Norway, and for another strain no travel information was given. All *Y. enterocolitica* isolates were tested for susceptibility to four antimicrobial groups (beta-lactams, quinolones, aminoglycosides, and trimethoprim-sulfamethoxazole) whereas only 18 strains were tested against all seven groups. The results are presented in Table 26 and Figures 37-38.

The crude number of isolates was considered low, and judgements should consequently be even more conservative regarding AMR results for *Y. enterocolitica* than for the other enteropathogenic bacteria.

TABLE 26. *Yersinia enterocolitica* serogroups O:3, O:9, biotype 2 and *Y. pseudotuberculosis* human cases in 2014 (n=65). Distributions (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	7.7	-	92.3
Cefotaxime	≤ 1	> 2	100.0	0.0	0.0
Ceftazidime	≤ 1	> 4	100.0	0.0	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin	≤ 0.5	> 1	98.5	0.0	1.5
Nalidixic acid [1]*	≥ 19	< 19	94.4	-	5.6
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Azithromycin [1] *	≤ 11	> 11	100.0	-	0.0
Tetracycline [1] *	≥ 13	< 13	100.0	-	0.0
Chloramphenicol *	≤ 8	> 8	100.0	-	0.0
Trimethoprim-sulfamethoxazole	≤ 2	> 4	95.4	1.5	3.1

[1] Epidemiological cut-off values based on zone distribution evaluations. * Only tested in 18/65 isolates.

RESULTS AND COMMENTS

Almost all isolates of pathogenic *Yersinia enterocolitica* expressed intrinsic resistance to ampicillin. In accordance with the data on zone diameters published from the EUCAST reference database, a small number of strains lacked this attribute. This was also in agreement with a study screening for *blaA* genes *(Sharma S. et al. FEMS Microbiol Lett 2006;257:319-327)*. The prevalence of resistance to other antimicrobial agents appeared stable during the years 2001-2013. However, when EUCAST establishes clinical breakpoints or epidemiological cut-off values for *Y. enterocolitica*, it may be possible to judge with more weight on this matter.

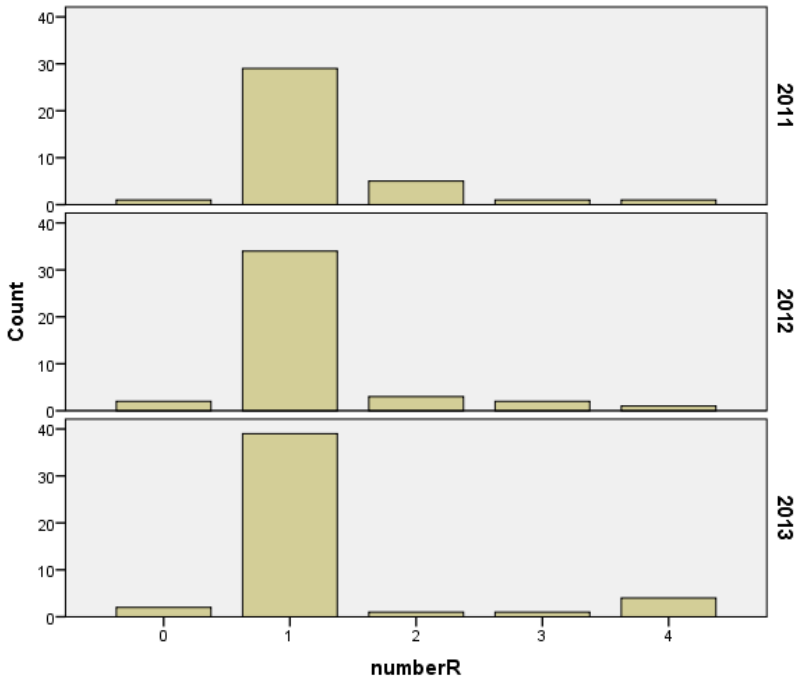


FIGURE 37. Distribution of number of antimicrobials that *Yersinia enterocolitica* isolates were resistant to; by year.

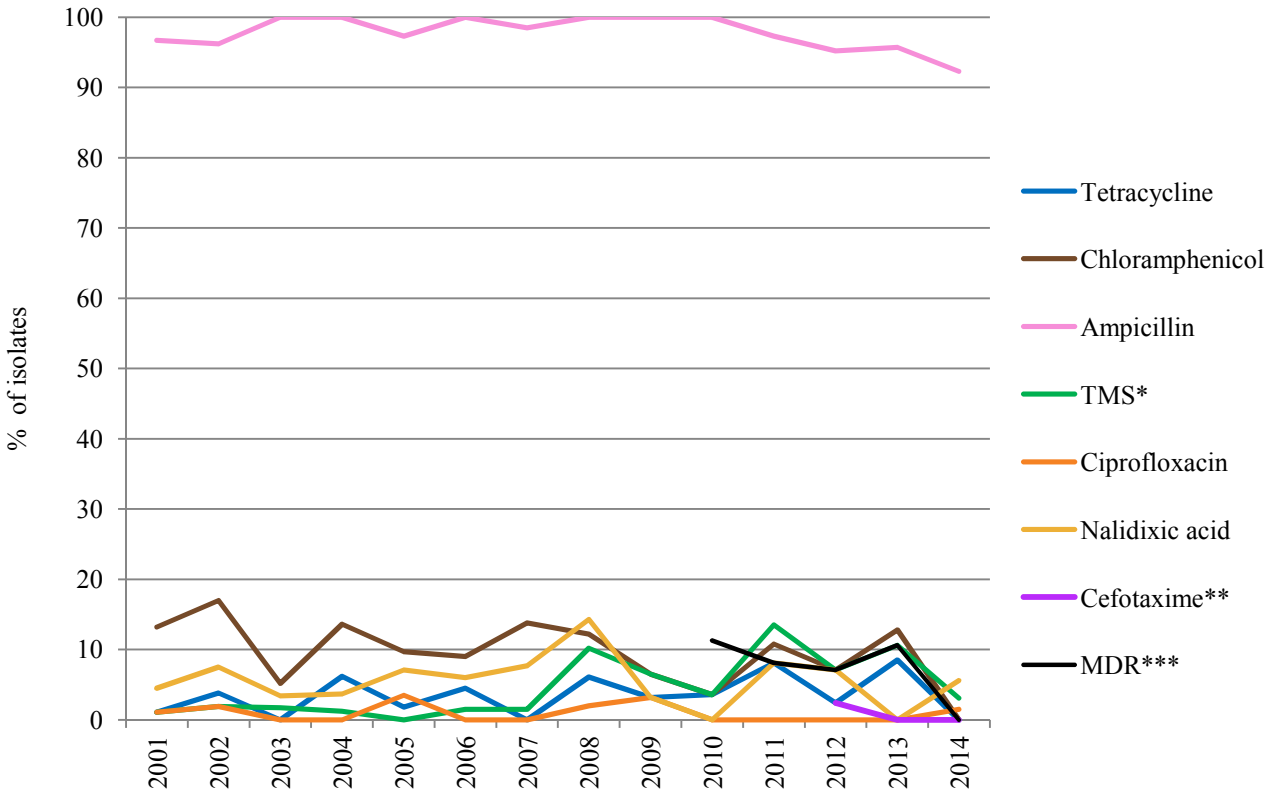


FIGURE 38. Prevalence of resistance to various antimicrobials in *Yersinia enterocolitica* from humans in Norway 2001-2014. *TMS=Trimethoprim-sulfamethoxazole. **Before 2014 cefpodoxime was tested. ***MDR testing 2011-2013 was calculated for five groups of antibiotics, whereas from 2014 onwards MDR results shown are for seven groups.

Shigella spp. from human clinical cases

In 2014, 16 (16.7%) of the 96 unique isolates *of Shigella* were domestically acquired. However, the domestically acquired strains were considered secondary to imported cases. Thus, the prevalence of resistance presented in this report predominantly relates to isolates originating from other countries. The species distribution of the 96 *Shigella* isolates that were tested for drug susceptibility was as follows: *S. sonnei* 62 (64.6%); *S. flexneri* 27 (28.1%); *S. boydii* 6 (6.3%); and *S. dysenteriae* 1 (1.0%, serotype 2). The number of antimicrobial agents that *Shigella* isolates were resistant to are shown in Figure 39. All isolates were tested for resistance against four different antibiotic groups: beta-lactams (ampicillin, cefotaxim, ceftazidime, and meropenem), ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole. In addition, one third of the isolates were tested for nalidixic acid, azithromycin, tetracycline, and chloramphenicol. Multi-resistance was defined as resistance to three or more antimicrobial categories, calculated on the basis of the isolates tested for all seven antibiotic groups. The results for *S. sonnei* and *S. flexneri* are presented in Table 27 and Figure 40, and in Table 28 and Figure 41, respectively.

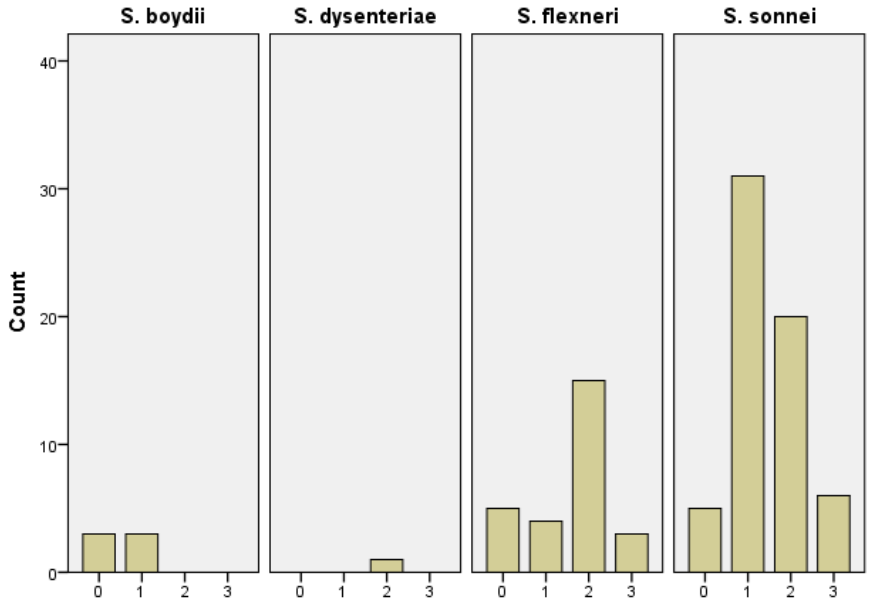


FIGURE 39. Distribution of number of antimicrobials that *Shigella* isolates were resistant to; by species, within all isolates (tested against four antibiotic groups).

TABLE 27. *Shigella sonnei* isolates from human cases in 2014 (n=62). Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	77.4	-	22.6
Cefotaxime	≤ 1	> 2	88.7	0.0	11.3
Ceftazidime	≤ 1	> 4	98.4	0.0	1.6
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin	≤ 0.5	> 1	72.6	0.0	27.4
Nalidixic acid [1]*	≥ 19	< 19	50.0	-	50.0
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Azithromycin [1] *	≤ 11	> 11	100.0	-	0.0
Tetracycline [1] *	≥ 13	< 13	21.4	-	78.6
Chloramphenicol *	≤ 8	> 8	100.0	-	0.0
Trimethoprim-sulfamethoxazole	≤ 2	> 4	8.1	4.8	87.1

[1] Epidemiological cut-off values based on zone distribution evaluations. * Only tested in 14/62 isolates.

TABLE 28. *Shigella flexneri* isolates from human cases in 2014 (n=27). Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	29.6	-	70.4
Cefotaxime	≤ 1	> 2	100.0	0.0	0.0
Ceftazidime	≤ 1	> 4	100.0	0.0	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin	≤ 0.5	> 1	85.2	0.0	14.8
Nalidixic acid [1]*	≥ 19	< 19	66.7	-	33.3
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Azithromycin [1] *	≤ 11	> 11	100.0	-	0.0
Tetracycline [1] *	≥ 13	< 13	22.2	-	77.8
Chloramphenicol *	≤ 8	> 8	55.6	-	44.4
Trimethoprim-sulfamethoxazole	≤ 2	> 4	25.9	0.0	74.1

[1] Epidemiological cut-off values based on zone distribution evaluations. * Only tested in 9/27 isolates.

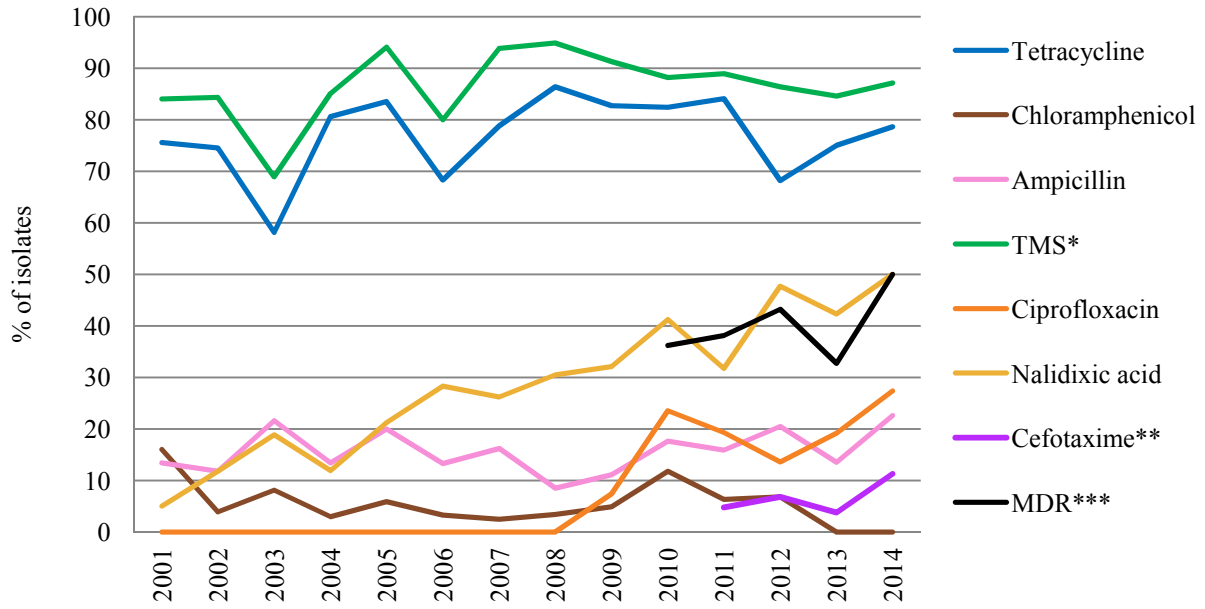


FIGURE 40. Prevalence of resistance to various antimicrobial agents in *Shigella sonnei* from humans in Norway 2001-2014. *TMS=Trimethoprim-sulfamethoxazole. **Before 2014 cefpodoxime was tested. ***MDR testing 2011-2013 was calculated for five groups of antibiotics, whereas from 2014 onwards MDR results shown are for seven groups.

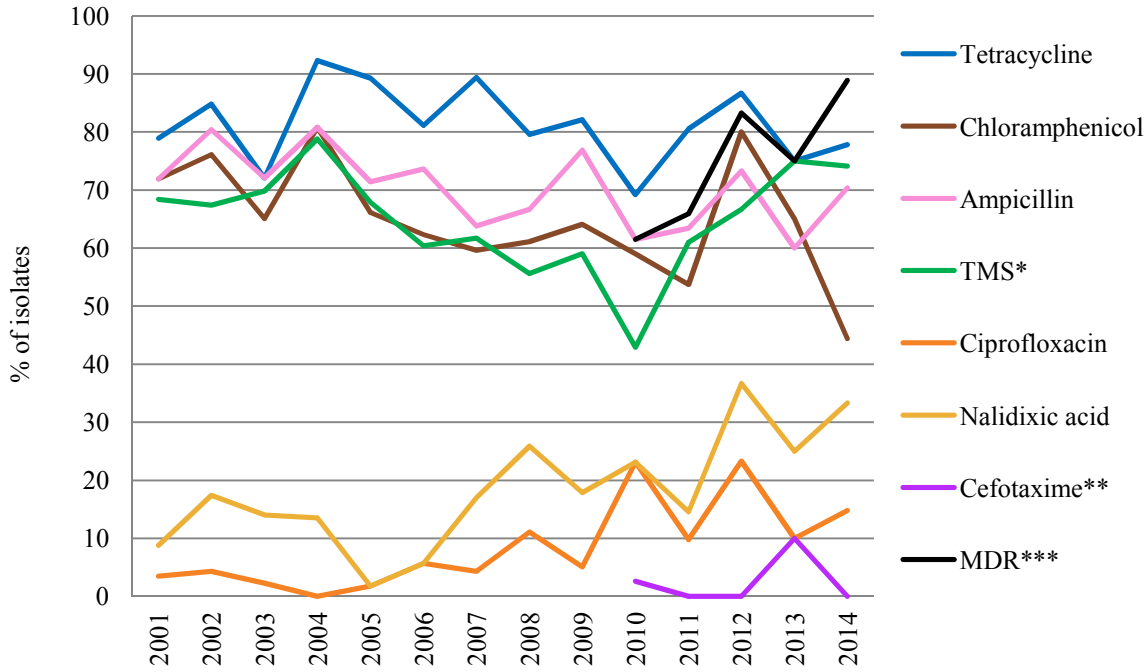


FIGURE 41. Prevalence of resistance to various antimicrobial agents in *Shigella flexneri* from humans in Norway 2001-2014. *TMS=Trimethoprim-sulfamethoxazole. **Before 2014 cefpodoxime was tested. ***MDR testing 2011-2013 was calculated for five groups of antibiotics, whereas from 2014 onwards MDR results shown are for seven groups.

RESULTS AND COMMENTS

The resistance patterns for *S. sonnei* have been fairly stable during the period 2001-2014. Resistance to nalidixic acid, however, has steadily increased since 2001, and resistance to ciprofloxacin now seems to have stabilised at around 20% after increasing during 2008/2009. A similar development appears for resistance to nalidixic acid and ciprofloxacin in *S. flexneri* isolates. The proportion of multi-drug resistance in both *S. sonnei* and *S. flexneri* (50.0% and 88.9%, respectively) was higher than in *Salmonella* as a whole (10.7%). One of the six *S. boydii* isolates displayed MDR. The single strain of *S. dysenteriae* was MDR.

Seven strains (7.3%), all *S. sonnei*, had reduced susceptibility to cefalosporins. All seven were phenotypically $ESBL_A$ producers with inhibitory effect of clavulanic acid.

HUMAN CLINICAL ISOLATES

Cecilie Torp Andersen, Trude Margrete Arnesen, Elisabeth Astrup, Dominique Caugant, Kjersti Wik Larssen, Andreas Radtke, Gunnar Skov Simonsen, Dagfinn Skaare, Martin Steinbakk, Gaute Syversen, Didrik Vestrheim, Frode Width-Gran

Distribution of bacterial species in blood cultures

Surveillance of antimicrobial resistance is usually based on prevalences of reduced susceptibility and resistance to certain combinations of antimicrobials and bacterial species in clinical samples. The NORM surveillance programme generally follows this approach. However, there is a serious limitation to the model because a transition in the distribution from susceptible bacterial species to inherently more resistant ones will represent de facto emergence of resistance which is not easily detected. In order to complement the surveillance of individual species, NORM collects data on all positive blood cultures from the laboratory information systems of the participating institutions. A patient with a given microbial isolate was excluded from registration with a new isolate of the same species within a month from the first entry. This rule was applied irrespectively of changes in the organism´s susceptibility pattern. Isolates of a different species were included in the surveillance. It proved difficult to systematically evaluate the clinical significance of species which are commonly part of the normal skin flora. In Table 29, proportions are therefore estimated for all isolates and for all isolates excluding species considered to be common skin contaminants such as coagulase negative staphylococci, *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp. and *Propioni-bacterium* spp. This does not imply that such isolates cannot cause infections, but only that it was not possible to enforce a standardised protocol for inclusion of the clinically significant isolates. Similarly, all isolates were registered as individual findings although polymicrobial bloodstream infections are regularly detected in some patients. Limitations in the data extraction procedure prohibited in-depth analysis of these parameters.

TABLE 29. Number of blood culture isolates in 2014, proportion of all isolates, and proportion of isolates excluding possible skin contaminants (coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp.) 2010-2014. The table is based on data from the information systems of all laboratories in Norway.

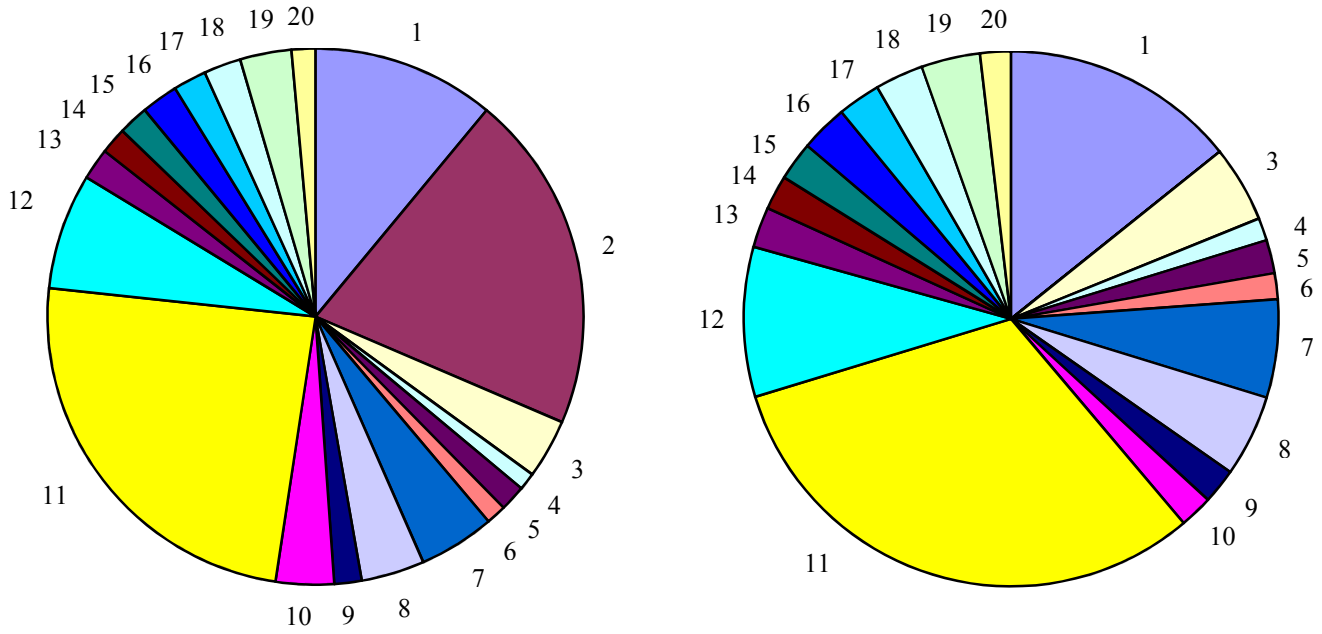
Species	No. of isolates 2014	% of all isolates					% of all isolates excluding skin flora				
		2010	2011	2012	2013	2014	2010	2011	2012	2013	2014
Staphylococcus aureus	1,693	11.4	11.0	11.3	11.5	11.0	14.5	14.2	15.0	14.3	14.2
Coagulase negative staphylococci	3,142	19.3	20.6	22.5	17.4	20.4	-	-	-	-	-
Streptococcus pneumoniae	550	5.7	5.3	4.2	4.2	3.6	7.3	6.8	5.6	5.2	4.6
Streptococcus pyogenes	162	1.3	1.4	1.0	1.3	1.1	1.6	1.8	1.3	1.6	1.4
Streptococcus agalactiae	240	1.5	1.6	1.6	1.7	1.6	1.9	2.1	2.1	2.1	2.0
Beta-haemolytic streptococci group C and G	185	1.3	1.2	1.2	1.2	1.2	1.7	1.6	1.6	1.5	1.6
Viridans- and non-haemolytic streptococci	705	4.7	4.1	3.8	5.5	4.6	5.9	5.2	5.1	6.8	5.9
Enterococcus faecalis	589	4.6	4.1	4.0	4.1	3.8	5.9	5.2	5.3	5.1	5.0
Enterococcus faecium	251	1.7	1.8	1.5	1.8	1.6	2.1	2.3	2.0	2.2	2.1
Other Gram positive aerobic and facultative bacteria	541	2.8	2.9	3.1	3.3	3.5	1.4	1.6	1.9	2.0	2.0
Escherichia coli	3,739	23.4	24.0	23.9	24.4	24.4	29.6	30.9	31.4	30.4	31.5
Klebsiella spp.	1,075	6.8	6.1	6.5	6.8	7.0	8.7	7.9	8.6	8.4	9.0
Enterobacter spp.	292	1.6	1.8	1.9	1.9	1.9	2.1	2.3	2.5	2.4	2.5
Proteus spp.	246	1.7	1.7	1.3	1.7	1.6	2.2	2.2	1.8	2.1	2.1
Other *Enterobacteriaceae*	340	2.3	2.2	2.0	2.3	2.2	2.9	2.9	2.7	2.9	2.9
Pseudomonas spp.	278	1.8	1.5	1.7	1.7	1.8	2.2	2.0	2.2	2.1	2.3
Other Gram negative aerobic and facultative bacteria	308	2.3	2.2	2.0	2.1	2.0	2.9	2.8	2.6	2.6	2.6
Bacteroides spp.	346	2.0	2.2	2.3	2.4	2.2	2.6	2.9	3.0	3.0	2.9
Other anaerobic bacteria	475	2.3	2.8	2.8	3.2	3.1	2.6	3.4	3.3	3.5	3.6
Yeasts	222	1.5	1.5	1.4	1.5	1.4	1.9	1.9	2.0	1.8	1.8
Total	**15,379**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

As seen in Table 29 and Figure 42, aerobic and facultative Gram-positive and Gram-negative bacteria represented 52.4% and 40.9% of all isolates, respectively. The predominance of Gram-positives among all isolates was at the same level as in previous years. The most common Gram-positive species were coagulase negative staphylococci which represented 20.4% of all isolates. This was an increase from 17.4% in 2013, but these fluctuations may result from inconsistent reporting from the laboratories. The difference between aerobic Gram-positives and Gram-negatives was reversed when species of the skin flora (coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp.) were excluded with 38.8% aerobic Gram-positives and 52.9% aerobic Gram-negatives.

Among aerobic Gram-positives, the prevalence of *S. pneumoniae* has steadily declined from 12.1% in 2005 to 4.6% in 2014 (skin contaminants are excluded), following the introduction of the conjugate pneumococcal vaccine in the national childhood immunisation programme. The proportions accounted for by viridans- and nonhaemolytic streptococci reverted to 5.9% of isolates (skin contaminants excluded) which is at the same level as previous years.

E. coli (31.5%) and other *Enterobacteriaceae* (16.5%) accounted for the vast majority of aerobic Gram-negative isolates, but the proportions have remained relatively unchanged over the years. *Pseudomonas* spp. (2.3%) has been fairly stable after a peak in 2005 (2.8%), all figures excluding skin flora.

Anaerobic bacteria and yeasts were less prevalent. Anaerobes accounted for 5.3% (6.5% excluding skin flora) which is a slight decrease from 2013 (5.6%; 6.5% excluding skin flora). Yeasts accounted for 1.4% (1.8% excluding skin flora) which is unchanged from earlier years. The major pathogens among anaerobes were members of *Bacteroides* spp. (2.2%/2.9%) and among yeasts *Candida albicans* (0.9%/1.2%). However, a multitude of other species was also represented.



1. *Staphylococcus aureus*
2. Coagulase negative staphylococci
3. *Streptococcus pneumoniae*
4. *Streptococcus pyogenes*
5. *Streptococcus agalactiae*
6. Betahaemolytic streptococci group C and G
7. Non-haemolytic and viridans streptococci
8. *Enterococcus faecalis*
9. *Enterococcus faecium*
10. Other Gram-positive bacteria
11. *Escherichia coli*
12. *Klebsiella* spp.
13. *Enterobacter* spp.
14. *Proteus* spp.
15. Other *Enterobacteriaceae*
16. *Pseudomonas* spp.
17. Other Gram-negative bacteria
18. *Bacteroides* spp.
19. Other anaerobic bacteria
20. Yeasts

FIGURE 42. Distribution of all blood culture isolates (left, n=15,379) and blood culture isolates excluding common skin contaminants (right, n=11,882) such as coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp. Data for 2014 were retrieved from the information systems of all Norwegian laboratories.

Escherichia coli in blood cultures

TABLE 30. *Escherichia coli* blood culture isolates in 2014 (n=1,645). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	57.4	-	42.6
Piperacillin-tazobactam	≤ 8	> 16	93.3	3.8	2.9
Cefuroxime	≤ 8	> 8	92.0	-	8.0
Cefotaxime	≤ 1	> 2	93.7	0.2	6.1
Ceftazidime	≤ 1	> 4	93.5	1.3	5.2
Cefepime	≤ 1	> 4	94.4	1.2	4.4
Meropenem	≤ 2	> 8	99.9	0.1	0.0
Gentamicin	≤ 2	> 4	91.4	0.9	7.7
Ciprofloxacin	≤ 0.5	> 1	86.4	1.0	12.6
Tigecycline	≤ 1	> 2	99.9	0.0	0.1
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	71.2	1.0	27.8
ESBL	Negative	Positive	94.2	-	5.8

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The NORM results are interpreted according to the breakpoints of the Norwegian Working Group for Antibiotics (NWGA) at the time of analysis. Norwegian breakpoints for Enterobacteriaceae presently correspond to EUCAST breakpoints. All results from previous years have been recategorised to comply with the 2015 EUCAST protocol.

The vast majority of isolates remained fully susceptible to broad-spectrum antimicrobial agents such as cefotaxime (93.7%), ceftazidime (93.5%), cefepime (94.4%), gentamicin (91.4%), piperacillin-tazobactam (93.3%), tigecycline (99.9%) and meropenem (99.9%) (Table 30). From 2013 to 2014 there was a reduction in the prevalence of susceptibility of 0.5-1.0% points for all these agents except meropenem and tigecycline.

The prevalence of non-susceptibility to gentamicin increased significantly from 0.2% I and 5.6% R in 2013 to 0.9% I and 7.7% R in 2014 (Figure 43). The prevalence of gentamicin resistance is approximately eight times higher than at the turn of the century. A high proportion of gentamicin non-susceptible isolates (49/142, 34.5%) also produced ESBL enzymes. They were retrieved from 18 different laboratories across the country. The prevalence at individual laboratories varied widely due to relatively small numbers. When aggregated by region, the prevalence of gentamicin non-susceptibility was higher in the South-East (10.9%) and West (7.6%) regions compared to the Middle (5.8%) and North (3.2%) regions.

The prevalence of non-suscpetibility to ciprofloxacin was 13.6% (1.0% I and 12.6% R) in 2014 compared to 12.3% in 2013 (0.5% I and 11.8% R). This is the highest rate of quinolone resistance ever recorded in NORM. The steadily increasing proportion of non-susceptibility to ciprofloxacin in *E. coli* blood culture isolates corresponds to the situation in almost all other European countries. The temporal association between ciprofloxacin non-susceptibility and ciprofloxacin usage is depicted in Figure 44. A similar association between quinolone use and resistance in systemic *E. coli* isolates is also reported internationally. The resistance rates for ampicillin (42.6% in 2014, 42.7% in 2013) and trimethoprim-sulfamethoxazole (27.8% in 2014, 25.4% in 2013) are relatively stable.

In 2014, detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefotaxime and ceftazidime. All isolates with reduced susceptibility to ceftazidime and/or cefotaxime were further characterised by combination MIC gradient tests. A total of 95 isolates (5.8%) were reported as ESBL positive which is a slight increase from 5.5% in 2012 and 5.0% in 2013 (Figure 46). The isolates originated from all 19 participating laboratories across the country. Estimates at laboratory level are uncertain due to small numbers. When aggregated at regional level there were only minor geographical differences in the prevalence of ESBL (South-East 6.2%, West 6.0%, Middle 3.1% and North 7.0%). Almost all ESBL isolates were non-susceptible to ampicillin (95/95), cefuroxime (94/95) and cefotaxime (94/95), and most of them were also non-susceptible to ceftazidime (85/95) and cefepime (91/95). Many isolates were intermediately (21/95) or even fully susceptible (65/95) to piperacillin-tazobactam. Most displayed some level of co-resistance to ciprofloxacin (74/95), gentamicin (49/95) and/or trimethoprim-sulfamethoxazole (73/95). All were fully susceptible to tigecycline, and only a single isolate displayed reduced susceptibility to meropenem. Twenty-four additional isolates were reported as non-susceptible to cefotaxime (n=11) and/or ceftazidime (n=23) without being confirmed as ESBL producers.

Ninety-seven *E. coli* ESBL isolates were molecularly characterised which revealed a predominance of CTX-M groups 1 (n=60) and 9 (n=29). The remaining eight isolates harboured plasmid encoded CMY (n=6), derepressed chromosomally encoded AmpC (n=1) and SHV-ESBL (n=1) enzymes.

Two isolates were reported as meropenem non-susceptible, but none of them were verified as carbapenemase producers by the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance. The meropenem non-susceptibility was interpreted to be caused by the combination of membrane impermeability and the presence of an $ESBL_A$ enzyme, and membrane impermeability in combination with hyperproduction of the chromosomal AmpC in each of the two isolates, respectively. However, two carbapenemase positive isolates were identified among the isolates initially susceptible according to the clinical breakpoints, but with zone diameters below the EUCAST screening breakpoint (S ≥ 27 mm). Whole genome sequencing of the isolates showed that one isolate contained a metallo-beta-lactamase (IMP-26) while the other isolate contained a class D carbapenemase (OXA-181). Both isolates were also $ESBL_A$ positive containing CTX-M-15 enzymes. In addition, the OXA-181 isolate harboured CMY-2, OXA-1 and TEM-1, whereas the IMP-26 isolate had TEM-1.

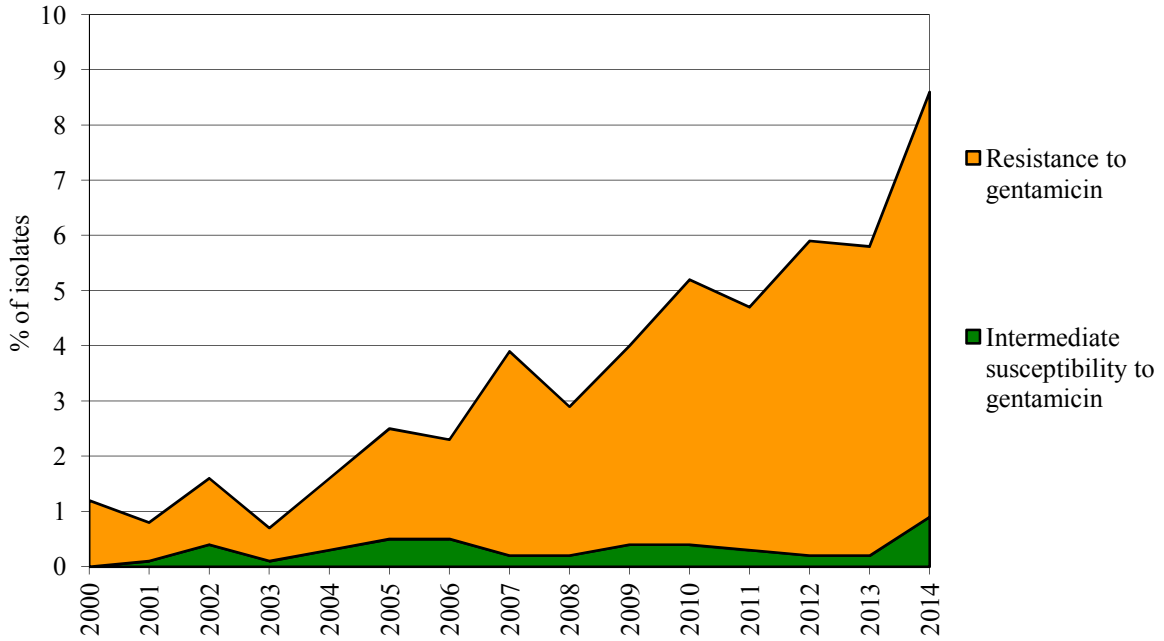


FIGURE 43. Prevalence of intermediate susceptibility and resistance to gentamicin in *Escherichia coli* blood culture isolates 2000-2014.

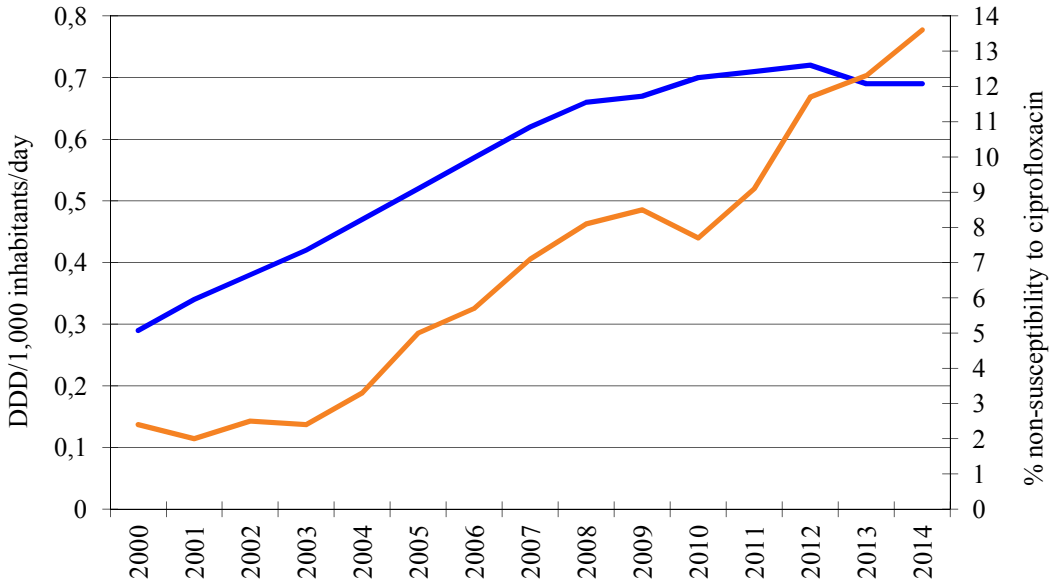


FIGURE 44. Usage of ciprofloxacin (blue) and prevalence of ciprofloxacin non-susceptibility in *Escherichia coli* blood culture isolates as defined by the 2015 breakpoints (orange) 2000-2014.

Escherichia coli in urine

TABLE 31. *Escherichia coli* urinary tract isolates in 2014 (n=965). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	61.2	-	38.8
Mecillinam	≤ 8	> 8	94.0	-	6.0
Amoxicillin-clavulanic acid*	≤ 32	> 32	92.1	-	7.9
Cefuroxime	≤ 8	> 8	94.3	-	5.7
Cefotaxime	≤ 1	> 2	95.5	0.6	3.9
Ceftazidime	≤ 1	> 4	95.0	1.1	3.9
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	95.8	0.3	3.9
Ciprofloxacin	≤ 0.5	> 1	90.6	0.7	8.7
Nitrofurantoin	≤ 64	> 64	98.8	-	1.2
Trimethoprim	≤ 2	> 4	71.2	0.3	28.5
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	72.5	0.9	26.6
ESBL	Negative	Positive	96.2	-	3.8

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Urinary tract isolates of *E. coli* have been included in the surveillance programme every year since NORM was established in 2000. The prevalence of resistance for 2014 is shown in Table 31 and the results for 2000-2014 are shown in Figure 45. All results since 2000 are categorised according to the 2015 EUCAST breakpoint protocol.

The resistance rates among urinary tract isolates have remained relatively stable over the last ten years, but are slowly drifting upward for most antibiotics. The prevalence of resistance to ampicillin has gradually increased from approximately 25% to nearly 40%. Around 25-30% of *E. coli* isolates are resistant to trimethoprim and trimethoprim-sulfamethoxazole. The prevalence of resistance to mecillinam increased from 4.2% in 2012 and 5.0% in 2013 to 6.0% in 2014, but susceptibility test results are notoriously difficult to reproduce for this agent and the observed changes may thus not reflect real differences in prevalence.

Ciprofloxacin is used as a second-line agent for urinary tract infections in Norway. The prevalence of non-susceptibility was previously stable around 3.5-5.5% but increased to 7.3% in 2013 and 9.4% in 2014, with 0.7% intermediate susceptibility and 8.7% resistance. The corresponding rates for blood culture isolates were 1.0% intermediate susceptibility and 12.6% resistance in 2014. The persistent discrepancy between urinary tract isolates and isolates from bloodstream infections suggests that systemic infections are caused by selected pathogenic lineages with increased virulence and accumulation of mutations in gyrase and/or topoisomerase genes, whereas urinary tract isolates are more representative of the wild type normal flora. The steadily increasing resistance rates are obviously a cause for great concern. The prevalence of resistance to amoxicillin-clavulanic acid decreased slightly from 8.1% in 2013 to 7.9% in 2014. The breakpoints used are only valid for uncomplicated urinary tract infections. Almost all isolates (98.8%) remained fully susceptible to nitrofurantoin.

In total, 37 isolates (3.8%) were reported as ESBL producers. This is a significant increase from 2.1% in 2013. As seen in Figure 46, the prevalence of *E. coli* ESBL is still lower in urine than in blood culture isolates (5.8%), but there is an increasing trend in both specimen types. The ESBL positive isolates were retrieved from fifteen different laboratories in all parts of the country. Fifteen isolates were retrieved from samples submitted by general practitioners, while the others were found in hospitalised patients (n=12), patients visiting outpatient clinics (n=5), patients in nursing homes (n=4) and a patient at an unspecified location (n=1). The ESBL strains were generally resistant to ampicillin (37/37), cefuroxime (36/37) and cefotaxime (36/37), and non-susceptible to ceftazidime (36/37). Most isolates were registered as *in vitro* susceptible to mecillinam (35/37). The clinical relevance of this finding is doubtful, since mecillinam is not stable for most beta-lactamases. Many of the ESBL isolates were non-susceptible to quinolones (24/37) and trimethoprim-sulfamethoxazole (25/37), but remained susceptible to nitrofurantoin (33/37) and gentamicin (19/37). All isolates were susceptible to carbapenems.

Molecularly characterisation revealed a predominance of CTX-M groups 1 (n=23) and 9 (n=10), or a combination of the two (n=1). Single isolates harboured CTX-M group 2, SHV-ESBL, or plasmid encoded CMY (n=1).

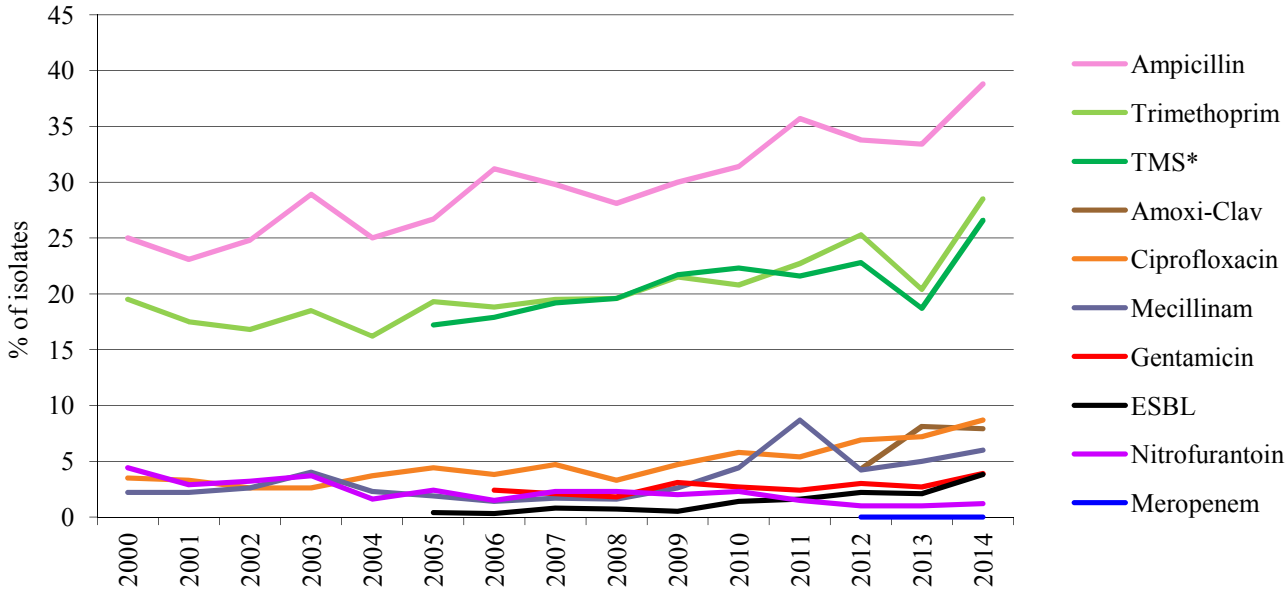


FIGURE 45. Prevalence of resistance to various antimicrobial agents in urinary tract *Escherichia coli* isolates 2000-2014 categorized according to the 2015 EUCAST guidelines. *TMS=Trimethoprim-sulfamethoxazole.

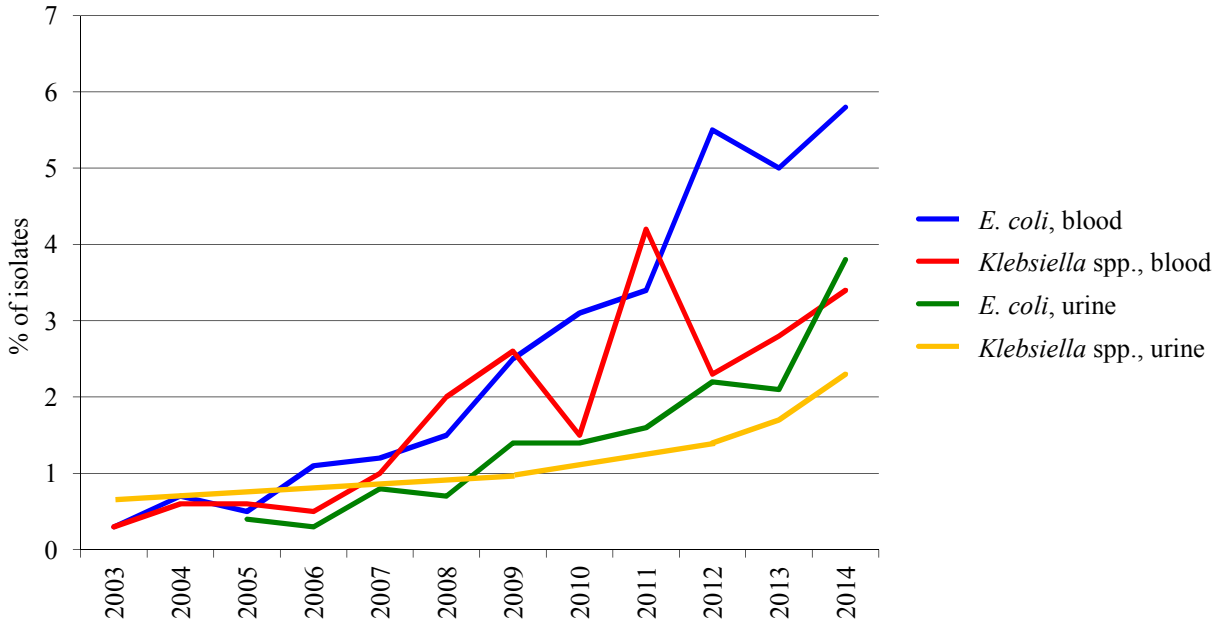


FIGURE 46. Prevalence of ESBL production among *Escherichia coli* and *Klebsiella* spp. isolates from blood and urine 2003-2014.

Klebsiella spp. in blood cultures

TABLE 32. *Klebsiella* spp. blood culture isolates in 2014 (n=754). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Piperacillin-tazobactam	≤ 8	> 16	90.0	6.2	3.8
Cefuroxime	≤ 8	> 8	91.0	-	9.0
Cefotaxime	≤ 1	> 2	95.9	0.5	3.6
Ceftazidime	≤ 1	> 4	94.1	2.1	3.8
Cefepime	≤ 1	> 4	95.6	0.8	3.6
Meropenem	≤ 2	> 8	99.9	0.1	0.0
Gentamicin	≤ 2	> 4	95.9	1.6	2.5
Ciprofloxacin	≤ 0.5	> 1	94.1	1.9	4.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	88.4	2.1	9.5
ESBL	Negative	Positive	96.6	-	3.4

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 33. *Klebsiella pneumoniae* blood culture isolates in 2014 (n=618). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Piperacillin-tazobactam	≤ 8	> 16	89.8	7.3	2.9
Cefuroxime	≤ 8	> 8	91.3	-	8.7
Cefotaxime	≤ 1	> 2	95.9	0.2	3.9
Ceftazidime	≤ 1	> 4	93.4	2.4	4.2
Cefepime	≤ 1	> 4	95.5	0.5	4.0
Meropenem	≤ 2	> 8	99.8	0.2	0.0
Gentamicin	≤ 2	> 4	95.4	1.5	3.1
Ciprofloxacin	≤ 0.5	> 1	93.0	2.3	4.7
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	86.4	2.6	11.0
ESBL	Negative	Positive	96.1	-	3.9

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 34. *Klebsiella oxytoca* blood culture isolates in 2014 (n=125). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Piperacillin-tazobactam	≤ 8	> 16	90.4	1.6	8.0
Cefuroxime	≤ 8	> 8	89.6	-	10.4
Cefotaxime	≤ 1	> 2	96.0	2.4	1.6
Ceftazidime	≤ 1	> 4	97.5	0.9	1.6
Cefepime	≤ 1	> 4	96.8	2.4	0.8
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	98.4	1.6	0.0
Ciprofloxacin	≤ 0.5	> 1	99.2	0.0	0.8
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	97.6	0.0	2.4
ESBL	Negative	Positive	99.2	-	0.8

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The surveillance of *Klebsiella* spp. in blood cultures included 618 *K. pneumoniae* (81.9%), 125 *K. oxytoca* (16.6%), and 11 (1.5%) isolates not identified to the species level, giving a total of 754 *Klebsiella* spp. isolates (Tables 32-34). There was a higher number of *Klebsiella pneumoniae* and fewer unspeciated isolates in 2014 than in 2013. As for *E. coli*, the breakpoint protocol of the Norwegian Working Group for Antibiotics (NWGA) has been in accordance with EUCAST since 2014.

The majority of *Klebsiella* spp. isolates remains susceptible to aminoglycosides, but the prevalence of non-suscpetibility increased from 1.0% in 2012 and 1.7% in 2013 to 4.1% in 2014. *K. oxytoca* isolates are more often susceptible to aminoglycosides (98.4%) than *K. pneumonia* isolates (95.4%). Aminoglycoside resistance in common Enterobacteriaceae species is a cause for great concern as these antimicrobials have traditionally been used in the empirical regimen for treatment of septiceamia in Norway.

The overall prevalence of resistance to ciprofloxacin has been stable at 3-4% when taking into account the changes in breakpoints and interpretive criteria. The 5.9% non-susceptibility (1.9% intermediate susceptibility and 4.0% resistance) observed in 2014 is an increase from previous years. Non-susceptibility to ciprofloxacin is much more common in *K. pneumoniae* (7.0%) than in *K. oxytoca* (0.8%). Non-susceptibility to trimethoprim-sulfamethoxazole remained stable at 11.6% in 2014 compared to 12.1% in 2013. As for ciprofloxacin, the prevalence of resistance to trimethoprim-sulfamethoxazole was significantly lower in *K. oxytoca* (2.4%) than in *K. pneumoniae* (11.0%).

A comparison of ESBL rates and non-susceptibility to beta-lactam antibiotics between *Klebsiella* species is complicated by the diagnostic challenges of the chromosomal K1 beta-lactamase in *K. oxytoca*. Most *Klebsiella* spp. isolates were susceptible to cefotaxime (95.9%), ceftazidime (94.1%) and the beta-lactam/beta-lactamase inhibitor combination piperacillin-tazobactam (90.0%), see Figure 47. The rates of non-susceptibility to third generation cephalosporins were at the same level as in previous years.

As for *E. coli*, the detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefotaxime and ceftazidime disks. Isolates with reduced zone diameters were further characterised by combination MIC gradient tests. The prevalence of phenotypically confirmed ESBL isolates increased to 3.4% in 2014 compared to 2.8% in 2013 (Figure 46). The 26 ESBL isolates originated from 13 different laboratories and were identified as *K. pneumoniae* (n=24, 3.9%), *K. oxytoca* (n=1) or *Klebsiella* sp. (n=1). The ESBL isolates were generally non-susceptible to cefuroxime (25/26), ceftazidime (26/26) and cefotaxime (25/26), and co-resistance was frequently seen to ciprofloxacin (19/26), trimethoprim-sulfamethoxazole (21/26) and gentamicin (18/26). Many isolates were intermediately (10/26) or even fully (7/26) susceptible to piperacillin-tazobactam.

Molecular characterisation of the reported ESBL isolates at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-Res) confirmed the presence of CTX-M groups 1 (n=22), SHV-ESBL (n=2) and SHV-1 hyperproduction in *K. pneumonia*. A single *K. oxytoca* isolate only contained the chromosomally encoded wildtype K1 beta-lactamase.

A single *K. pneumoniae* isolate was reported as intermediately susceptible to meropenem and a number of isolates had meropenem zone diameters below the EUCAST screening breakpoint (S ≥ 27 mm), but none were confirmed as true carbapenemase producers.

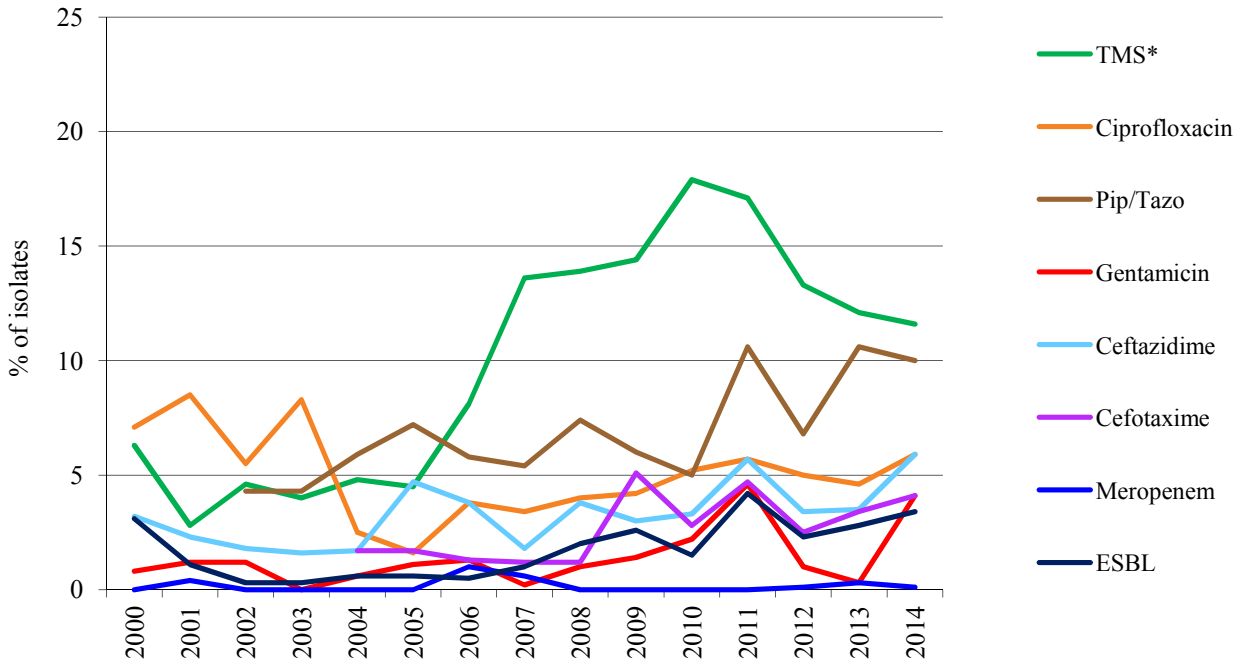


FIGURE 47. Prevalence of non-susceptibility to various antimicrobial agents in *Klebsiella* spp. blood culture isolates 2000-2014. *TMS=Trimethoprim-sulfamethoxazole.

Klebsiella spp. in urine

TABLE 35. *Klebsiella* spp. urinary tract isolates in 2014 (n=950). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Mecillinam	≤ 8	> 8	88.7	-	11.3
Amoxicillin-clavulanic acid*	≤ 32	> 32	93.8	-	6.2
Piperacillin-tazobactam	≤ 8	> 16	90.5	5.8	3.7
Cefuroxime	≤ 8	> 8	92.5	-	7.5
Cefotaxime	≤ 1	> 2	97.3	0.5	2.2
Ceftazidime	≤ 1	> 4	95.7	1.8	2.5
Meropenem	≤ 2	> 8	99.9	0.0	0.1
Gentamicin	≤ 2	> 4	98.8	0.6	0.6
Ciprofloxacin	≤ 0.5	> 1	95.3	1.5	3.2
Trimethoprim	≤ 2	> 4	81.1	1.7	17.2
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	86.5	1.6	11.9
ESBL	Negative	Positive	97.7	-	2.3

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Klebsiella spp. urinary tract isolates have previously been included in the NORM surveillance programme in 2001, 2003, 2009, 2012 and 2013. Due to methodological changes and adjustment of breakpoints it is not possible to directly compare the results from 2009 and 2012 with the ones from 2001 and 2003. There are no *Klebsiella* spp. breakpoints for nitrofurantoin.

In general, the rates of resistance to urinary tract antibiotics were slightly lower in *Klebsiella* spp. than in *E. coli* isolates (Tables 35-37). The vast majority of isolates are still susceptible to gentamicin (98.8% compared to 97.9% in 2013), ciprofloxacin (95.3% compared to 96.1% in 2013), and meropenem (99.9%). The comparable rates for *E. coli* were 95.8% for gentamicin, 90.6% for ciprofloxacin and 100% for meropenem. Susceptibility to trimethoprim (81.1% compared to 82.9% in 2013) and trimethoprim-sulfamethoxazole (86.5% compared to 87.2% in 2013) were higher than in *E. coli* (71.2% and 72.5%, respectively).

All *Klebsiella* isolates are inherently resistant to ampicillin due to the chromosomal SHV beta-lactamase. Wild type strains are since 2014 categorised as susceptible to cefuroxime in accordance with the EUCAST protocol. As for *Klebsiella* spp. blood culture isolates, ESBL detection in urinary tract isolates was based on non-susceptibility to cefotaxime and/or ceftazidime and subsequent confirmatory ESBL MIC gradient tests. Only 22 isolates were reported as ESBL positive of which 17 were *K. pneumonia*, two were *K. oxytoca* and three were unspeciated. The 22 ESBL isolates were retrieved from twelve different laboratories and originated from general practices (n=12), hospitals (n=6), outpatient clinics (n=2), nursing homes (n=1) and an unknown location (n=1). The 2.3% ESBL rate (2.7% in *K. pneumoniae*) represented a modest increase from 1.7% in 2013 and was lower than the 3.4% rate (3.9% in *K. pneumoniae*) found in blood culture isolates. The 22 ESBL isolates were generally non-susceptible to trimethoprim (n=16), trimethoprim-sulfamethoxazole (n=15) and ciprofloxacin (n=10), but many remained susceptible to gentamicin (n=17), mecillinam (n=15) and piperacillin-tazobactam (n=10). Molecular characterisation of 17 *K. pneumonia* isolates confirmed the presence of CTX-M groups 1 (n=10) and 9 (n=2) in addition to SHV-ESBL (n=3), an SHV-1 hyperproducer and a wildtype strain. The collection also included two *K. oxytoca* isolates (one CTX-M 1 and one SHV-ESBL) as well as a *Raoultella ornithinolytica* strain containing an SHV-ESBL enzyme.

A single *K. pneumoniae* isolate was reported as meropenem resistant, but carbapenemase production was not confirmed.

TABLE 36. *Klebsiella pneumoniae* urinary tract isolates in 2014 (n=625). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Mecillinam	≤ 8	> 8	89.1	-	10.9
Amoxicillin-clavulanic acid*	≤ 32	> 32	95.0	-	5.0
Piperacillin-tazobactam	≤ 8	> 16	90.4	7.0	2.6
Cefuroxime	≤ 8	> 8	92.3	-	7.7
Cefotaxime	≤ 1	> 2	97.1	0.5	2.4
Ceftazidime	≤ 1	> 4	95.2	1.9	2.9
Meropenem	≤ 2	> 8	99.8	0.0	0.2
Gentamicin	≤ 2	> 4	98.6	0.8	0.6
Ciprofloxacin	≤ 0.5	> 1	93.9	1.6	4.5
Trimethoprim	≤ 2	> 4	78.4	2.1	19.5
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	84.3	2.4	13.3
ESBL	Negative	Positive	97.3	-	2.7

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 37. *Klebsiella oxytoca* urinary tract isolates in 2014 (n=108). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Mecillinam	≤ 8	> 8	82.4	-	17.6
Amoxicillin-clavulanic acid*	≤ 32	> 32	85.2	-	14.8
Piperacillin-tazobactam	≤ 8	> 16	87.1	0.9	12.0
Cefuroxime	≤ 8	> 8	87.0	-	13.0
Cefotaxime	≤ 1	> 2	96.3	0.9	2.8
Ceftazidime	≤ 1	> 4	95.3	2.8	1.9
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	99.1	0.0	0.9
Ciprofloxacin	≤ 0.5	> 1	99.1	0.9	0.0
Trimethoprim	≤ 2	> 4	89.8	0.0	10.2
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	93.5	0.0	6.5
ESBL	Negative	Positive	98.1	-	1.9

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

The $ESBL_{CARBA}$ (carbapenemase) situation in Norway

The rapid global increase and dissemination of multi-drug resistant and in particular $ESBL_{CARBA}$-producing Gram-negative pathogens is considered a global public health threat (1). $ESBL_{CARBA}$ or carbapenemases are beta-lactamases with the ability to hydrolyse and inactivate carbapenems which are considered last-line antibiotics for treatment of systemic infections caused by Gram-negative bacteria. In addition, the majority of $ESBL_{CARBA}$ beta-lactamases can inactivate other beta-lactams like penicillins and cephalosporins. $ESBL_{CARBA}$-producing isolates are frequently co-resistant to other important antibiotics such as aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole. Consequently, treatment options for infections caused by $ESBL_{CARBA}$-producing Gram-negative bacteria are therefore limited and experimental non-validated antibiotic-combinations are employed (1-3). Genes encoding $ESBL_{CARBA}$ beta-lactamases are frequently associated with other resistance genes on mobile genetic elements such as plasmids, increasing the efficiency of spread among Gram-negative bacteria.
$ESBL_{CARBA}$ beta-lactamases can be divided into three main groups (4, 5): (i) $ESBL_{CARBA-A}$ (e.g. KPC and IMI), (ii) $ESBL_{CARBA-B}$ (e.g. NDM, VIM and IMP) and (iii) $ESBL_{CARBA-D}$ (e.g. OXA-23, OXA-24, OXA-58 and OXA-48). OXA-23, OXA-24 and OXA-58 are mainly identified among *Acinetobacter baumannii,* while OXA-48 is mainly identified among Enterobacteriaceae.
In Norway, $ESBL_{CARBA}$-producing bacteria are mandatory to report to the public health authorities after confirmation at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-Res). This update describes a summary of $ESBL_{CARBA}$-producing Gram-negative bacteria in 2014. Isolates from the same patient are included if they were of different species and/or harboured different $ESBL_{CARBA}$ beta-lactamases.

In 2014 a total of 12 $ESBL_{CARBA}$-producing Enterobacteriaceae from 11 patients were identified (Figure 48A and B). One patient in 2014 was identified with both $ESBL_{CARBA}$-producing *Escherichia coli* and *Enterobacter* spp. Overall, *Klebsiella pneumoniae* (n=6) was the most common species followed by *E. coli* (n=4) and *Enterobacter* spp. (n=2). The diversity of $ESBL_{CARBA}$ variants, NDM (n=4), OXA-48-like (n=3), KPC (n=3), VIM (n=1) and IMP (n=1), reflects the global epidemiology (Figure 48B) (6).

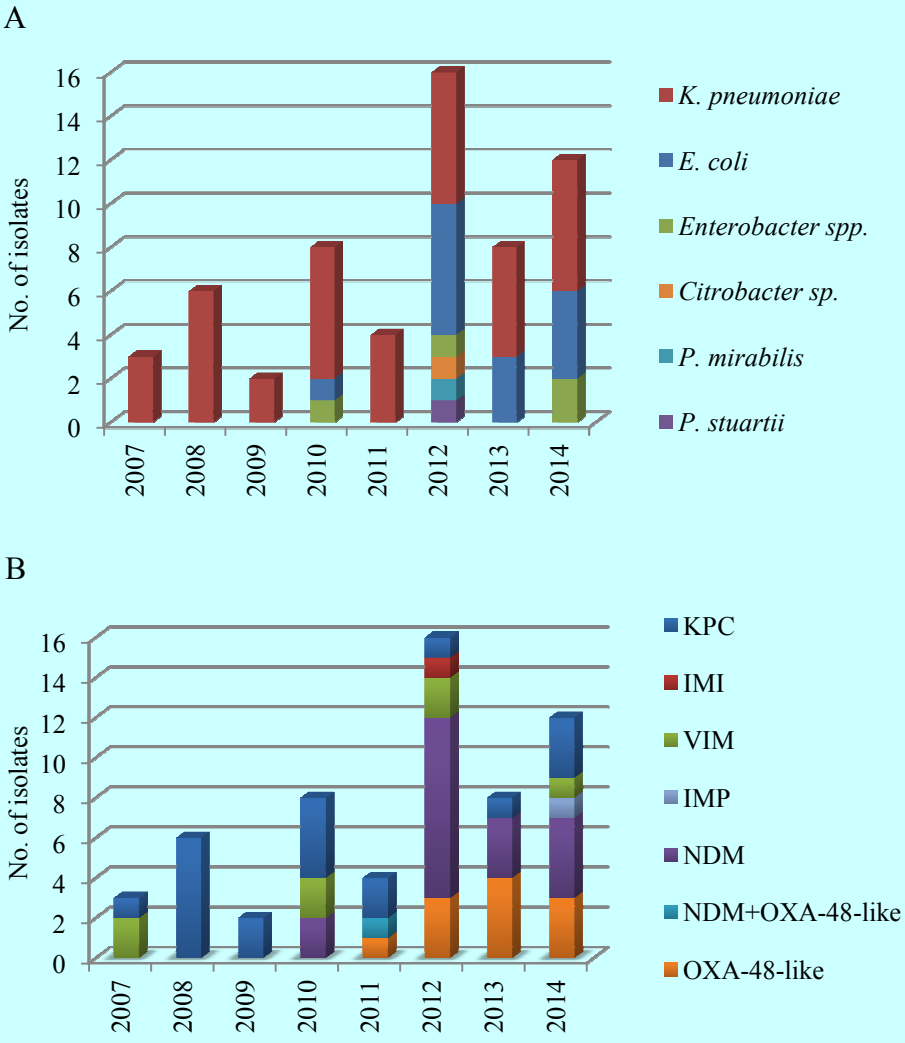


FIGURE 48. $ESBL_{CARBA}$-producing Enterobacteriaceae 2007-2014 by species (A) and $ESBL_{CARBA}$ variant (B).

The majority of cases are associated with import, illustrating that Norway takes part in the global dissemination of $ESBL_{CARBA}$-producing Gram-negative bacteria. The $ESBL_{CARBA}$ variant IMP has never previously been identified among Enterobacteriaceae in Norway. The majority of isolates was identified from clinical specimens (n=8) followed by faecal screening (n=4) which is in contrast to what is observed in other countries such as Sweden and France (7, Sjöström P. personal communication).

Six and 15 $ESBL_{CARBA}$-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates were identified in 2014, respectively (Figure 49).

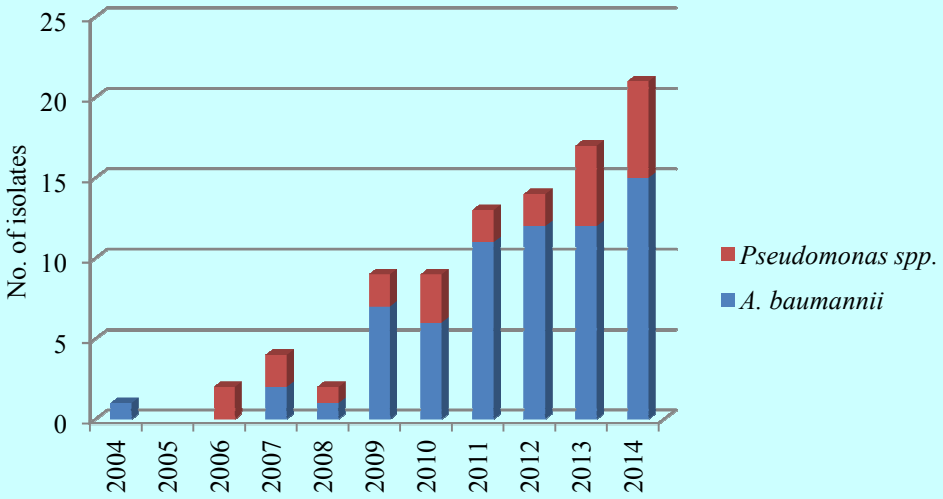


FIGURE 49. Identified $ESBL_{CARBA}$-producing *Pseudomonas* spp. and *A. baumannii* in Norway 2004-2014.

VIM (n=5) was the most common $ESBL_{CARBA}$ variant among *P. aeruginosa*. One isolate harboured the $ESBL_{CARBA}$ variant GES-5 which was identified in Norway for the first time. The majority of GES beta-lactamases are $ESBL_A$ beta-lactamases. However, some variants including GES-5 have activity against carbapenems and are classified as $ESBL_{CARBA-A}$ (7). OXA-23 was the dominant variant among *A. baumannii* identified in 12 isolates. OXA-24 and OXA-58 were identified in one isolate each. One *A. baumannii* isolate showed PCR-negative results for the most common $ESBL_{CARBA-D}$ and $ESBL_{CARBA-B}$ variants, but showed positive carbapenemase activity in biochemical assays. The dominance of VIM and OXA-23 among *P. aeruginosa* and *A. baumannii*, respectively, reflects the global epidemiology. Noteworthy, several patients were identified with both $ESBL_{CARBA}$-producing *P. aeruginosa* and *A. baumannii*.

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Outbreaks of resistant microbes in healthcare institutions

Outbreaks of antibiotic resistant microbes pose a major threat to society and health services. In countries such as Norway with low rates of sporadic multi-drug resistant organism (MDRO) infections, outbreaks (and the way they are controlled) may be the tipping point towards endemicity. Examples of this have been seen previously in other countries. For example, VRE has gone from a relatively rare finding amongst ICU patients to becoming the third most common cause of nosocomial bacteremia in the US health system [1]. The goal of the Norwegian MRSA infection control guidelines and other recommendations directed against resistant bacteria is to prevent the secondary spread of these organisms in our healthcare institutions, where the dissemination of these bacteria can have even more serious consequences than out in the general population due to selection pressure and a vulnerable population.

Definition of an outbreak

We define an outbreak of an infectious disease as follows:

- two or more cases of the same disease, suspected to have a common source, or
- a number of cases that clearly exceeds what one would expect (the endemic level) within an area in a given period.

Outbreak notification

According to the MSIS white paper, outbreaks in hospitals must be notified to the Norwegian Institute of Public Health (NIPH) and the Municipal Health Officer with a copy sent to the Regional Center of Excellence for Hospital Hygiene. Outbreaks in long-term healthcare facilities (LTCFs) must be notified to the Municipal Health Officer, whose responsibility is to notify the NIPH and the County Officer. Outbreaks must be notified immediately when suspicion arises – which can be prior to confirmation of the outbreak. In 2005, the NIPH in collaboration with the Norwegian Food Safety Authority (Mattilsynet) introduced an online notification system for outbreak notification [2] (Vesuv). Vesuv can also be used as both a channel of communication and method of registering changes in the outbreak status.

Outbreak statistics

Between 2010 and 2014 the NIPH received 475 notifications of suspected or verified outbreaks in healthcare facilities (79 in 2014). However, most of these were not due to MDROs. The number of MRSA, VRE and ESBL outbreaks (based on the above definition) in healthcare institutions are presented in Table 38. Table 39 shows the number of outbreaks by institution type revealing a difference in the distribution of reported outbreaks by microbe type. Table 40 contains information on the size of the reported outbreaks and, where available, the ratio of colonisation to clinical infections reported for the outbreaks.

TABLE 38. Number of outbreaks, reported by year.

Year	MRSA	VRE	ESBL
2010	9	1	2
2011	4	1	0
2012	12	1	1
2013	9	2	4
2014	10	4	3

MRSA=Methicillin resistant *Staphylococcus aureus*, VRE=Vancomycin resistant enterococci, ESBL=Extended spectrum beta-lactamase producing Enterobacteriaceae

TABLE 39. Number of outbreaks by type of healthcare institution 2010-2014.

Aetiology	Hospital	LTCF	Other
MRSA	12	32	0
VRE	9	0	0
ESBL	6	3	1

LTCF=Long-term healthcare facility

TABLE 40. Size of reported outbreaks and colonisation to infection ratio in 2014.

Aetiology	Outbreak size: n Median (range)	Colonisation/infection ratio
MRSA	3 (2 – 18)	2.8
VRE	8 (2 – 320)	8.4
ESBL	4.5 (2 – 63)	Not available

Outbreaks of MRSA

Notified individual cases of MRSA have increased each year in MSIS, but these are predominantly cases where the person is believed to have been infected outside Norway and for cases detected in the community. The number of MRSA outbreaks in healthcare facilities in 2014 was at the same level as in previous years with the exception of 2011 when there were only four notified outbreaks. In previous years most outbreaks of MRSA have come from nursing homes, however, in 2014 for the first time more outbreaks were reported from hospitals. Maternity/neonatal wards [3] have been over-represented amongst the hospital-based outbreaks, possibly due to high risk of transmission in these settings. This can be seen in the high numbers of 0-1 year old inpatients notified to MSIS (Figure 50). However, overall outbreaks of MRSA have still been more common in nursing homes with Steen et al. [4] reporting on twenty outbreaks in nursing homes between 2005-11 in Oslo alone.

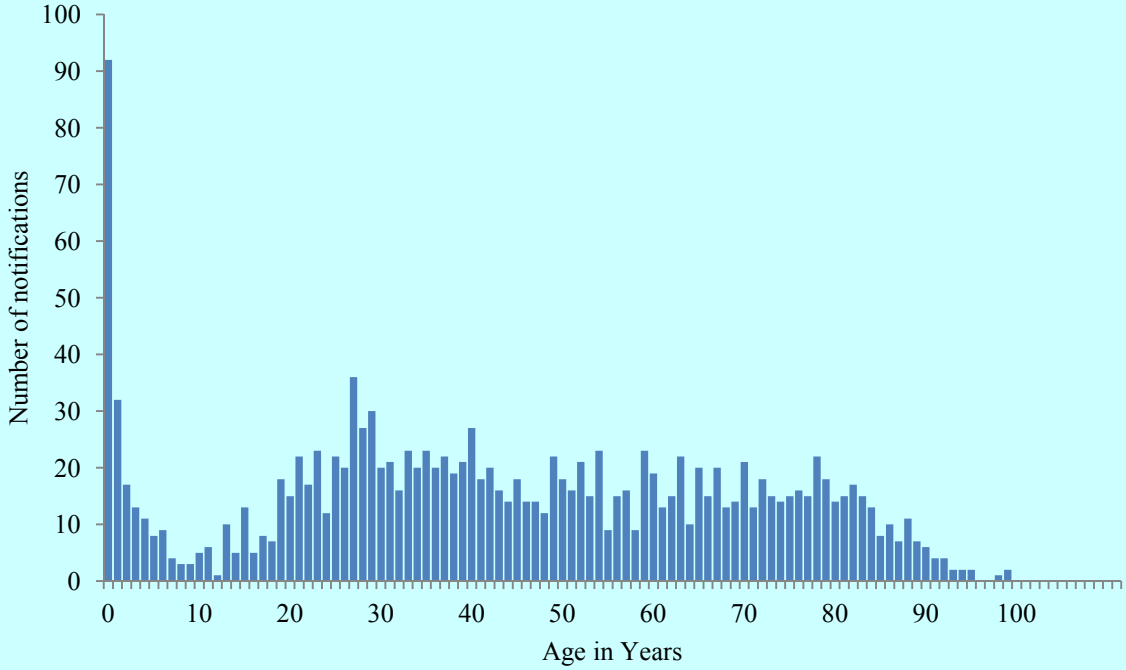

FIGURE 50. Age distribution amongst in-patients notified with MRSA 2010-2014.

Outbreaks of VRE

Before 2010, outbreaks of VRE were unprecedented in Norway. In recent years there has been at least one outbreak in hospitals each year, this increased to two in 2013 and four in 2014. So far there are no reports of outbreaks in nursing homes. In 2014 there were a total of 66 patients registered as part of VRE outbreaks in healthcare institutions of which seven had active infections. As reported in the previous NORM report, at present it appears that outbreaks of VRE are the driving factor for the increase in the detection of these bacteria in Norway.

Outbreaks of ESBL-producing bacteria

In 2014, three outbreaks of $ESBL_{A/M}$-producing bacteria were notified, two from nursing homes and one from a hospital. The two outbreaks in nursing homes were caused by *E. coli*. There was also a large outbreak of an $ESBL_A$-carrying *Klebsiella pneumoniae,* centered on one hospital with over 60 patients detected from many different wards. The lack of good baseline data on the prevalence of this type of organism made the initial declaration of a hospital-based outbreak difficult. However, once this was understood, comprehensive infection control measures were implemented, including screening of contaminated sinks, to prevent further spread.

Of the 10 registered outbreaks of ESBL-producing bacteria since 2010, only one has thus far involved a carbapenemase producing organism. This was due to *Acinobacter baumannii* in an intensive care facility and was driven by within-unit spread of an imported organism through indirect patient-to-patient transmission. Following the outbreak, control measures were evaluated to prevent this from occurring again. However, this is not the only such reported outbreak. Between 2007 and 2010 another hospital reported an outbreak of KPC with probable intergenus spread and long-term consequences for the patients involved [5].

Discussion

In countries such as Norway with low endemic levels of antimicrobial resistant organisms, outbreaks in healthcare institutions may play an especially important role in the rise of their prevalence. From the notifications registered in MSIS there is evidence to suggest that for both VRE and ESBL producing bacteria we are seeing a transition from random sporadic cases to ever more common healthcare outbreaks. It is of concern that our hospitals may become net-exporters of MDROs into the wider community and especially into nursing homes. However, these outbreaks also provide us with an opportunity to halt their spread. It is within these walls (the healthcare institution) that we can implement our most effective control and detection measures and restrict (or guide) our prescribing of antibiotics. At least one study has shown that for VRE in ICUs it is possible, through effective infection control programmes, to reduce the basic reproductive number from 3 down to 0.7 [6].

It is worth noting the difference in the colonisation to infection ratios reported in MRSA and VRE outbreaks. There are several possible explanations including that it is an artefact of the reporting method. However, we may also consider that it is due to a difference in the virulence of MRSA and VRE or patient centered factors. This not only affects the ratio directly but also has implications in terms of early detection of the outbreak and infection prevention measures around detected cases.

From the data collected since 2010 in Vesuv it would appear that efforts to stop outbreaks of MDROs in Norway should have a special focus on MRSA in nursing homes, VRE in hospitals and Gram-negative ESBL producing bacteria in both settings. There is an ever increasing literature on these types of outbreaks; however, publication bias leads to only a small number of strategies and possibly lucky results being published. Effective early communication (including reporting) and handling of healthcare-associated outbreaks appears to be an increasingly important element in the fight against antibiotic resistance.

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Haemophilus influenzae in blood cultures and cerebrospinal fluids

TABLE 41. *Haemophilus influenzae* in blood cultures and cerebrospinal fluids in 2014 (n=69). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 1	> 1	79.7	-	20.3
Amoxicillin-clavulanic acid	≤ 2	> 2	94.2	-	5.8
Cefuroxime	≤ 1	> 2	69.6	17.4	13.0
Cefotaxime	≤ 0.125	> 0.125	98.6	-	1.4
Ceftriaxone	≤ 0.125	> 0.125	100.0	-	0.0
Ciprofloxacin	≤ 0.5	> 0.5	98.6	-	1.4
Chloramphenicol	≤ 2	> 2	100.0	-	0.0
Tetracycline	≤ 1	> 2	98.6	0.0	1.4
Trimethoprim-sulfamethoxazole*	≤ 0.5	> 1	87.0	2.9	10.1
Penicillin G (mm)	≥ 12	< 12	73.9	-	26.1
Cefaclor (mm)	≥ 23	< 23	71.0	-	29.0
Beta-lactamase	Negative	Positive	87.0	-	13.0

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 42. *Haemophilus influenzae* in blood cultures and cerebrospinal fluids in 2014 (n=69). Distribution (%) of MICs (mg/L) and zone diameters for penicillin G and cefaclor (mm).*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Ampicillin				1.4		1.4	42.0	31.9	2.9	7.2		1.4	1.4	1.4		8.7
Amoxi-clav**						1.4	2.9	40.6	40.6	8.7	4.3	1.4				
Cefuroxime						1.4		10.1	57.9	17.4	7.2	5.8				
Cefotaxime		1.4	7.2	23.2	56.5	10.1	1.4									
Ceftriaxone	7.2	46.4	33.3	10.1	1.4				1.4							
Ciprofloxacin	2.9	17.4	50.7	26.1	1.4				1.4							
Chloramph.			1.4				1.4	11.6	81.2	4.3						
Tetracycline					1.4		31.9	62.3	2.9			1.4				
TMS***			10.1	46.4	24.6	2.9	1.4	1.4	2.9		1.4	1.4		7.2		
	< 11	11	12	13	14	15	16	17	18	19	20	21	22	23	24	≥ 25
Penicillin G	26.1			7.2	7.2	8.7	10.1	17.4	7.2	7.2	4.3		1.4	1.4		1.4
Cefaclor	8.7					1.4	1.4	1.4		2.9	1.4	1.4	10.1	4.3	10.1	56.5

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **Amoxi-clav=Amoxicillin-clavulanic acid. ***TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Systemic isolates of *H. influenzae* were first included in the NORM programme in 2013 and resistance data are now provided by the Reference Laboratory at the Norwegian Institute of Public Health on a yearly basis. *H. influenzae* isolates from respiratory tract specimens have been surveyed by NORM in 2001, 2004, 2007, 2011 and 2014, and the test panels for isolates originating from systemic and localised infections are similar to facilitate comparison.

In 2014, a total of 69 isolates were recovered from blood cultures (n=67) or cerebrospinal fluids (n=2), all representing unique patients (Tables 41-42). Beta-lactamase production was detected in 13.0% which is at the same level as 15.2% in 2013 and slightly lower than 17.3% in respiratory tract isolates. A total of 14/69 isolates were resistant to ampicillin, and beta-lactamase production was present in nine of them. Two of these isolates were concomitantly non-susceptible to amoxicillin-clavulanic acid and cefuroxime, thus indicating a combination of beta-lactamase production and chromosomal resistance mechanisms. The five beta-lactamase negative, ampicillin resistant strains were all cefuroxime resistant suggesting a chromosomal basis for beta-lactam resistance. Two of them were also resistant to amoxicillin-clavulanic acid.

Cefuroxime MIC > 2 mg/L has been suggested as the most precise indicator for alterations in the wild type PBP3 sequence. A total of nine isolates (13.0%) displayed this phenotype compared to 19.0% in 2013 and 13.4% in respiratory tract isolates in 2014. Some of these isolates remained susceptible to ampicillin (3/9) and amoxicillin-clavulanic acid (6/9). One blood culture isolates displayed

resistance to third-generation cephalosporins with an MIC to cefotaxime of 0.25 mg/L. The isolate was beta-lactamase negative but resistant to ampicillin and cefuroxime. The ceftriaxone MIC was 0.03 mg/L and thus well within the susceptible range.

Different substrates have been suggested for screening of beta-lactam resistance in *H. influenzae*. The penicillin G 1U disk (PCG1) successfully identified all ampicillin and amoxicillin-clavulanic acid resistant isolates, but missed one cefuroxime resistant strain. Nine out of 60 (15.0%) beta-lactamase negative isolates were resistant to PCG1; five of these were resistant to ampicillin and 8/9 were non-susceptible to cefuroxime. The breakpoint for the cefaclor disk test is calibrated for beta-lactamase positive isolates; cefaclor correctly identified two cefuroxime non-susceptible isolates in addition to seven isolates where cefuroxime resistance was not verified. The results illustrate the continuing challenges in defining the optimal algorithm for beta-lactam susceptibility testing in *H. influenzae*.

As previously seen in respiratory tract isolates, resistance to ciprofloxacin (1.4%), chloramphenicol (0.0%) and tetracycline (1.4%) was at a very low level. The 10.1% resistance to trimethoprim-sulfamethoxazole was at the same level as 13.9% in systemic isolates in 2013, but lower than 19.0% seen in respiratory tract isolates in 2014.

Haemophilus influenzae in respiratory tract specimens

TABLE 43. *Haemophilus influenzae* in respiratory tract specimens in 2014 (n=463). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 1	> 1	80.3	-	19.7
Amoxicillin-clavulanic acid	≤ 2	> 2	90.9	-	9.1
Cefuroxime	≤ 1	> 2	76.2	10.4	13.4
Cefotaxime	≤ 0.125	> 0.125	99.4	-	0.6
Ciprofloxacin	≤ 0.5	> 0.5	99.6	-	0.4
Chloramphenicol	≤ 2	> 2	99.1	-	0.9
Tetracycline	≤ 1	> 2	98.5	0.2	1.3
Trimethoprim-sulfamethoxazole*	≤ 0.5	> 1	78.0	3.0	19.0
Penicillin G (mm)	≥ 12	< 12	60.0	-	40.0
Cefaclor (mm)	≥ 23	< 23	64.1	-	35.9
Beta-lactamase	Negative	Positive	82.7	-	17.3

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 44. *Haemophilus influenzae* in respiratory tract specimens in 2014 (n=463). Distribution (%) of MICs (mg/L) and zone diameters for penicillin G and cefaclor (mm).*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Ampicillin				0.2	0.9	7.8	41.3	17.1	13.2	3.2	1.5	0.9	1.5	2.2	1.5	8.9
Amoxi-clav**				0.4	0.6	2.6	8.4	32.2	38.4	8.2	5.8	1.5	0.9	0.2		0.6
Cefuroxime						0.9	6.5	38.9	30.0	10.4	5.8	4.5	2.1	0.2		0.6
Cefotaxime	1.5	3.8	26.6	43.8	16.0	7.8	0.2		0.4							
Ciprofloxacin	6.9	23.5	59.8	8.0	0.6	0.4		0.2	0.4							
Chloramph.					0.2	0.2	1.9	44.7	48.6	3.5			0.2	0.4		0.2
Tetracycline				0.2		12.1	62.6	23.3	0.2	0.2	0.6	0.4	0.2			
TMS***	3.0	11.0	28.9	20.3	4.8	3.0	3.2	3.7	3.0	3.5	1.9	0.9	0.2	12.5		
	< 11	11	12	13	14	15	16	17	18	19	20	21	22	23	24	≥ 25
Penicillin G	38.2	1.7	4.8	3.7	3.9	7.8	7.1	11.4	8.0	5.2	5.4	0.4	0.6	0.9	0.2	0.6
Cefaclor	3.9	0.2	0.9	0.2	1.7	1.9	1.5	2.2	2.6	3.2	5.8	3.9	6.5	5.6	10.2	48.4

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **Amoxi-clav=Amoxicillin-clavulanic acid. ***TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

A total of 463 *H. influenzae* isolates were recovered from respiratory tract specimens including eye and middle ear during 2014 (Tables 43-44). Beta-lactamase production was detected in 17.3% which confirms the increasing trend from 10.5% in 2007 and 12.3% in 2011 (Figure 51). The 19.7% ampicillin resistance rate was comparable to 18.3% in 2011, and beta-lactamase production was present in 75/91 (82.4%) of these isolates. Nineteen of the 75 isolates were concomitantly non-susceptible to amoxicillin-clavulanic acid and/or cefuroxime, thus indicating a combination of beta-lactamase production and chromosomal resistance mechanisms. Five isolates were reported as ampicillin susceptible in spite of being beta-lactamase positive. The 16 beta-lactamase negative, ampicillin resistant strains were, with two exceptions, cefuroxime resistant, suggesting a chromosomal basis for beta-lactam resistance. Thirteen of them were also resistant to amoxicillin-clavulanic acid.

A total of 62 isolates (13.4%) displayed resistance to cefuroxime (MIC > 2 mg/L) compared to 16.3% in 2007 and 17.0% in 2011. The rate of PBP-mediated beta-lactam resistance may thus be declining as opposed to the dissemination of beta-lactamase production, but the change may also reflect differences in test methodology. Some of the cefuroxime resistant isolates remained susceptible to ampicillin (44/62) and amoxicillin-clavulanic acid (30/62). Three isolates displayed resistance to cefotaxime (MIC 0.25 – 1 mg/L). They were all beta-lactamase negative and resistant to cefuroxime, but one of them remained susceptible to ampicillin and amoxicillin-clavulanic acid. Respiratory tract isolates were not tested for ceftriaxone susceptibility.

The penicillin G 1U disk (PCG1) successfully identified most isolates resistant to ampicillin (90/91), amoxicillin-clavulanic acid (40/42) and cefuroxime (62/62). Conversely, 106/383 (27.7%) beta-lactamase negative isolates were resistant to PCG1; 16 of these were resistant to ampicillin and 99 were non-susceptible to cefuroxime. Cefaclor correctly identified all 11 beta-lactamase positive, cefuroxime non-susceptible isolates in addition to 22 isolates where cefuroxime resistance was not confirmed.

As seen in blood cultures and previous surveys of respiratory tract isolates, resistance to ciprofloxacin (0.4%), chloramphenicol (0.9%) and tetracycline (1.3%) was at a very low level. The prevalence of resistance to trimethoprim-sulfamethoxazole was 19.0% which is higher than in blood cultures (10.1%), but comparable to the finding in respiratory tract isolates in 2011 (22.0%). There was no significant difference in resistance to non-beta-lactam antibiotics between beta-lactamase positive and negative isolates.

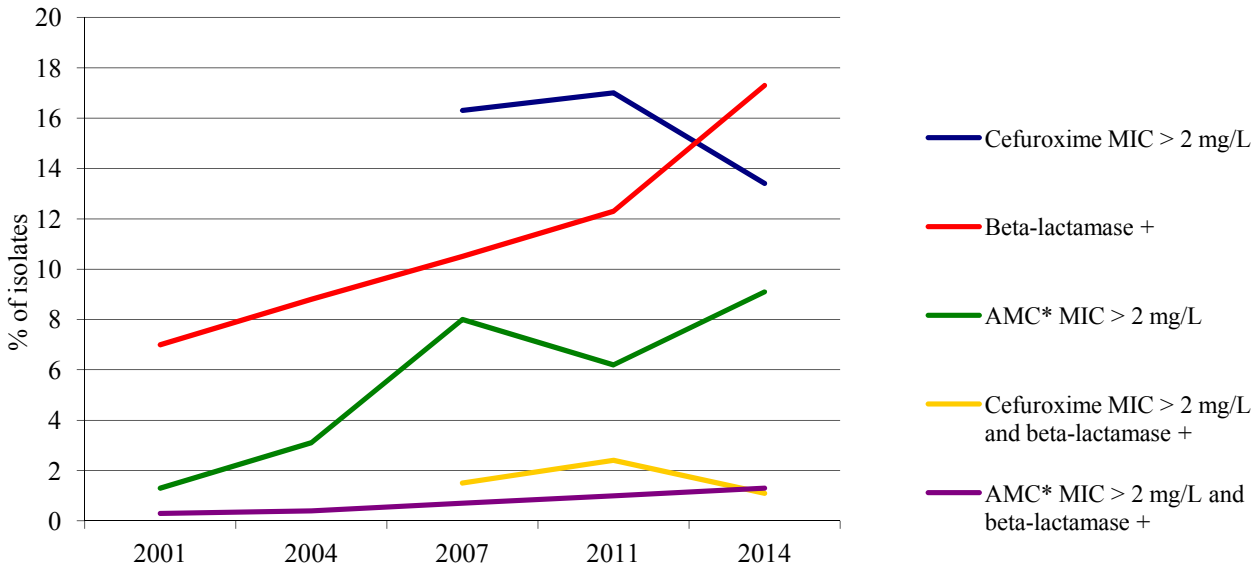

FIGURE 51. Prevalence of beta-lactamase production, chromosomally encoded beta-lactam resistance, and combination of both mechanisms in *Haemophilus influenzae* respiratory tract isolates 2001-2014. The time intervals on the x-axis are not identical. *AMC=Amoxicillin-clavulanic acid.

Neisseria meningitidis in blood cultures and cerebrospinal fluids

TABLE 45. *Neisseria meningitidis* in blood cultures and cerebrospinal fluids in 2014 (n=13). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.06	> 0.25	7.7	84.6	7.7
Ceftriaxone	≤ 0.125	> 0.125	100.0	-	0.0
Ciprofloxacin	≤ 0.03	> 0.03	100.0	-	0.0
Chloramphenicol	≤ 2	> 4	100.0	0.0	0.0
Rifampicin	≤ 0.25	> 0.25	100.0	-	0.0

TABLE 46. *Neisseria meningitidis* in blood cultures and cerebrospinal fluids in 2014 (n=13). Distribution (n) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G					1	8	3	1								
Ceftriaxone	13															
Ciprofloxacin	3	10														
Chloramph.								1	12							
Rifampicin	4	4	3	2												
Azithromycin							5	5	3							
Tetracycline						1	5	7								
Sulfonamide										2	1	3			2	5

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. Non-shaded rows indicate that breakpoints have not been defined.

RESULTS AND COMMENTS

N. meningitidis from blood cultures and cerebrospinal fluids were first included in NORM in 2013. As for systemic *H. influenzae* isolates the Reference Laboratory at the Norwegian Institute of Public Health now provides data on *N. meningitidis* on a yearly basis. The results are presented in Tables 45-46.

A total of 13 isolates were recovered from cerebrospinal fluids (n=5) and blood cultures (n=8). All isolates were from unique patients. The isolates belonged to serogroups B (n=5), C (n=1), W (n=2) and Y (n=5). Elleven isolates displayed penicillin G MICs of 0.125 - 0.25 mg/L and were thus intermediately susceptible, whereas a single isolate was resistant with an MIC of 0.5 mg/L. The genetic basis for non-susceptibility was not determined, but was most likely caused by alterations in the penicillin-binding protein 2 (PBP2) encoded by *penA*. Sulfonamide resistance has been widespread in *N. meningitidis* since the 1960s. EUCAST has not defined clinical breakpoints for this agent, but the MIC distributions clearly demonstrate a high prevalence of acquired resistance among Norwegian isolates.

Neisseria gonorrhoeae

TABLE 47. *Neisseria gonorrhoeae* from all specimen types in 2014 (n=255). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.06	> 1	3.9	49.4	46.7
Ceftriaxone	≤ 0.125	> 0.125	98.8	-	1.2
Cefixime	≤ 0.125	> 0.125	96.1	-	3.9
Azithromycin	≤ 0.25	> 0.5	62.8	29.0	8.2
Ciprofloxacin	≤ 0.03	> 0.06	24.3	0.4	75.3
Tetracycline	≤ 0.5	> 1	22.4	22.4	55.2
Spectinomycin	≤ 64	> 64	100.0	-	0.0
Beta-lactamase	Negative	Positive	69.4	-	30.6

TABLE 48. *Neisseria gonorrhoeae* from all specimen types in 2014 (n=255). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G**		0.4	2.4	1.2		11.4	16.5	11.0	10.6	14.5	8.6	4.3	2.7	16.5		
Ceftriaxone	14.9	23.9	12.5	19.2	22.0	6.3	1.2									
Cefixime			49.8	21.6	15.3	9.4	3.9									
Azithromycin				1.6	10.2	18.0	32.9	29.0	4.3	0.8	1.2	0.4	1.2			0.4
Ciprofloxacin	9.4	10.2	2.7	2.0	0.4	0.4	0.8		1.2	8.6	13.3	9.4	4.7	36.9		
Tetracycline						3.5	5.9	12.9	22.4	18.4	3.9	0.8	9.0	16.1	3.5	3.5
Spectinomycin												11.0	72.9	16.1		

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **Pen G=Benzylpenicillin.

RESULTS AND COMMENTS

Neisseria gonorrhoeae was surveyed in NORM in 2003 and 2010. Since 2013, Oslo University Hospital has provided resistance data for Norwegian *N. gonorrhoeae* isolates on a yearly basis. All isolates from all specimen types were included in the survey, but only a single isolate was accepted from each patient. The microbiological data could not be linked to information from the Norwegian Surveillance System for Communicable Diseases (MSIS).

In 2014, a total of 255 isolates were available for analysis. The isolates were reported to originate from urethra (n=175), cervix uteri (n=39), anus (n=21), throat (n=3), eye (n=1) or "uknown/others" (n=16). A total of 203 isolates (79.6%) originated from men, 47 (18.4%) from women and five isolates (2.0%) were not specified to gender. The geographical location where the infection was acquired was in most cases unknown to the laboratory. From MSIS it is known that gonococcal infections frequently are acquired abroad with secondary transmission in sexual networks within Norway. There is an ongoing outbreak among men who have sex with men, but the strains linked to this outbreak could not be identified within the NORM protocol.

The results from susceptibility testing are presented in Tables 47-48. A majority of isolates were intermediately susceptible (50.2% in 2013, 49.4% in 2014) or resistant (48.0% in 2013, 46.7% in 2014) to penicillin G. Seventy-eight isolates (30.6%) produced beta-lactamase and were phenotypically resistant to penicillin G. This is at the same level as in 2013 (32.4%). Most beta-lactamase positive isolates (74/78, 94.9%) were also non-susceptible to ciprofloxacin. In addition, 43 isolates were resistant and 124 were intermediately susceptible to penicillin G in spite of being beta-lactamase negative. This may be caused by alterations in penicillin binding proteins (PBPs) and/or reduced permeability through the outer cell membrane.

Three isolates (1.2%) were categorised as resistant to ceftriaxone and displayed an MIC of 0.25 mg/L. Ceftriaxone resistant isolates from Norway have previously been linked to treatment failure. Ten (3.9%) isolates were resistant to the oral cephalosporin cefixim which is no longer recommended for empirical treatment in Norway. The decrease from 10.7% resistance in 2013 may be due to technical reasons. The results confirm the emergence of cephalosporin non-susceptible gonococci in Norway, which is of course extremely alarming from both a clinical and a public health perspective. The current European treatment guidelines consist of a combination of ceftriaxone and azithromycin. It should be noted that 37.2% of the isolates were categorised as non-susceptibilible to azithromycin including two of the three ceftriaxone resistant isolates which had MICs for azithromycin of 0.5 and 1 mg/L, respectively.

Ciprofloxacin was previously used for empirical treatment of gonorrhoeae acquired outside South-East Asia. The prevalence of ciprofloxacin resistance remained stable at 75.3% in 2014. Ciprofloxacin is consequently not a viable alternative except in cases where susceptibility has been documented. All strains were susceptible to the aminoglycoside spectinomycin.

Chlamydia trachomatis and *Mycoplasma genitalium* infections: Treatment failure and antibiotic resistance

Chlamydia trachomatis (CT) and *Mycoplasma genitalium* (MG) are often categorised together because of resemblance in the clinical disease they produce. Also, they are difficult to retrieve, labor intensive and time consuming to culture. Diagnosing infection relies upon nucleic acid amplification tests. Culture is done only by a few laboratories in Europe, and mainly for research purposes. This in turn means that very few isolates undergo susceptibility testing. When testing is performed, there are concerns about reproducibility (1). Despite these "at a glance" similarities, CT and MG are unrelated and very different microbes with major differences in prevalence of antibiotic resistance and treatment outcome.

Treatment failure and antibiotic resistance in *Chlamydia trachomatis* infections

Concerns have been raised about declining cure rates for genital *Chlamydia trachomatis* infections (CTI). The concerns are based on comparison between cure rates in current studies and studies performed in the late 90s and early 2000s. A meta-analysis from 2002 including 13 trials between 1990 and 1999 showed combined cure rates of 97% for single dose azithromycin and 98% for a 7 day course of doxycycline (2). Two current and frequently cited studies found microbiological cure rates of 77.4% and 86.3% for azithromycin and 94.8 % and 90.0% for doxycycline. The two newer studies had only 41-58 patients in each arm (3,4). Only one of the studies in the meta-analysis from 2002 used PCR-based methods for test-of-cure (TOC), the other relied on less sensitive methods which might have overestimated the microbiological cure rates. All in all, it may be too early to draw the conclusion that cure rates have actually been declining over the last decade.

The metabolically active forms of *Chlamydia* spp. are strictly intracellular, and are therefore an unlikely target for horizontal interspecies transfer of antibiotic resistance genes. Despite this, tetracycline resistance has emerged in several *Chlamydia suis* strains (a pig pathogen) based on resistance genes originated from Gram-negative bacteria (5). There is so far no evidence for this in human *Chlamydia* species, but when/if the genes should emerge in human *Chlamydia*, it might spread fast since interstrain gene transfer is quite frequent between *Chlamydia* (6). Mutation based resistance can easily be induced *in vitro* for a variety of antibiotics. *In vitro* resistant isolates are generally heteroresitant, and seem to easily lose their resistant phenotype and/or viability (1). There are no larger studies which have investigated the prevalence of mutation based resistance as the cause for treatment failure in CTI. A handful of studies have investigated a few clinical isolates each where treatment has failed. The findings of these studies are contradicting; some concluding that there are elevated Minimum Inhibitory Concentrations (MIC) / Minimum Bactericidal Concentrations (MBC) and molecular evidence of mutation based resistance, while others cannot reproduce these findings (7, 8).

Treatment failure and antibiotic resistance in *Mycoplasma genitalium* infections

In contrast to CTI, there is clear evidence for a high proportion of treatment failure due to antibiotic resistance when treating *Mycoplasma genitalium* infection (MGI). For macrolides and quinolones, resistance is conferred by mutation based altering of antibiotic binding sites, namely the 23S part of the ribosome and in the topoisomerase/gyrases. The mutations associated with resistance are well characterised, and for macrolides there are several publications on how to assess macrolide susceptibility by molecular testing directly on clinical samples (9). Rates of treatment failure for azithromycin are usually reported between 20-50%, and a large proportion develops resistance during treatment. In a study from Greenland, 100% (n=29) of cases of MGI were macrolide resistant. Some argue that development of resistance is less frequent when azithromycin is given as a multi-day course compared to a single dose. However; there are no randomised clinical trials confirming this suggestion, and a recent observational study showed no difference in treatment outcome between single-dose and multi-day treatment with azithromycin (10). Moxifloxacin is the only quinolone to have acceptable treatment outcomes and is now used as a second line treatment if azithromycin fails. Several reports of resistance to moxifloxacin are now being published. Some centers use pristinamycin, an antibiotic composed of a macrolide and stretogramin A moiety, as a third line treatment if moxifloxacin fails (11). Again; reports of treatment failure using pristinamycin are surfacing. Several studies have confirmed low success rates using doxycycline as treatment for MGI, but the reason for this is not evident since isolates retrieved after treatment failure have MICs for doxycycline similar to doxycycline susceptible species. There are no published reports of antibiotic resistance based on horizontal gene transfer in MG, but is has been described in other *Mycoplasma* species.

In summary, MG seems to have a remarkable ability to develop mutation based resistance, but the reason for the low success rate of doxycycline treatment remains unknown. New regimes for treating infections without inducing antibiotic resistance are warranted, and might come to rely on combination therapy. Not only do we need an answer to how we should treat to achieve acceptable cure rates and avoid development of antibiotic resistance. We also lack a description of the natural course of the infection, and an answer to whether we always should treat MGI. A current meta-analysis indicates a two-fold increase in risk of cervicitis, pre-term birth, spontaneous abortion and PID among women infected with MG. However, the association with infertility was not significant, and no studies have determined whether the risks for complications are reduced by MGI treatment. The latter should be kept in mind by both clinicians and authors of guidelines when considering broad-spectrum antibiotics for treating MGI (12).

Summary

There are clear differences in the prevalence of antibiotic resistance and treatment failure between MG and CT infections. Treatment should therefore be based on laboratory confirmation of infection, and laboratories should be encouraged to provide pre-treatment determination of macrolide, and preferably, quinolone resistance mutation in MG positive samples. New regimes for treating MGI without inducing antibiotic resistance are warranted, and might come to rely on combination therapy.

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Beyond Resistance Mechanisms: Can bacterial adaptation be used to our advantage?

Antimicrobial resistance (AMR) is a well-known threat to public health worldwide and is attributed to an estimated 25,000 deaths and in excess of €1.5 billion in increased costs annually in Europe alone [1]. Bacteria acquire AMR either by spontaneous mutation or acquisition of mobile genetic elements such as plasmids and transposons. From an evolutionary perspective, the emergence of AMR is an inevitable outcome due to our use and high consumption of antimicrobials. A confounding issue is that there have been limited novel antimicrobials in the pharmaceutical pipeline [2]. The lack of novel therapeutics and increase in AMR has had crippling effects on our ability to treat certain infections. Currently, there is a resurgence of interest in new antimicrobial development, which stems primarily from increased government funding for such research [3,4]. However, while we wait for the new miracle drugs to reach the clinic, we must consider alternative strategies for antimicrobial stewardship, to extend the life of currently used antimicrobials or, alternatively, to revive older neglected drugs.

In 2001, the WHO published recommendations to reduce the emergence of AMR. They include improved surveillance, infection control, and public awareness, as well as, reduced use in animals [5]. Additional antimicrobial stewardship practices include general reduction of antimicrobial consumption, and coordinated changes to empirical treatments, such as antimicrobial cycling or mixing and combination therapy [6,7]. Whereas these strategies are important at the community level, there is an emerging realisation that AMR is both an ecological and evolutionary problem. Using insights from these fields could improve efforts to reduce the emergence and spread of AMR.

Mutation or alteration of bacteria can lead to improved survival of the organism under selective antimicrobial pressure, however, this adaptation initially comes at some cost from a bacterial perspective [8]. As a result, phenotypic changes including reduced growth rate, virulence or altered drug susceptibilities can be observed. This “biological cost of resistance” is the most important factor controlling the reversibility of AMR in the absence of antibiotic selective pressures [9, 10]. This principle is the basis of current drug cycling recommendations. However, several recent studies suggest novel models for targeted drug therapy, including exploiting suppressive drug pairs [11] and altered drug cycling based on collateral sensitivity (CS) networks [12]. Currently the CS approach appears to be more promising and aims to improve on current drug cycling strategies.

CS was first defined in 1952 by Szybalski and Bryson as when “a strain made more resistant to one antibiotic becomes considerably more sensitive to another” [13]. The authors observed this phenomenon, as well as cross-resistance (also designated collateral resistance; CR), in a collection of laboratory selected AMR *E. coli* isolates. They found a complex network of CS and CR exemplified by a mutant selected on chloromycetin that was 80 times less sensitive to penicillin, but 50 times more sensitive to circulin than the wild-type (WT) [13]. Variation in CR has been observed both within and between antibiotic classes [12], although the strength of CR within a specific drug class is greater than between classes [14]. CR can be

due to general mechanisms of resistance, such as reduced membrane uptake, increased efflux, metabolic changes or enzymatic inactivation or modification of similarly structured drugs. Contrarily, the mechanism of CS is not as easily explained and there could be multiple mechanisms depending on the observed CS network. In 2013 Lazar et al. suggested a specific mechanism of CS. They found that *E. coli* resistant to aminoglycosides had reduced membrane potential, which likely caused reduced function of the endogenous AcrAB efflux pump [15]. This efflux pump has many clinically relevant substrates including chloramphenicol, fluoroquinolones, beta-lactams, erythromycin and fusidic acid [16].

Szybalski and Bryson originally suggested that the identification of mutually exclusive AMR traits would facilitate reversion to a sensitive phenotype, and the exploration of collateral networks would facilitate the design of appropriate treatments [13]. While there has been some research on CS related to multi-drug resistant cancer [17], CS was largely disregarded in microbiology until recently. In 2013, Imamovic and Sommer performed similar studies to those of Szybalski, and selected for AMR *E. coli* with a large collection of 23 antimicrobials [12]. Based on their observations, they suggest that more strategic planning and effective antimicrobial use could slow the development of AMR. The authors predict that specific sequences of antimicrobial usage, based on "reciprocal collateral sensitivity profiles", would force the evolution of bacteria into a predictable trajectory. This would allow for anticipation of resistance development and informed selection of the next antibiotic to be used. Ultimately, treatment would return to the initial drug of choice, to which the bacteria will be susceptible once again, then completing the CS cycle [12].

Specifically, Imamovic noted near-universal CR to antibiotics of the same class, and 17 instances of CS within the 23 AMR *E. coli* isolates [12]. The authors did not, however, observe as high levels of CS as Szybalski, they reported decreases in MIC between 2 to 32 fold as compared to 1.5 to 300 fold [13], respectively. Imamovic identified 207 possible CS cycles in which, as few as two drugs, or as many as four, could be used sequentially to select against resistance. Further, they demonstrated enhanced susceptibility of resistant isolates as compared to the WT strain in mixed cultures *in vitro*.

While these results are promising, there are a few challenges that should be addressed before such approaches are applied clinically. *In vitro* testing and comparison of CS networks should be further explored in diverse collections of *E. coli* clinical isolates and other pathogenic bacteria [18]. AMR mechanisms selected *in vitro* may not well represent what is found to occur *in vivo*, as selective pressures and environmental conditions may change the evolution of resistance. Thus, it will be important to test how CS networks change dependent on the resistance mechanism, both by chromosomal mutation and by horizontal gene transfer, such as the acquisition of multidrug resistant plasmids. We hope that continued attention and research in this field will validate the implementation of evolutionarily-informed treatment strategies to reduce the burden of AMR.

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Staphylococcus aureus in blood cultures

TABLE 49. *Staphylococcus aureus* blood culture isolates in 2014 (n=1,163). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	95.8	0.2	4.0
Clindamycin	≤ 0.25	> 0.5	98.9	0.3	0.8
Fusidic acid	≤ 1	> 1	95.6	-	4.4
Ciprofloxacin	≤ 1	> 1	97.2	-	2.8
Gentamicin	≤ 1	> 1	99.6	-	0.4
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	99.3	0.3	0.4
Tetracycline	≤ 1	> 2	95.4	0.4	4.2
Tigecycline	≤ 0.5	> 0.5	99.4	0.0	0.6
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	100.0	0.0	0.0
Beta-lactamase	Negative	Positive	27.1	-	72.9
Cefoxitin screen	Negative	Positive	99.2	-	0.8
MRSA (*mecA*)	Negative	Positive	99.2	-	0.8

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Nine methicillin resistant *S. aureus* (MRSA) isolates were detected in the NORM surveillance system in 2014 (Table 49) corresponding to a prevalence of 0.8%. This is at the same level as in 2011 (0.5%), 2012 (1.0%) and 2013 (0.3%). The resistance phenotype was confirmed by *mecA* PCR in all cases. The isolates originated from seven different hospitals.

Laboratory screening for MRSA in NORM is performed using cefoxitin disks. All MRSA isolates had cefoxitin zone diameters below the screening breakpoint. Some MRSA isolate were concomitantly resistant to erythromycin (3/9), tetracycline (3/9), gentamicin (2/9), ciprofloxacin (3/9) and/or fucidic acid (2/9). All MRSA isolates were susceptible to clindamycin, trimethoprim-sulfamethoxazole, linezolid, rifampicin and tigecycline. The results from susceptibility testing of all Norwegian MRSA isolates are presented in Table 51 on page 86. No methicillin susceptible *S. aureus* (MSSA) isolates were reported to have cefoxitin zone diameters below the screening breakpoint.

The NORM findings are a bit lower than the reports from the databases of the participating laboratories where 24 out of 1,701 (1.4%) *S. aureus* blood culture isolates were MRSA. None of the 19 *S. aureus* isolates recovered from cerebrospinal fluids were methicillin resistant, thus bringing the total number of systemic MRSA isolates to 24/1,720 (1.4%).

A total of 49 *S. aureus* isolates (4.2%) were non-susceptible to erythromycin. This is at the same level as 3.7% in 2012 and 4.1% in 2013. The macrolide resistance phenotypes of the isolates were determined by the double disk diffusion (DDD) test. Six isolates (12%) were constitutively MLS_B resistant, 28 (57%) were inducibly MLS_B resistant and 15 (31%) displayed efflux mediated M type resistance. These figures represent 0.5%, 2.4% and 1.3% of all *S. aureus* isolates from blood cultures, respectively. The distribution of MLS phenotypes was unchanged from 2013 to 2014.

The prevalence of resistance to fusidic acid at 4.4% was slightly lower than 5.9% in 2012 and 4.8 % in 2013. The 2.8% prevalence of ciprofloxacin resistance was at the same level as 2.5% in 2012 and 2.9% in 2013. There were no significant changes for gentamicin, rifampicin or tigecycline. All isolates were susceptible to trimethoprim-sulfamethoxazole and linezolid. The general test panel for *S. aureus* did not include vancomycin in 2014.

Figure 52 shows the prevalence of non-susceptibility to various antimicrobials. A total of 72.9% of the isolates were beta-lactamase positive which is unchanged from previous years. Beta-lactamase positive isolates were more likely to be resistant to erythromycin (4.8%) and tetracycline (5.1%) compared to beta-lactamase negative isolates (1.9% and 1.9%, respectively). For the other antimicrobials there were only minor differences.

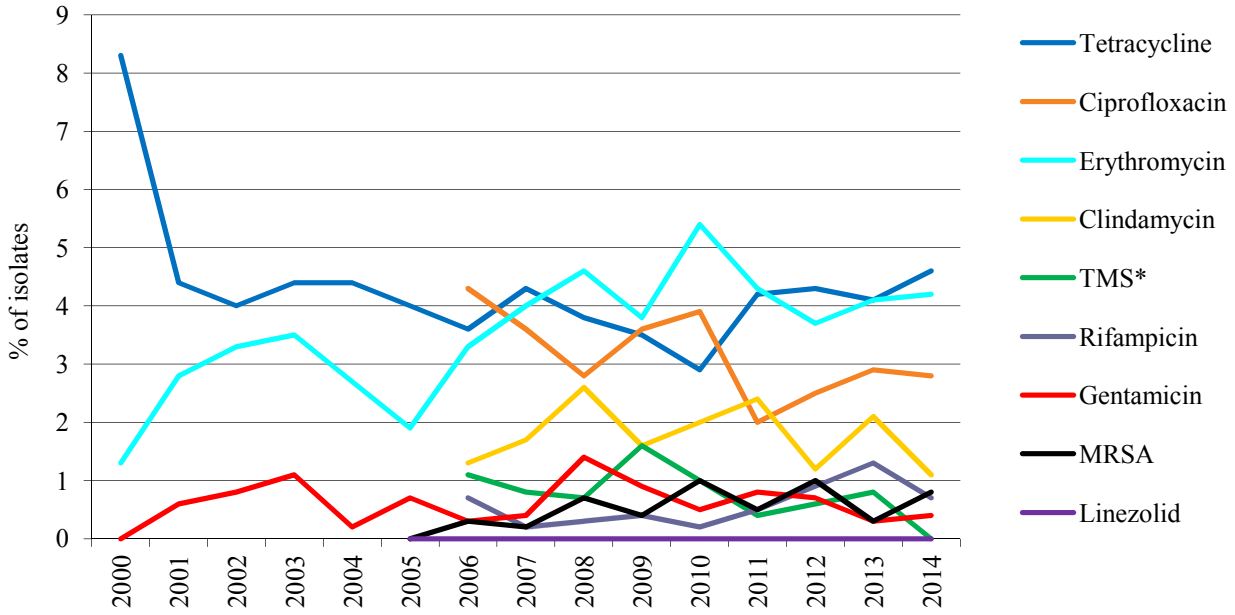

FIGURE 52. Prevalences of non-susceptibility to various antimicrobial agents among *Staphylococcus aureus* blood culture isolates 2000-2014. Doxycycline was replaced by tetracycline in 2006. *TMS=Trimethoprim-sulfamethoxazole.

Staphylococcus aureus in wound specimens

TABLE 50. *Staphylococcus aureus* isolates from wound specimens in 2014 (n=955). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	94.2	0.3	5.5
Clindamycin	≤ 0.25	> 0.5	98.2	0.5	1.3
Fusidic acid	≤ 1	> 1	91.4	-	8.6
Ciprofloxacin	≤ 1	> 1	97.7	-	2.3
Gentamicin	≤ 1	> 1	99.4	-	0.6
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	99.0	0.4	0.6
Tetracycline	≤ 1	> 2	94.4	0.3	5.3
Tigecycline	≤ 0.5	> 0.5	99.7	0.0	0.3
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.5	0.2	0.3
Beta-lactamase	Negative	Positive	23.6	-	76.4
Cefoxitin screen	Negative	Positive	98.7	-	1.3
MRSA (*mecA*)	Negative	Positive	98.7	-	1.3

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

S. aureus from wound specimens were screened for methicillin resistance by the cefoxitin disk method in the same way as blood culture isolates. Twelve out of 955 (1.3%) isolates were confirmed as MRSA by *mecA* PCR. The prevalence was at the same level as in 2011 (1.3%), 2012 (0.7%) and 2013 (1.2%), and also as in blood cultures (0.8%, see above). The MRSA isolates originated from patients admitted to hospital (n=3), outpatient clinics (n=3), general practitioners (n=5) and an unknown location (n=1) in different parts of the country. Four MRSA isolates were only resistant to beta-lactam antibiotics. The remaining eight isolates displayed reduced susceptibility or co-resistance to tetracycline (n=6), erythromycin (n=5), gentamicin (n=4), fusidic acid (n=3), ciprofloxacin (n=2), clindamycin (n=1), rifampicin (n=1) and trimethoprim-sulfamethoxazole (n=1) in different combinations. All MRSA isolates were fully susceptible to tigecycline and linezolid. No isolates were reported with zone diameters below the cefoxitin screening breakpoint without being confirmed as MRSA by *mecA* PCR. This indicates high specificity of the cefoxitin screen as well as a low prevalence of *mecC* MRSA (see page 86).

The prevalence of resistance to fusidic acid in *S. aureus* wound isolates remained essentially unchanged at 8.6% in 2014 compared to 9.0% in 2013 (Table 50 and Figure 53). This confirms that the gradually declining prevalence of fusidic acid resistance has now levelled off after the epidemic which peaked at 25.0% in 2004. The prevalence of resistance to fusidic acid is still significantly lower in blood culture isolates (4.4%).

For other antimicrobial agents such as trimethoprim-sulfamethoxazole, gentamicin, rifampicin, and tetracycline there were only minor changes from 2013 to 2014, and the prevalence of non-susceptibility was in general similar for blood culture isolates and isolates from wound specimens. All isolates were susceptible to linezolid.

A total of 55 (5.8%) isolates were non-susceptible to erythromycin which is at the same level as 6.1% in 2013. The isolates were further examined for determination of resistance phenotype. The majority (35/55, 64% of macrolide resistant isolates) were inducibly resistant to clindamycin, thus representing the $iMLS_B$ phenotype. Minor proportions were either constitutively resistant to clindamycin (n=10) or low-level resistant to erythromycin only (n=10), expressing efflux mediated M type resistance. The findings are in accordance with the results from previous years.

A total of 76.4% of the isolates were beta-lactamase positive compared to 76.2% in 2013. There were no significant differences in the prevalence of resistance to other antimicrobial agents between beta-lactamase positive and beta-lactamase negative isolates.

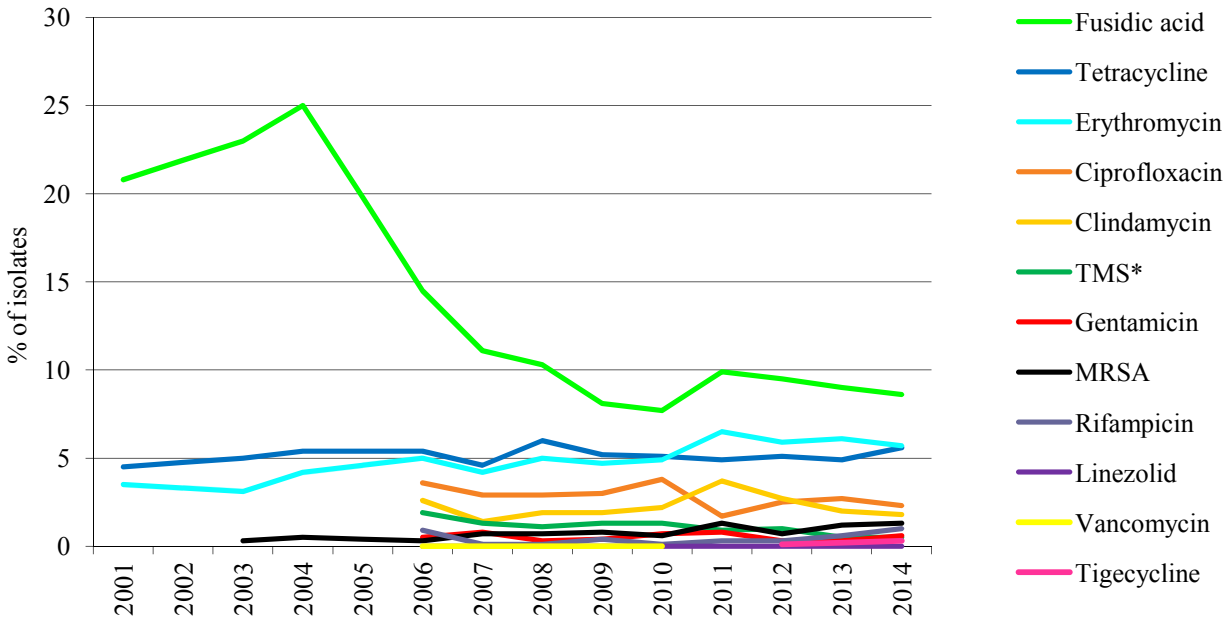

FIGURE 53. Prevalence of non-susceptibility to various antimicrobial agents among *Staphylococcus aureus* wound isolates 2001-2014. Doxycycline was replaced by tetracycline in 2006. *TMS=Trimethoprim-sulfamethoxazole.

Methicillin resistant *Staphylococcus aureus* (MRSA) infections in Norway 2014

Infections with methicillin resistant *Staphylococcus aureus* (MRSA) have been notifiable in Norway since 1995 and colonisation since 2005. A total of 1,867 cases of MRSA were reported in 2014 (37 per 100,000 person-years). 832 (45%) of the cases had an infection while 1,035 were colonised, Figure 54.

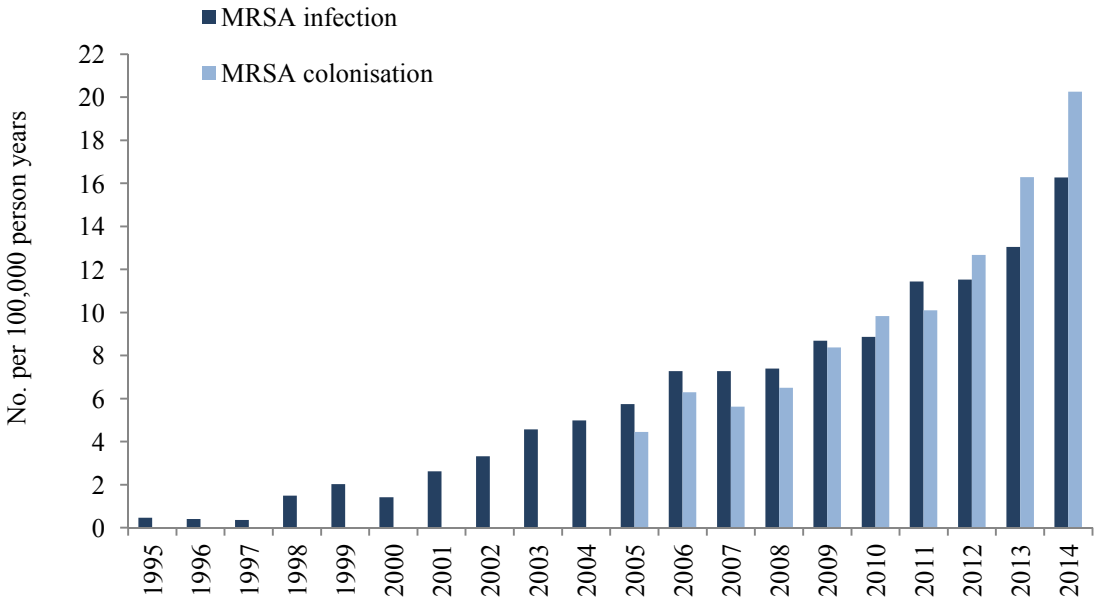

FIGURE 54. Number of MRSA cases per 100,000 person-years in Norway 1995-2014, by infection and colonisation.

The notification rate of MRSA increased with 26% from 2013 to 2014, compared with a mean increase per year of 18% in the former five years. The increase last year is mainly seen among notified cases of MRSA colonisation. The main objective of the Norwegian MRSA infection control measures is to prevent MRSA from becoming endemic in healthcare institutions. 362 (19%) of persons notified with MRSA in 2014 were inpatients at the time of diagnosis, while 56 (3%) were residents in nursing homes and 1,379 (74%) were diagnosed in the community. These numbers only reflect where MRSA infection/colonisation was diagnosed, not where it was acquired.

Sixty of the reported MRSA cases in 2014 were healthcare workers. While we have seen a significant increase in healthcare associated cases over the last two years, the greatest proportion of the total increase is seen in the community and in persons infected abroad (Figure 55).

Livestock associated MRSA (LA-MRSA) was found in Norwegian swine herds for the first time in 2013. While outbreak investigations were still ongoing in 2014, LA-MRSA is so far a limited problem in Norway. In total, 23 cases of LA-MRSA (CC 398) were reported in 2014. Of these, only three were diagnosed during contact tracing around farms with known LA-MRSA. Seven persons were probably infected in Norway, but had no known connection to outbreaks, and the remaining 13 were either reported or presumed to be infected abroad.

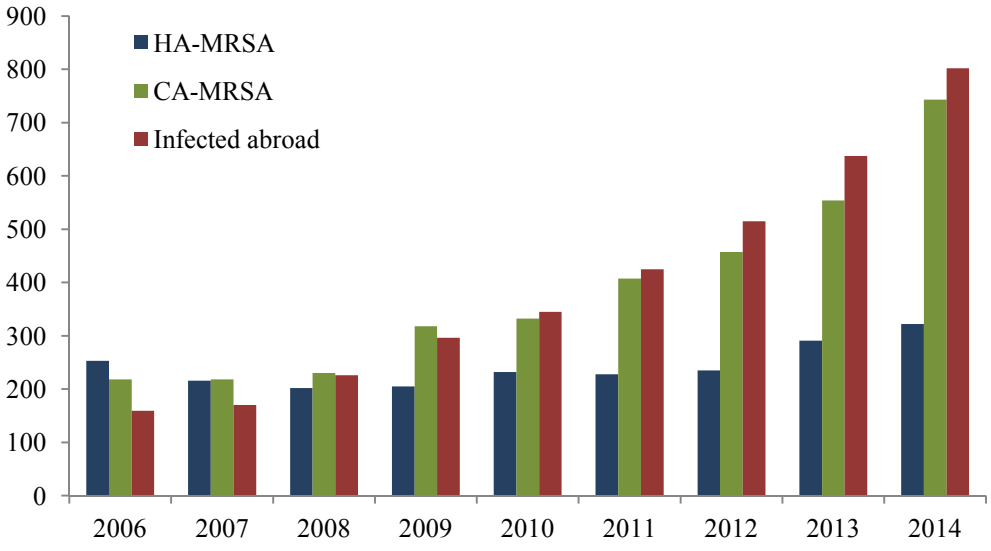

FIGURE 55. Reported cases of MRSA infections and colonisations in Norway 2006-2014, by healthcare associated (HA), community associated (CA) and imported cases.

The Reference Laboratory for MRSA, St. Olav University Hospital, Trondheim, received 1,890 MRSA isolates from different patients in 2014. 294 different spa-types were identified and the six most frequent were (spa-type, n (%)): t002, n=178 (9.4%), t019, n=156 (8.3%), t008, n=124 (6.6%), t127, n=114 (6.0%), t223, n=92 (4.9%) and t044, n=76 (4.0%). 140 spa-types were reported as single events.

Based on spa-type, all isolates were characterised in MLST clonal complex. 1,418 isolates (75.2%) occurred in the six most prevalent clusters (CC, n (%)): CC5, n=373 (19.8%), CC8, n=268 (14.2%), CC30, n=239 (12.7%), CC1, n=212 (11.2%), CC22, n=194 (10.3%), and CC88, n=132 (7.0%).

The Reference Laboratory found 28 LA-MRSA (CC398) in humans (20 spa-type t034, 5 t011 and 1 each of t12359, t4571, t2974) and 2 isolates positive for *mecC* (spa-type t6902 and t843).

Susceptibility testing was performed on 1,871 MRSA isolates collected in 2014 with the EUCAST 2014 disk diffusion method and analysed with breakpoints from NordicAST 2014. 581 strains (31.1%) were sensitive to all antibiotics tested except beta-lactams. The highest proportions of resistance were found for erythromycin (31.5%) followed by tetracycline (24.6%) and norfloxacin (22.1%). 21.7% of the strains were resistant to clindamycin, of which 64.2% were inducibly resistant. The lowest rates of resistance were found towards trimethoprim-sulfamethoxazole (1.0%), mupirocin (0.5%) and rifampicin (1.0%). No strains were resistant to linezolid.

TABLE 51. Susceptibility characterisation of methicillin resistant *Staphylococcus aureus* (MRSA) from 2014 (n=1,871). Standard agar diffusion method ad modum EUCAST 2014. Breakpoints from NordicAST 2014.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	68.4	0.1	31.5
Clindamycin	≤ 0.25	> 0.5	76.9	1.4	21.7
Fusidic acid	≤ 1	> 1	85.5	-	14.5
Norfloxacin	≤ 4	> 4	77.9	-	22.1
Gentamicin	≤ 1	> 1	87.8	-	12.2
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	98.8	0.2	1.0
Tetracycline	≤ 1	> 2	75.3	0.1	24.6
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	96.7	2.3	1.0
Mupirocin	≤ 1	> 256	84.8	14.8	0.4

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

Enterococcus spp. in blood cultures

TABLE 52. *Enterococcus* spp. blood culture isolates in 2014 (n=606). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 4	> 8	75.2	0.0	24.8
Imipenem	≤ 4	> 8	73.9	0.7	25.4
Gentamicin*	≤ 128	> 128	-	75.6	24.4
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	99.8	0.2	0.0
Vancomycin (any genotype)	≤ 4	> 4	98.3	-	1.7
Vancomycin (Van A or VanB)	Negative	Positive	99.5	-	0.5

*The wild type is defined as intermediately susceptible.

TABLE 53. *Enterococcus faecalis* blood culture isolates in 2014 (n=407). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 4	> 8	100.0	0.0	0.0
Imipenem	≤ 4	> 8	99.3	0.2	0.5
Gentamicin*	≤ 128	> 128	-	81.3	18.7
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	100.0	0.0	0.0
Vancomycin (VanA or VanB)	Negative	Positive	100.0	-	0.0

*The wild type is defined as intermediately susceptible.

TABLE 54. *Enterococcus faecium* blood culture isolates in 2014 (n=174). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 4	> 8	15.5	0.0	84.5
Imipenem	≤ 4	> 8	13.2	1.7	85.1
Gentamicin*	≤ 128	> 128	-	59.2	40.8
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	99.4	0.6	0.0
Vancomycin (VanA or VanB)	Negative	Positive	98.3	-	1.7

*The wild type is defined as intermediately susceptible.

RESULTS AND COMMENTS

As in previous years, enterococci were analysed both as a group and separately for *E. faecalis* and *E. faecium*. The results for each species are microbiologically more valid as resistance rates differ significantly between *E. faecalis* and *E. faecium*. However, overall rates of resistance are of interest when formulating empirical treatment strategies because they include the probability of systemic infections with each enterococcal species. The overall results for enterococci are presented in Table 52. The surveillance in NORM 2014 included 407 (67.2%) *E. faecalis* isolates (66.4% in 2013), 174 (28.7%) *E. faecium* isolates (27.5% in 2013) and 25 (4.1%) unspeciated enterococcal isolates (6.1% in 2013). The ratio of *E. faecalis* to *E. faecium* isolates has declined in many countries as the incidence of *E. faecium* bacteremia has increased. In Norway this ratio was 2.3 in 2014 which is comparable to previous years. The number of isolates not speciated to the genus level or identified as *E. faecalis* or *E. faecium* has decreased over the last five years. The panel of antimicrobial agents examined and the breakpoints for interpretation remained unchanged from 2013 to 2014.

E. faecalis was universally susceptible to ampicillin (Table 53). The prevalence of resistance to ampicillin in *E. faecium* remained relatively stable at 84.5% compared to 86.7% in 2013 (Table 54). As expected, the results for imipenem closely mirrored those for ampicillin.

The prevalence of high-level gentamicin resistance (HLGR) in *E. faecalis* was 18.7% which is a decrease from 25.6% in 2012 and 23.6% in 2013 (Figure 56). The prevalence of HLGR in *E. faecium* also decreased from 46.8% in 2013 to 40.8% in 2014, the lowest level recorded in a decade. Almost all (70/71) HLGR *E. faecium* isolates were concomitantly non-susceptible to ampicillin. Conversely, 70 of 147 (47.6%) ampicillin non-susceptible *E. faecium* also displayed HLGR. These findings are similar to the results from previous years. The strong linkage between ampicillin resistance and HLGR may indicate the continuing presence of the internationally disseminated *E. faecium* clonal complex (CC) 17 which is non-susceptible to ampicillin and often harbours high-level resistance to aminoglycosides and vancomycin. The wide dissemination of high-level gentamicin resistance in enterococci is of great concern as it abolishes the bactericidal synergy between aminoglycosides and beta-lactams often used for treatment of severe enterococcal infections.

Transferable vancomycin resistance has not yet been endemically established in clinical enterococcal isolates in Norway, but recent outbreaks have occurred in different parts of the country (see page 71). Ten blood culture isolates were reported as vancomycin resistant in NORM 2014 (1.7%), but only three *E. faecium* (2 VanB, 1 VanA) isolates contained transferable glycopeptide resistance confirmed by positive PCRs. The remaining seven vancomycin resistant isolates were registered as *E. gallinarum* which is inherently low-level resistant to vancomycin due to expression of the VanC ligase. All enterococcal isolates in NORM 2014 were fully susceptible to linezolid as opposed to previous years when several resistant isolates have been detected.

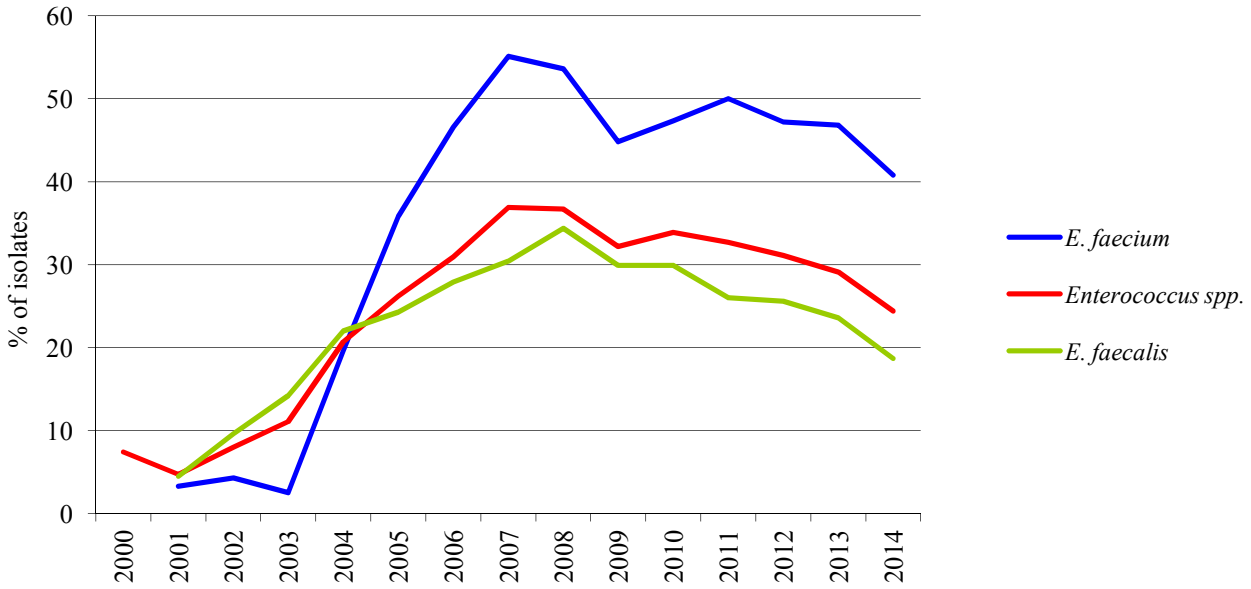

FIGURE 56. Prevalence of high-level resistance to gentamicin in blood culture isolates of *Enterococcus faecalis*, *E. faecium* and all enterococci combined during 2000-2014. The breakpoint for high-level resistance was decreased from R ≥ 1,024 mg/L to R > 128 mg/L in 2004.

Streptococcus pneumoniae in blood cultures and cerebrospinal fluids

TABLE 55. *Streptococcus pneumoniae* in blood cultures and cerebrospinal fluids in 2014 (n=561). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.06	> 2	94.5	5.3	0.2
Cefotaxime	≤ 0.5	> 2	99.3	0.7	0.0
Ceftriaxone	≤ 0.5	> 2	99.8	0.2	0.0
Erythromycin	≤ 0.25	> 0.5	95.5	0.2	4.3
Clindamycin	≤ 0.5	> 0.5	96.8	-	3.2
Tetracycline	≤ 1	> 2	94.8	0.9	4.3
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	91.5	2.3	6.2
Chloramphenicol	≤ 8	> 8	99.5	-	0.5
Oxacillin screen (mm)	≥ 20	< 20	92.0	-	8.0

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 56. *Streptococcus pneumoniae* in blood cultures and cerebrospinal fluids in 2014 (n=561). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G			19.6	70.1	4.8	1.1	2.1	1.1	1.1		0.2					
Cefotaxime		0.2	17.6	71.7	2.9	4.1	2.1	0.7	0.7							
Ceftriaxone		2.0	48.5	40.5	1.8	4.5	1.4	2.1	0.2							
Erythromycin					5.0	78.6	11.9	0.2		0.4	0.5	0.2				3.2
Clindamycin					8.4	71.1	17.1	0.2		0.2						3.0
Tetracycline					3.0	87.0	4.8			0.9		0.9	2.3	0.9	0.2	
TMS**						2.3	59.2	26.2	3.7	2.3	1.2	0.9	0.7	3.4		
Chloramph.							0.2		0.2	41.0	58.1		0.5			
Norfloxacin										7.3	61.5	30.1	0.7	0.2		0.2

	< 20	20	21	22	23	24	25	26	27	28	29	30	31	32	33	≥ 34
Oxacillin disk	6.1	0.7	0.4	0.5	0.7	5.0	11.4	11.2	21.6	22.1	8.6	7.8	1.2	0.7		

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. Non-shaded rows indicate that breakpoints have not been defined. **TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The results are summarised in Tables 55-56 and Figures 57-58. All systemic *S. pneumoniae* isolates submitted to the National Reference Laboratory for Pneumococci at the Norwegian Institute of Public Health during 2014 were included in the surveillance protocol. Twenty-three isolates were recovered from cerebrospinal fluids, and three of these were found in patients who concomitantly had positive blood cultures. Both blood culture isolates and isolates from cerebrospinal fluids were included from patients with positive cultures from both specimen types.

Norwegian breakpoints for pneumococci are in accordance with EUCAST, and these remained unchanged in 2014 as defined by MIC values. The results for penicillin G, cefotaxime and ceftriaxone were interpreted according to the general breakpoints for pneumococci (R > 2 mg/L for all three substances; S ≤ 0.06, S ≤ 0.5 and S ≤ 0.5 mg/l, respectively). The isolates from cerebrospinal fluids were in addition categorised according to breakpoints for meningitis (R > 0.064, R > 0.5 and R > 0.5 mg/L, respectively).

A total of 5.3% (30/561) of *S. pneumoniae* isolates were intermediately susceptible to penicillin G (MIC 0.125-1 mg/L), and a single blood culture isolate (0.2%) was classified as resistant with an MIC of 4 mg/L. This isolate also displayed intermediate susceptibility to cefotaxime and ceftriaxone (both MIC 1 mg/L). Three penicillin intermediate blood culture isolates (MIC 1 mg/L) were non-suscpetible to cefotaxime (MIC 1 mg/L) but remained susceptible to ceftriaxone. Three of the isolates intermediately susceptible to penicillin were recovered from cerebrospinal fluid, and as they displayed MIC values of 0.125-0.5 mg/L they should clinically be categorised as resistant. Non-susceptibility to penicillin G was at the same level as in 2012 (6.3%) and 2013 (3.2%).

The oxacillin screening disk is often used to discriminate between penicillin susceptible and non-susceptible isolates. All penicillin G non-susceptible isolates were resistant to oxacillin. Conversely, 14/530 penicillin G susceptible isolates were oxacillin resistant. The sensitivity and specificity of the screening test was thus

100.0% and 97.4%, respectively. Many of the penicillin non-susceptible *S. pneumoniae* isolates were also non-susceptible to erythromycin (16/31), tetracycline (15/31) and trimethoprim-sulfamethoxazole (17/31).

The prevalence of macrolide non-susceptibility remained stable at 4.5% compared to 6.0% in 2012 and 3.6% in 2013 (Figure 57). Most of these isolates (18/25, 72% of macrolide non-susceptible isolates, 3.2% of all isolates) were concomitantly high-level resistant to erythromycin and clindamycin which is compatible with a constitutive MLS_B phenotype. The remaining seven isolates (28% of macrolide non-suscpetible isolates, 1.2% of all isolates) were low-level resistant to erythromycin and susceptible to clindamycin as seen in the inducible MLS_B phenotype or efflux-based M-type resistance. The double disk diffusion (DDD) test was not performed in all isolates to separate these alternatives. The distribution of MLS phenotypes was not significantly altered from 2013 to 2014. The low number of isolates analysed precludes any firm conclusions, but the results may suggest a continuing predominance of *erm*-mediated macrolide resistance as opposed to the *mef*-dominated peak 2002-2009 (Figure 58).

The 8.5% prevalence of non-susceptibility to trimethoprim-sulfamethoxazole is a further increase from 6.5% in 2012 and 7.6% in 2013. The prevalence of non-susceptibility to tetracycline is stable at 5.2% (Figure 57). The vast majority of isolates (99.5%) remained susceptible to chloramphenicol which was earlier often used for empirical treatment of meningitis in Norway. The low prevalence of high-level norfloxacin resistance (Table 56) may reflect the very limited use of levofloxacin and moxifloxacin for respiratory tract infections in Norway.

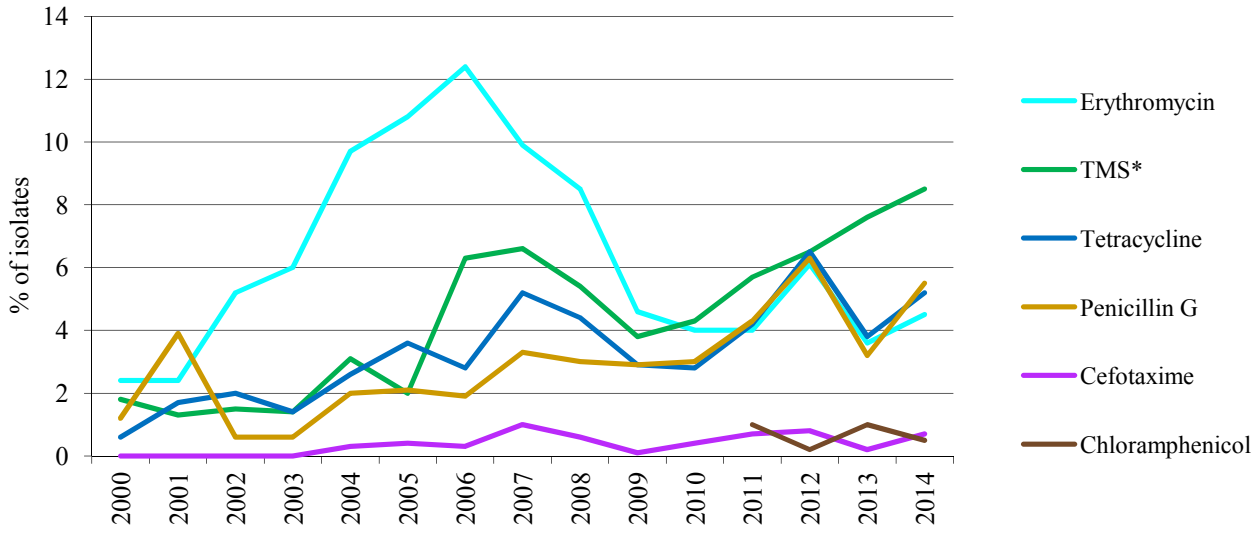

FIGURE 57. Prevalence (%) of non-susceptibility to various antimicrobial agents in *Streptococcus pneumoniae* blood culture isolates during 2000-2014. Doxycycline was substituted by tetracycline in 2005. All results are categorised according to the 2015 breakpoint protocol. *TMS=Trimethoprim-sulfamethoxazole.

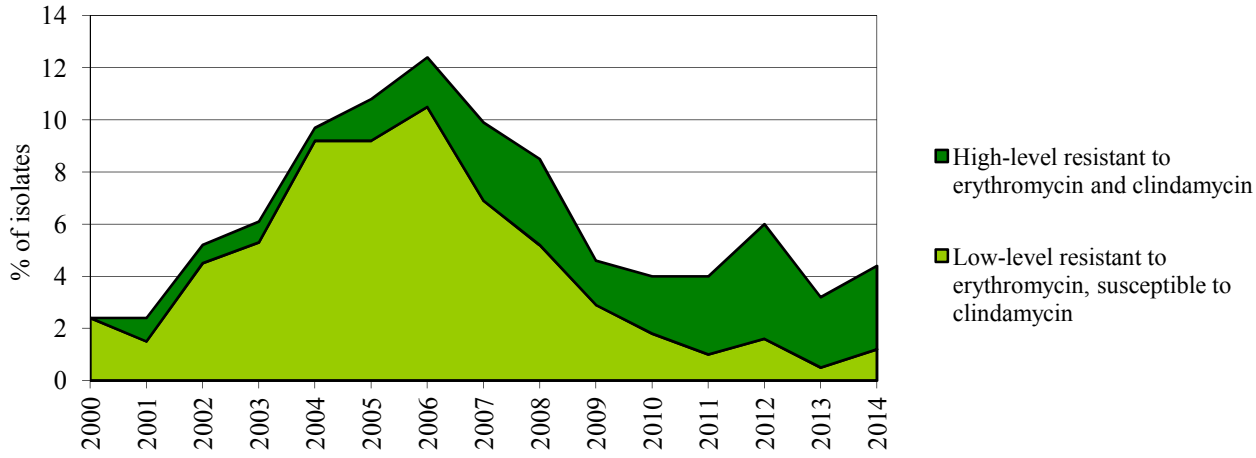

FIGURE 58. Prevalence (%) of non-susceptibility to erythromycin and clindamycin in *Streptococcus pneumoniae* blood culture isolates during 2000-2014.

Streptococcus pneumoniae in respiratory tract specimens

TABLE 57. *Streptococcus pneumoniae* in respiratory tract specimens in 2014 (n=303). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.06	> 2	94.1	5.9	0.0
Cefotaxime	≤ 0.5	> 2	98.7	1.3	0.0
Ceftriaxone	≤ 0.5	> 2	100.0	0.0	0.0
Erythromycin	≤ 0.25	> 0.5	92.1	0.3	7.6
Clindamycin	≤ 0.5	> 0.5	95.7	-	4.3
Tetracycline	≤ 1	> 2	89.7	2.0	8.3
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	93.1	2.6	4.3
Chloramphenicol	≤ 8	> 8	99.7	-	0.3
Oxacillin screen (mm)	≥ 20	< 20	90.8	-	9.2

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 58. *Streptococcus pneumoniae* in respiratory tract specimens in 2014 (n=303). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G	8.3	48.5	32.7	3.0	1.7	2.3	2.0	1.0	0.7							
Cefotaxime	3.3	8.6	41.3	33.7	5.0	3.3	2.0	1.7	1.0	0.3						
Ceftriaxone	4.3	39.6	42.9	5.3	3.6	1.0	1.7	1.7								
Erythromycin			0.3	1.7	10.2	41.3	38.6	0.3		0.3	1.0	1.3	1.0	0.3		3.6
Clindamycin			2.6	4.0	11.2	40.9	33.0	4.0	0.3	0.7			0.3			3.0
Tetracycline			0.3	1.7	39.3	46.5	1.3	0.7		2.0	1.0	1.7	3.3	2.3		
TMS**			1.3	7.9	28.7	41.3	8.6	4.0	1.3	2.6	1.3	1.3	0.7	1.0		
Chloramph.								0.3	5.9	56.4	35.6	1.3	0.3			
Norfloxacin										4.3	27.4	44.2	19.8	2.3	0.7	0.3

	< 20	20	21	22	23	24	25	26	27	28	29	30	31	32	33	≥ 34
Oxacillin disk	9.2	1.0	1.3	2.0	4.0	8.3	10.6	9.6	4.3	9.9	8.9	14.5	6.9	4.3	1.0	4.3

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. Non-shaded rows indicate that breakpoints have not been defined. **TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

S. pneumoniae isolates from respiratory tract specimens were last surveyed in NORM in 2012. The prevalence rates of non-susceptibility to various antimicrobials are shown in Tables 57-58 and Figure 59. As for blood culture and cerebrospinal fluid isolates, the prevalence of penicillin non-susceptibility was stable with 5.9% in 2014 compared to 6.2% in 2012. Similarly, the rate of non-susceptibility to erythromycin was 7.9% in 2014 compared to 7.6% in 2009 and 6.2% in 2012. Tetracycline non-susceptibility has increased from 6.6% in 2012 to 10.3% in 2014, whereas trimethoprim-sulfamethoxazole non-susceptibility (6.9%) was at the same level as in 2012 (6.2%).

A total of 18/303 isolates were intermediately susceptible to penicillin G, no penicillin resistant isolates were detected. Four of these isolates displayed reduced susceptibility to cefotaxime (MIC 1-2 mg/L). Seventeen of the 18 penicillin non-susceptible isolates were detected by the oxacillin screening test (sensitivity 94%), whereas 11 fully penicillin susceptible isolates were classified as oxacillin resistant (specificity 96.1%). Beta-lactam non-susceptible isolates were commonly cross-resistant to other antimicrobial agents such as trimethoprim-sulfamethoxazole (7/18), erythromycin (6/18) and tetracycline (8/18).

In 2014, 7.9% of respiratory tract isolates were non-susceptible to erythromycin compared to 4.5% in systemic isolates. The 24 erythromycin non-susceptible isolates in 2014 comprised ten M-type isolates (42% of erythromycin non-susceptible isolates, 3.3% of all isolates) as opposed to 11 constitutively resistant (46% of erythromycin non-susceptible isolates, 3.6% of all isolates) and three inducibly resistant (13% of erythromycin non-susceptible isolates, 1.0% of all isolates) MLS_B isolates. A total of six isolates (2.0%) were concomitantly non-susceptible to penicillin G and erythromycin. This is a minor decrease from 3.1% in 2012.

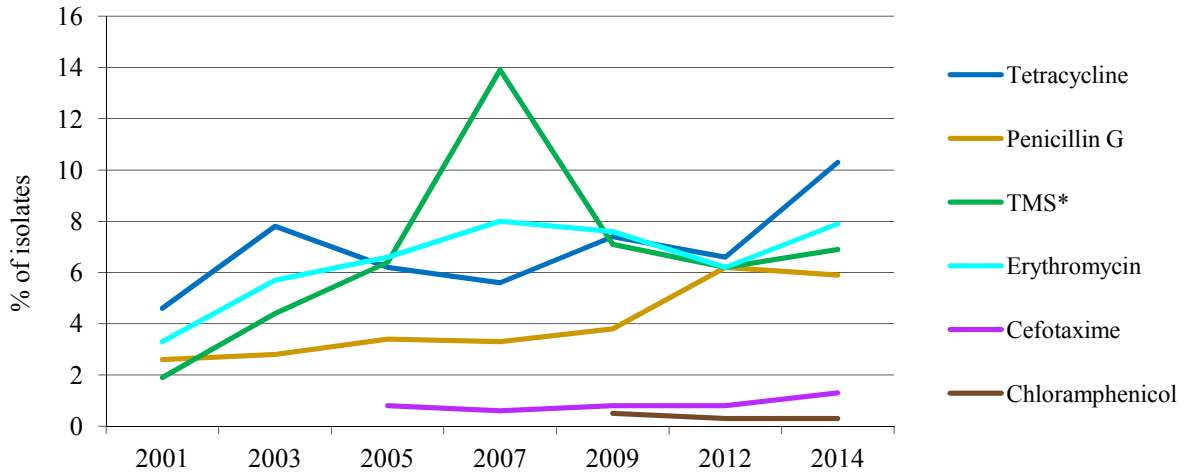

FIGURE 59. Prevalence of non-susceptibility to various antimicrobials in *Streptococcus pneumoniae* from respiratory tract samples 2001-2014. Doxycycline was replaced by tetracycline in 2005. *TMS=Trimethoprim-sulfamethoxazole. Please note that the x-axis is not to scale.

Streptococcus pyogenes in blood cultures

TABLE 59. *Streptococcus pyogenes* in blood cultures in 2014 (n=188). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	96.3	0.0	3.7
Clindamycin	≤ 0.5	> 0.5	97.9	-	2.1
Tetracycline	≤ 1	> 2	83.5	0.0	16.5
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	96.2	1.1	2.7

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 60. *Streptococcus pyogenes* in blood cultures in 2014 (n=188). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G		0.5	44.7	52.1	2.1	0.5										
Erythromycin					32.4	63.3	0.5				0.5		0.5			2.7
Clindamycin			0.5	2.1	45.2	49.5		0.5								2.1
Tetracycline					13.8	60.1	9.6					1.1	5.3	6.4	3.7	
TMS**				0.5	5.9	30.9	36.7	17.0	5.3	1.1	0.5			2.1		

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The Reference Laboratory at the Norwegian Institute of Public Health provides resistance data for systemic *S. pyogenes* isolates on a yearly basis. The Norwegian breakpoints for haemolytic streptococci are in accordance with EUCAST. All comparisons in this report are based on interpretations using the present recommendations.

As expected, all isolates were fully susceptible to penicillin G (Tables 59-60). The prevalence of resistance to erythromycin (3.7%) and clindamycin (2.1%) was slightly increased from 2013 (1.9% and 0.6%, respectively). Four of the seven macrolide resistant isolates were concomitantly high-level resistant to clindamycin. The prevalence of tetracycline resistance has increased from 8.6% in 2006 and 14.8% in 2013 to 16.5% in 2014, whereas the prevalence of non-susceptibility to trimethoprim-sulfa-methoxazole has remained relatively stable at 3.8% in 2014 compared to 4.9% in 2006 and 3.9% in 2013.

Streptococcus agalactiae in blood cultures and cerebrospinal fluids

TABLE 61. *Streptococcus agalactiae* isolates from sterile sites in 2014 (n=243). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.25	> 0.25	100.0	-	0.0
Cefotaxime	≤ 0.5	> 0.5	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	79.0	2.9	18.1
Clindamycin	≤ 0.5	> 0.5	87.2	-	12.8
Tetracycline	≤ 1	> 2	25.5	0.8	73.7
Vancomycin	≤ 2	> 2	100.0	-	0.0

TABLE 62. *Streptococcus agalactiae* isolates from sterile sites in 2014 (n=243). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G			2.1	12.3	77.8	7.4	0.4									
Cefotaxime				6.2	64.6	28.4	0.8									
Erythromycin					3.2	23.0	52.7	2.9	0.4	1.6	2.5	3.3	2.9	0.4		7.0
Clindamycin				0.4	3.7	52.3	29.2	1.6	2.1	0.4		0.8				9.5
Tetracycline				1.6	16.5	6.2			1.2	0.8	0.8	8.6	34.6	26.7	2.1	0.8
Vancomycin						0.8	30.0	63.8	5.3							

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark).

RESULTS AND COMMENTS

Streptococcus agalactiae (beta-haemolytic group B streptococci) has previously been included in NORM in 2006, 2009 and 2012. All systemic isolates in Norway are referred to the National Reference Laboratory at St. Olavs Hospital in Trondheim where confirmatory identification and susceptibility testing is performed. From 2014, the reference laboratory will provide resistance data for all invasive *S. agalactiae* isolates on a yearly basis.

Relevant breakpoints have remained unchanged since 2009. A total of 243 strains were included in 2014. Fifty-three isolates originated from neonates and small children < 1 year of age. In three cases the age of the patient was not recorded. Most isolates (97.5%) were recovered from blood cultures, but there were also isolates from cerebrospinal fluids (n=2) and from other specimen types (n=4). Only one isolate was included from each patient.

As seen in Tables 61-62 there were no isolates with reduced susceptibility to penicillin G, cefotaxime or vancomycin. A total of 44 isolates (18.1%) were resistant to erythromycin compared to 7.7% in 2009 and 12.0% in 2012. In addition, a variable number of intermediately susceptible isolates were detected in 2009 and 2014. The increasing prevalence of macrolide resistance since 2009 is statistically significant and will be followed in the surveillance programme in the coming years.

Thirty-two erythromycin non-susceptible isolates were analysed for MLS resistance phenotype. Twenty-five displayed constitutive (n=14) or inducible (n=11) MLS_B resistance indicating the presence of an *erm* determinant. The remaining seven isolates had a resistance pattern in accordance with an efflux-mediated M phenotype encoded by a *mef* gene. Three isolates were recorded as clindamycin resistant (MIC 1-8 mg/L) in spite of erythromycin susceptibility (MIC 0.125 mg/L).

The prevalence of resistance to tetracycline (73.7%) was at the same level as in 2009 (75%) and 2012 (76.0%) with the majority of isolates displaying MIC values of 16-64 mg/L (Table 62).

Anaerobic bacteria in blood cultures

TABLE 63. Anaerobic Gram-negative bacteria in blood culture 2014 (n=214). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.25	> 0.5	16.7	1.9	81.5
Piperacillin-tazobactam	≤ 8	> 16	76.2	5.1	18.7
Meropenem	≤ 2	> 8	96.7	2.3	0.9
Clindamycin	≤ 4	> 4	81.9	-	18.1
Metronidazole	≤ 4	> 4	97.7	-	2.2

TABLE 64. Distribution (n) of MICs (mg/L) for *Bacteroides fragilis* (n=95), *Bacteroides* non-*fragilis* (n=72) and *Fusobacterium* spp. (n=21) from blood culture 2014.*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Bacteroides fragilis (n=95)																
Penicillin G							1	1			2	5	18	19	47	2
Pip-Tazo**							6	18	28	31	4	5		1		2
Meropenem				5	12	39	27	6			4			2		
Clindamycin				1	10	7	21	12	16	11	3	1	2	1	1	9
Metronidazole						3	8	23	35	22	3					
Bacteroides non-*fragilis* (n=72)																
Penicillin G								1	1	4	2	2	10	19	30	3
Pip-Tazo**								2	2	6	8	13	11	14	3	13
Meropenem				3	4	11	28	20	4	1	1					
Clindamycin		1	6	6		2	2	6	7	11	9	1		1		20
Metronidazole					1	1	4	16	26	20	3					
Fusobacterium spp. (n=21)																
Penicillin G	2	7	10	1	1						1					
Pip-Tazo**		4	5	8	3		1									
Meropenem	6	14				1										
Clindamycin			5	6	7	3										
Metronidazole		4	3	3	3	2	4	2								

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **Pip-Tazo=Piperacillin-Tazobactam

TABLE 65. Anaerobic Gram-positive bacteria in blood culture 2014 (n=120). *Propionibacterium* spp. are not included. Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.25	> 0.5	61.7	13.3	25.0
Piperacillin-tazobactam	≤ 8	> 16	87.3	5.1	7.6
Meropenem	≤ 2	> 8	98.3	1.7	0.0
Clindamycin	≤ 4	> 4	85.2	-	17.5
Metronidazole	≤ 4	> 4	93.2	-	6.8

TABLE 66. Distribution (n) of MICs (mg/L) for *Clostridium* spp. (n=63) and *Propionibacterium* spp. (n=29) from blood culture 2014.*

	≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Clostridium spp. (n=63)																
Penicillin G			2	8	13	10	10	8	8	2	1					1
Pip-Tazo**			4	4	10	8	7	5	6	6	6	4	3			
Meropenem	7	8	8	3	5	9	5	8	4	4	1	1				
Clindamycin				4	5	6	10	5	2	13	4	6	3			5
Metronidazole			1	2	5	2	11	3	18	18	2					1
Propionibacterium spp. (n=29)																
Penicillin G	6	6	12	4		1										
Pip-Tazo**		1	7	5	4	2	3	3	4							
Meropenem	1	6	5	6	8	2		1								
Clindamycin		1	2	9	10	6	1									
Metronidazole																29

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **Pip-Tazo=Piperacillin-Tazobactam

RESULTS AND COMMENTS

Anaerobic bacteria from blood cultures were surveyed in NORM for the first time in 2014, and there are no previous nationwide studies of antimicrobial resistance in Norway for these pathogens. There are a number of methodological challenges with surveillance of anaerobes as detailed in the textbox below (see page 96). In order to ensure clinical relevance of the material, only blood culture isolates were included. Furthermore, only laboratories using MALDI-TOF and/or 16S rDNA sequencing for identification submitted data, as inconsistent speciation is a major obstacle for meaningful interpretation of data. Seven clinical laboratories at major hospitals participated in the survey.

The data for Gram-negative and Gram-positive bacteria are presented in Tables 63-64 and 65-66, respectively. The SIR distributions are given for all genera/species in the group except for *Propionibacterium* spp. which is excluded from Table 65. The MIC distribution data in Tables 64 and 66 are only presented for selected genera/species as specified.

A total of 214 Gram-negative isolates were included in the survey. The majority belonged to *Bacteroides fragilis* (n=95), *Bacteroides* non-*fragilis* (n=72) and *Fusobacterium* spp. (n=21). The remaning isolates were identified as members of the genera *Bilophila* (n=2), *Desulfovibrio* (n=1), *Dialister* (n=5), *Leptotrichia* (n=1), *Porphyromonas* (n=2), *Prevotella* (n=8), *Veillonella* (n=3), *Odoribacter* (n=1), *Butyricimonas* (n=1) or unspecified (n=4). Beta-lactamase testing was not included in the protocol, but as seen in Tables 63-64 the vast majority of isolates were clearly resistant to penicillin G including practically all *Bacteroides* spp. strains. 76.2% of the Gram-negative isolates were susceptible to piperacillin-tazobactam, and as seen in Table 64 *B. fragilis* appears more sensitive to this combination drug than other members of the same genus. Clindamycin resistance was detected in 18.1% of the isolates including *Bacteroides* spp. *Fusobacterium* spp. were generally suscpeptible to all these agents. Seven isolates were non-suscpetible to meropenem, six *B. fragilis* and one *B. stercoris*, displaying MICs of 4-32 mg/L. Five isolates (three *Dialister*, one *Leptotrichia* and one *Prevotella*) were reported as resistant to metronidazole (MIC 8-256 mg/L).

Among the 149 Gram-positive isolates included in the survey, the majority belonged to the genera *Clostridium* (n=63) and *Propionibacterium* (n=29). The latter is not generally considered a true anaerobe and the isolates are therefore omitted from Table 65. The remaning 58 isolates were identified as *Actinomyces* (n=2), *Bifidobacterium* (n=6), *Eubacterium* (n=2), *Lactobacillus* (n=1), *Peptostreptococcus* (n=2), *Ruminococcus* (n=2), *Tissierella* (n=2), *Anaerococcus* (n=2), *Finegoldia* (n=1), *Parvimonas* (n=9), *Peptoniphilus* (n=9), *Catabacter* (n=1) and *Eggerthella* (n=18). As expected, *Propionibacterium* spp. isolates were highly resistant to metronidazole and susceptible to all other agents (Table 66). The remaning eight metronidazole resistant isolates (MIC 8-256 mg/L) were identified as *Bifidobacterium* (n=6), *Lactobacillus* (n=1) and *Clostridium* (n=1). Two *Clostridium* isolates displayed meropenem MICs of 4 and 8 mg/L and were thus classified as intermediately susceptible. Piperacillin-tazobactam generally had high activity among all anaerobic Gram-positive species, whereas many species have a high prevalence of inherent or acquired resistance to penicillin G. Clindamycin resistance at 17.5% is similar to the findings in other surveys.

Antibiotic susceptibility testing of anaerobic bacteria

Susceptibility testing of anaerobic bacteria is difficult for several reasons:

- Different anaerobic bacteria often have different requirements for growth
- Many anaerobic bacteria need more than 24-48 h for significant (primary) growth
- Not all laboratories have access to anaerobic chambers and part of the process of susceptibility testing needs to be done outside such cabinets or anaerobic jars
- Even though the Clinical and Laboratory Standards Institute (3) anaerobic working group has established a reference agar dilution method, there is no internationally agreed standard for susceptibility testing of anaerobic bacteria

Most laboratories find the agar dilution method too cumbersome for routine susceptibility testing and will use gradient strips on Brucella agar made according to the CLSI recommendation (3). Some authors have reported major differences in growth rate among four different commercial Brucella agar media (4). The Brucella agar is not optimised and standardised for susceptibility testing. Suitable control strains must be included on a daily basis or when anaerobic susceptibility testing is done.

Do we really need to do susceptibility testing of anaerobic bacteria?
Inappropriate therapy may result in poor clinical response and increased mortality (5,6). In Europe there has been an increase in clindamycin resistance among *Bacteroides* spp. from less than 10% in the 1980ies to more than 40% 20 years later (2). A recent report from Norway (7) has found 12-29% resistance towards clindamycin in different *Bacteroides* spp. and 31% in *Clostridium* spp. They also reported that 2-7% of *Bacteroides* spp were resistant to meropenem (7). The increasing rates of antibiotic resistance among anaerobic bacteria towards commonly used antibiotics necessitate good regional/national data.

Which method to choose for susceptibility testing of anaerobic bacteria?
There is an ISO standard for susceptibility testing of rapidly growing aerobic bacteria (9). There is not yet such a reference method for susceptibility testing of anaerobic bacteria. However, the Clinical and Laboratory Standards Institute (3) has developed an approved standard for testing of anaerobic bacteria based on Brucella agar with haemin (5 µg/L), vitamin K1 (1 µg/L) and 5% laked sheep blood. Recommended incubation time is 42-48 h.
Incubation time for most *Bacteroides* spp. and *Prevotella* spp. and *Clostridum* spp. is 20-24 h, while many other anaerobic bacteria will need 40-48 h of incubation. McFarland 0.5 is equvalent to 1-2 $x10^8$ cfu/ml *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741. However, this corresponds to only 1-4 x 10^7 cfu/ml of *C. difficile* ATCC 700057. The manufacturers of gradient strips recommend an inoculum of McFarland 1 for testing anaerobic bacteria. When using agar dilution, an inoculum of 10^5 per spot is recommended for both *B. fragilis* and *B. thetaiotaomicron*. It is important to use proper anaerobic incubation atmosphere (ie. 10% CO_2, 10% H_2 and 80% N_2).
Agar gradient diffusion is probably the most used method for susceptibility testing of anaerobic bacteria and a good correlation has been shown with the "reference" agar dilution method (8). Another problem is that some anaerobic bacteria are aero-tolerant making it more difficult to detect suboptimal anaerobic conditions and interpret the result for metronidazole. However, this problem can be controlled for by using suitable control strain (in the same cabinet or jar as the test strain).

EUCAST has established breakpoints for anaerobic bacteria, but not yet developed a disk diffusion methodology for susceptibility testing of anaerobic bacteria. The Clinical Breakpoint Table v. 5.0, states (10): "Disk diffusion criteria for antimicrobial susceptibility testing of anaerobes have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions." EUCAST has issued different tables for Gram-positive anaerobes and Gram-negative anaerobes, as well as a separate table for *Clostridium difficile*.

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Mycobacterium tuberculosis

A total of 327 cases of tuberculosis disease were reported to the Norwegian Surveillance System for Communicable Diseases (MSIS) in 2014. Of these, 23 cases were born in Norway. Fifteen of the cases had been treated with anti-TB drugs previously. Six additional cases had previous TB but had not been treated.

There were ten multi-drug resistant TB isolates (MDR-TB) as defined by resistance to at least rifampicin and isoniazid. Three of the ten MDR-TB cases were previously treated for TB, the remaining seven had TB for the first time. One MDR-TB isolate was also resistant to moxifloxacin and amikacin, and the case was thus categorised as extensively drug-resistant tuberculosis (XDR-TB). XDR-TB isolates are resistant to at least rifampicin and isoniazid plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).

Two hundred and sixty-five cases were confirmed infections with *M. tuberculosis* complex by culture followed by susceptibility testing of the strain isolated. The results are presented in Table 67. The cases were registered in MSIS the year in which the first positive culture was taken.

TABLE 67. Antimicrobial susceptibility of 265 isolates of *Mycobacterium tuberculosis* complex (not *M. bovis* (BCG)) from human infections in 2014. Figures from 2013 are shown in parentheses.

Origin of birth	No. of cases	No. of isolates	Resistance to antimicrobial agents (No. of isolates)					
			Isoniazid	Rifampicin	Ethambutol	Streptomycin	Pyrazinamid	MDR-TB*
Norway	23 (53)	14 (35)	0 (0)	0 (0)	0 (0)	0 (8)	0 (0)	0 (0)
Europe excl. Norway	29 (29)	25 (28)	5 (3)	2 (2)	2 (1)	4 (3)	2 (2)	2 (2)
Asia	98 (135)	78 (106)	8 (15)	2 (2)	2 (2)	7 (13)	8 (8)	2 (2)
Africa	175 (177)	146 (142)	21 (13)	6 (3)	3 (0)	17 (17)	9 (4)	6 (2)
America	2 (4)	2 (4)	0 (0)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)
Unknown	0 (3)	0 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	327 (401)	265 (318)	34 (31)	10 (7)	7 (3)	28 (43)	19 (14)	10 (6)
Proportion of resistant isolates (%)			12.9 (9.7)	3.8 (2.2)	2.6 (0.9)	10.6 (13.5)	7.2 (4.4)	3.8 (2.5)

* MDR-TB: Multi-drug resistant tuberculosis, resistant to at least rifampicin and isoniazid.

Candida spp. in blood cultures

TABLE 68. Antimicrobial susceptibility of *Candida albicans* blood culture isolates (n=146). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	99.3	0.7	0.0
Voriconazole*	≤ 0.12	> 0.12	100.0	-	0.0
Anidulafungin*/**	≤ 0.03	> 0.03	98.6	-	1.4
Micafungin*/**	≤ 0.016	> 0.016	97.9	-	2.1

* Recommended breakpoints by the European Commitee on Antimicrobial Susceptibility Testing – EUCAST.
** There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin as well as micafungin are considered susceptible.

TABLE 69. *Candida albicans* blood culture isolates (n=113). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B					0.7	3.4	41.8	54.1									
Fluconazole						14.4	65.8	19.2			0.7						
Voriconazole	20.5	67.1	12.3														
Anidulafungin	89.0	8.9	0.7		1.4												
Micafungin	1.4	37.7	58.9		0.7	1.4											
Caspofungin**			5.5	8.9	45.2	27.4	11.6		1.4								

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
**There are no European breakpoints for caspofungin. Strains susceptible to anidulafungine and micafungin are considered susceptible.

TABLE 70. Antimicrobial susceptibility of *Candida glabrata* blood culture isolates (n=33). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 0.002	> 32	0.0	75.7	24.3
Anidulafungin*/**	≤ 0.06	> 0.06	100.0	-	0.0
Micafungin*/**	≤ 0.03	> 0.03	100.0	-	0.0

* Recommended breakpoints by the European Commitee on Antimicrobial Susceptibility Testing - EUCAST.
** There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin as well as micafungin are considered susceptible.
There is insufficient evidence that *C. glabrata* is a good target for therapy with voriconazol and no clinical breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.

TABLE 71. *Candida glabrata* blood culture isolates (n=33). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B						6.1	15.2	57.6	21.2								
Fluconazole							3.0		9.1	3.0	6.1	27.3	18.2	9.1	6.1	3.0	15.2
Voriconazole**	3.0		3.0	3.0	6.1	9.1	24.2	24.2	9.1	6.1				12.1			
Anidulafungin	15.2	30.3	54.5														
Micafungin		24.2	69.7	6.1													
Caspofungin***					3.0	27.3	69.7										

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
**There is insufficient evidence that *C. glabrata* is a good target for therapy with voriconazol and no breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.
***There are no European breakpoints for caspofungin. Strains susceptible to anidulafungine are considered susceptible.

TABLE 72. Antimicrobial susceptibility of *Candida tropicalis* blood culture isolates (n=9). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	100.0	0.0	0.0
Voriconazole*	≤ 0.12	> 0.12	100.0	-	0.0
Anidulafungin*/**	≤ 0.06	> 0.06	100.0	-	0.0

* Recommended breakpoints by the European Commitee on Antimicrobial Susceptibility Testing – EUCAST.
** There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin are considered susceptible to caspofungin.
There is insufficient evidence that *C. tropicalis* is a good target for therapy with micafungin and no breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.

TABLE 73. *Candida tropicalis* blood culture isolates (n=9). Distribution (n) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B								7	2								
Fluconazole							3	3	3								
Voriconazole			2	5	2												
Anidulafungin		1	8														
Micafungin**			5	4													
Caspofungin***						6	3										

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
**There is insufficient evidence that *C. tropicalis* is a good target for therapy with micafungin and no breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.
***There are no European breakpoints for caspofungin. Strains susceptible to anidulafungine are considered susceptible to caspofungin.

TABLE 74. Antimicrobial susceptibility of *Candida parapsilosis* blood culture isolates (n=9). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	90.0	-	10.0
Voriconazole*	≤ 0.12	> 0.12	100.0	-	0.0
Anidulafungin*	≤ 0.002	> 4	0.0	88.9	11.1
Micafungin*	≤ 0.002	> 2	0.0	100.0	0.0

* Recommended breakpoints by the European Commitee on antimicrobial susceptibility testing - EUCAST.
** There are no European breakpoints for caspofungin. Strains intermediate to anidulafungin and micafungin can be considered intermediate to caspofungin.

TABLE 75. *Candida parapsilosis* blood culture isolates (n=9). Distribution (n) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B								6	3								
Fluconazole						1	2	3	2			1					
Voriconazole	2	1	2	3			1										
Anidulafungin						1	1	3	1	2	1						
Micafungin							1	6	2								
Caspofungin**							2	4		3							

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
** There are no European breakpoints for caspofungin. Strains intermediate to anidulafungin and micafungin can be considered intermediate to caspofungin.

RESULTS AND COMMENTS

The National Mycology Reference Laboratory received 210 *Candida* isolates of nine different *Candida* species isolated from blood stream infections in 199 patients during 2014 compared to 176 isolates of eight different *Candida* species in 2013. Double infection was found in eight patients and persistent or recurrent infection with the same species more than one month apart, was observed in four patients in 2014.

The predominance of *Candida albicans* was unchanged, (n=146; 69.5%) followed by *Candida glabrata,* (n=33; 15.7%), *Candida tropicalis* (n=9; 4.3%) and *Candida parapsilosis* (n=9; 4.3%). The numbers of the five remaining species were low (n=13; 6.2%).

All isolates were susceptibility tested for amphotericin B, fluconazole, voriconazole, caspofungin, anidulafungin and micafungin by E-test according to the manufacturer's instructions (bioMérieux). The MIC distribution and antifungal susceptibility testing of the 4 most common species are shown in Tables 68-75.

All but one of the isolates were susceptible to amphotericin B. In 2014 84.3% of the isolates were susceptible to fluconazole. Acquired fluconazole resistance was rare and observed in only one *C. parapsilosis* isolate in a patient with persistent infection. One *C. albicans* isolate was categorised as intermediate (MIC 4 mg/l). Otherwise reduced susceptibility was due to intrinsic resistance in *C. krusei* (n=4), and found in *C. blankii* (n=1*), C. pelliculosa* (n=1) and *C. glabrata* (n=33). Breakpoints for fluconazole (S $\leq$ 0.002; R > 32) in *C. glabrata* categorise the wild type as intermediately susceptible. In 2014 24.3% (n=8) of the Norwegian *C. glabrata* isolates were categorised as resistant.

There is still insufficient evidence that *C. glabrata* and *C. krusei* are suitable targets for therapy with voriconazole, and no breakpoints are set. EUCAST recommends reporting the MIC value without categorisation of S, I and R. Except from this species, the *C. brakii* isolate and the *C. parapsilosis* with acquired azole resistance, all isolates were found susceptible to voriconazole.

Acquired echinocandin resistance was observed in three *C. albicans* isolates from three patients with persistent infection. One *C. parapsilosis* isolate was resistant to anidulafungin, the rest were as the wild type categorised as intermediately susceptible to micafungin and anidulafungin, but the number is low.

EUCAST breakpoints have not yet been established for caspofungin due to significant inter-laboratory variation in MIC ranges for caspofungin. Susceptibility testing of *C. parapsilosis* for caspofungin was previously not recommended. In 2014, EUCAST defined that *C. parapsilosis* isolates that are intermediately susceptible to anidulafungin and micafungin can be regarded as intermediate to caspofungin. Similarly, isolates that are susceptible to anidulafungin as well as micafungin should be considered susceptible to caspofungin, until caspofungin breakpoints have been established.

Appendix 1:
Collection of data on usage of antimicrobial agents in animals

Data sources

Feed additives

Until 2003, the Norwegian Agricultural Inspection Service was responsible for the collection of data on sales of antimicrobial growth promoters and coccidiostats as feed additives, while from that time the Norwegian Food Safety Authority has been in charge of collecting such data. Reliable data on the use of different substances and categories of feed additives was obtained from these sources.

Antimicrobial agents for therapeutic use

In Norway, veterinary antimicrobial agents for therapeutic use in domestic animals or farmed fish are prescription drugs only. Moreover, veterinary antimicrobial agents have to be dispensed through pharmacies, which are supplied by drug wholesalers only. An exemption from the pharmacy/wholesalers monopoly has been granted for medicated feeds (i.e. feeds into which drugs for therapeutic use are mixed prior to sales). Medicated feeds have to be prescribed by veterinarians and produced by feed mills authorized by the Norwegian Medicines Agency. In Norway, medicated feeds produced and supplied by feed mills are only used for farmed fish. Medicated feeds for livestock are not produced in feed mills due to the small size of livestock herds compared to most other European countries. However, herd/flock treatment of livestock with antimicrobial agents is possible, again subject to veterinary prescription, through the drinking water or as a top dressing on the feed. The sales figures for veterinary antimicrobial agents from wholesalers and feed mills are thought to roughly equal the use of these drugs. Sales figures for veterinary antimicrobial agents are therefore used as a synonym for usage of veterinary antimicrobial agents. Drug wholesalers and feed mills report their sales figures to the Norwegian Institute of Public Health. This reporting was made mandatory from January 1st 2002. Number of items sold for the various veterinary drug formulations, strengths and package sizes were obtained from the Norwegian Institute of Public Health and calculated to express kg active substance.

Drug classification system

The Anatomical Therapeutic Chemical vet (ATCvet) classification system was used to categorise veterinary medicinal products (http://www.whocc.no/atcvet).

Unit of measurement

The amount of active substance denoted in kilograms was chosen as the unit of measurement. Veterinary antimicrobial drug usage was calculated from sales figures for delivery of antimicrobials from wholesalers to pharmacies, and from feed mills. The data for benzyl penicillin salts and esters (procaine benzylpenicillin and penethamate hydriodide) were converted to the corresponding values for benzyl penicillin.

Inclusion criteria – veterinary drugs

The veterinary drugs included for terrestrial animals were all the approved veterinary antimicrobial products belonging to the following ATCvet groups: QA07AA (gastrointestinal infections) (no product in ATCvet group QA07AB on the market in Norway), QG01AA+AE (uterine infections) (no products in ATCvet groups QG51AC, -AE, -AX, -BA, -BC or -BE on the market in Norway), and QJ (antimicrobial agents for systemic use that includes intramammary dose applicators (QJ51)). Additionally, antimicrobial products sold on special exemption from market authorisation have been included following a case by case assessment. Sales of antimicrobial agents as medicated feeds and as premixes, both intended for use in farmed fish, belonging to QJ are presented separately. An exemption has been made for an antimicrobial premix approved for farmed fish only (trimethoprim-sulfadiazine 1:5) but sold solely for use in terrestrial animals since 1995 (unpublished data). Consequently, the sales of the antimicrobial agents in terrestrial animals reported for the years 1993-2005 were underestimated, although only slightly. However, the updated usage figures for 1995-2005 correlated positively ($r=0.998$) with the data reported previously for these years confirming the formerly reported reduction in the usage of antimicrobial agents in terrestrial animals. Dermatological preparations (QD) and preparations intended for sensory organs (QS) are not included in the material. Human antimicrobial preparations are used in small animal practice. However, data on the use of such antimicrobial preparations in animals are not included in this report as such sales cannot be separated from sales intended for use in humans.

Analysis of the data

In order to assess the overall use of antimicrobial VMPs for terrestrial food producing animals and for pets, sales of products approved for companion animals only (tablets and pasta) have been separated form the total sales.

Appendix 2:
Collection of data on human usage of antimicrobial agents

Data sources
In Norway, antimicrobials are prescription only medicines, and only allowed sold through pharmacies. These data are collected from three databases: the Norwegian drug wholesales statistics database, the hospital pharmacies drug statistics database, and the Norwegian prescription database (NorPD).

The Norwegian Institute of Public Health collects data on drug use from wholesalers. The wholesales database covers total sales of antimicrobials in Norway and is based on sales of medicaments from drug wholesalers to pharmacies and health institutions in Norway. The figures presented should be regarded as maximum figures based on the assumption that all medicaments sold are actually consumed. The actual drug consumption will probably be somewhat lower. Data are available since the beginning of the 1970s.

Data on antibacterial use in hospitals are retrieved from Sykehusapotekenes Legemiddelstatistikk (the hospital pharmacies drug statistics database) which is a cooperation of LIS (Legemiddelinnkjøpssamarbeid – Drug Procurement Cooperation) and the four regional pharmaceutical health trusts operating the hospital pharmacies in Norway. Sykehusapotekenes Legemiddelstatistikk collects sales data from each pharmacy delivering drugs to hospitals. Data are collected as sales from the pharmacy to hospital wards. Data are available since 2006. The Norwegian Advisory Unit for Antibiotic Use in Hospitals (Nasjonal kompetansetjeneste for antibiotikabruk i spesialisthelsetjenesten) has provided the statistics according to activity by number of admissions and bed days.

Population statistics per 1 January are collected from Statistics Norway. Information on bed days and admissions are collected from the Norwegian Patient Register at the Norwegian Directorate of Health. The definition of bed days is: "*the number of whole days an admitted patient disposes a bed*". An admission is defined as: "*admission of patient where the medical interventions usually are complex and requires hospitalisation for one or more days*" (2).

Data on the use in ambulatory care are retrieved from NorPD, a nation-wide prescription database situated at the Norwegian Institute of Public Health. This database includes all prescriptions being prescribed to out-patients in Norway. These data give us the exact population prevalence of antibacterial use in ambulatory care. More information is available at www.fhi.no. Data are available from 2004.

Drug Classification
The data are categorised according to the ATC classification system (1). Defined Daily Doses (DDDs) are employed as units of measurement. The ATC/DDD index of 2015 is used.

Unit of measurement
The ATC/DDD system is recommended by the WHO to serve as a tool for drug utilisation research in order to improve quality of drug use. One component of this is the presentation and comparison of drug consumption statistics at international and other levels.
The use of defined daily dose, DDD, as a unit of measurement, simplifies and improves the evaluation of drug consumption over time, nationally and internationally. The DDD is a theoretical unit of measurement, and does not necessarily reflect the recommended or Prescribed Daily Dose.

The basic **definition** of the unit is:
The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults.

The DDDs for antibacterials are as a main rule based on the use in infections of moderate severity. Some antibacterials are only used in severe infections and their DDDs are assigned accordingly. The DDDs assigned are based on daily treatment.

Inclusion criteria
The antibacterials for human use included in this report belong to ATC group J01 (Antibacterials for systemic use). Oral vancomycin (A07AA09), rifaximin (A07AA11) and oral and rectal metronidazole (P01AB01) are also included. For antifungals, only ATC-group J02 (Antimycotics for systemic use) is included. Of the antimycobacterials (ATC J04), only rifampicin is included. The content of rifampicin in plain products and in combinations is calculated and data are presented as total amount of rifampicin used. Antibacterials used in dermatological preparations (ATC group D) and preparations intended for sensory organs (ATC group S) are not included in the material, except for mupirocin, which is included in one table.

References
1. WHO Collaborating Centre for Drug Statistics Methodology. ATC index with DDDs 2015. WHO Collaborating Centre, Oslo
2. Definitions Norwegian Directorate of Health https://volven.helsedirektoratet.no/begrep.asp?id=452&catID=12.

Appendix 3:
Sampling, microbiological methods and data processing in NORM-VET

Sampling strategy
The indicator bacteria *Escherichia coli* and *Enterococcus* spp. from broiler flocks were collected at slaughter. From each broiler flock 10 caecal samples were collected and a total of 210 pooled samples were included. The samples were also used for selective isolation of extended-spectrum beta-lactamase (ESBL) producing and quinolone resistant *E. coli* (QREC), for vancomycin resistant *Enterococcus* spp. (VRE), as well as for quantification of ESBL producing *E. coli*. In addition, broiler meat samples bought at retail were included. A total of 201 samples were collected. Only one sample from each individual or production unit was included.

Indicator isolates of *E. coli*
Sample material, i.e. caecal content from 10 broilers per flock were mixed and plated directly onto MacConkey agar and incubated at 41±0.5°C for 24h. Typical colonies were subcultured on blood agar (Heart infusion agar, Difco) containing 5% bovine blood and lactose-bromthymol blue agar, and incubated at 37°C for 24h. Colonies were identified as *E. coli* by typical colony appearance, lactose fermentation, a positive indole reaction, negative oxidase and citrate.

ESBL producing *E. coli*
Sample material from broiler flocks used for *E. coli* isolation, was plated directly onto MacConkey agar (Difco) containing 1 mg/L cefotaxime and MacConkey agar containing 2 mg/L ceftazidime. In parallel, 1 g of caecal sample was mixed with 9 mL MacConkey broth containing 1 mg/L cefotaxime and incubated at 41±0.5°C for 24-48h. If negative growth after direct inoculation, 10 µL from the overnight selective broth were plated onto MacConkey agar containing 1 mg/L cefotaxime and MacConkey agar containing 2 mg/L ceftazidime. Meat samples from broiler of approximately 5 g were cultured in MacConkey broth for 24 h at 41±0.5°C before plating onto MacConkey agar with 1 mg/L cefotaxime and MacConkey agar with 2 mg/L ceftazidime. The agar plates were incubated at 41±0.5°C for 24-48h. Presumptive ESBL positive *E. coli* were subcultured on blood agar, confirmed as *E. coli* using MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) and further tested for ESBL production.

Quinolone resistant *E. coli*
Sample material from broiler flocks were plated directly onto MacConkey agar containing 0.12 mg/L ciprofloxacin. From meat samples, 10 µL of the overnight broth were plated onto the corresponding selective plate. Plates were incubated at 41±0.5°C for 24h and presumptive QREC were subcultured on blood agar and confirmed as *E. coli* using MALDI-TOF MS.

***Enterococcus* spp.**
Sample material from broiler flocks was plated directly onto Slanetz & Bartley agar (Oxoid) and incubated at 41±0.5°C for 24-48h. Typical positive colonies from each sample were selected, and the isolates confirmed as *Enterococcus* spp. by phenotypic characterisation and negative catalase test. The isolates were further identified to the species level as *E. faecalis* or *E. faecium* using PCR (Dutka-Malen et al. 1995) or MALDI-TOF MS.

Vancomycin resistant *Enterococcus* spp.
Caecal content was plated directly onto Slanetz & Bartley agar (Oxoid) with 4 mg/L vancomycin and incubated at 41±0.5°C for 48h. Presumptive positive colonies were selected, subcultured on blood agar and confirmed as *Enterococcus* spp. by phenotypic characterisation and negative catalase test. The isolates were identified to the species level as *E. faecalis* or *E. faecium* using PCR or MALDI-TOF MS.

Genotyping
For the presumptive ESBL positive *E. coli*, PCR was performed for the identification of $ESBL_M$ genes or real-time PCR for the bla_{CMY-2} gene (Pérez-Pérez et al, Schmidt et al). Presumptive VRE positive isolates were tested for *vanA* or *vanB* genes using a multiplex PCR (Clark et al. 1993).

Susceptibility testing
Isolates were tested for antimicrobial susceptibility at NVI, Oslo. MIC values were obtained using plates from Sensititre® (TREK Diagnostic LTD) with different panels depending on the bacteria to be tested.
Epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 19.05.2015) were used, except for azithromycin for *E. coli* and quinupristin-dalfopristin for *Enterococcus spp.* for which cut-off values are not defined. See Appendix 6 for definitions of cut offs values.

Quality assurance systems
The following susceptible bacteria were included as quality controls on a regular basis: *E. coli* ATCC 25922, *E. faecalis* ATCC 29212. The following resistant bacteria were tested on a regular basis: *E. coli* CCUG 37382, *E. coli* K8-1 (ESBL), *E. coli* K5-20 (AmpC), *E. faecium* CCUG 33829. The results were approved according to reference values given by EUCAST when available. Additional control strains were included when necessary. The laboratories at NVI are accredited according to the requirements of NS-EN ISO/IEC 17025 and participate in quality assurance programmes for veterinary pathogens (Veterinary Laboratories Agency Quality Assurance Unit. Loughborough, UK), and for resistance monitoring (EURL for Antimicrobial Resistance in Denmark).

Data processing
Susceptibility data were recorded and stored in the sample registration system at NVI as discrete values (MIC). Data management and analysis was performed in SAS-PC System® v 9.1.3 for Windows (SAS Institute Inc., Cary, NC, USA). The 95% confidence intervals were calculated by the exact binomial test using R version 3.02 for Windows (R Development Core Team, 2012).

Overview of antimicrobial groups and agents tested for in NORM-VET:

Antimicrobial group	Antimicrobial agents	*E. coli**	*Salmonella* sp.	*Enterococcus* sp.
Tetracyclines	Tetracycline	X	X	X
	Tigecycline	X	X	X
Amphenicols	Chloramphenicol	X	X	X
Penicillins with extended spectrum	Ampicillin	X	X	X
Second-generation cephalosporins	Cefoxitin	(X)		
Third-generation cephalosporins	Cefotaxime	X	X	
	Ceftazidime	X	X	
Fourth-generation cephalosporins	Cefepime	(X)		
Carbapenems	Meropenem	X	X	
	Ertapenem	(X)		
	Imipenem and enzyme inhibitor	(X)		
Trimethoprim and derivatives	Trimethoprim	X	X	
Sulfonamides	Sulfamethoxazole	X	X	
Macrolides	Erythromycin			X
	Azithromycin	X	X	
Streptogramins	Quinupristin and dalfopristin			X
Streptomycins	Streptomycin			
Other aminoglycosides	Gentamicin	X	X	X
Fluoroquinolones	Ciprofloxacin	X	X	X
Other quinolones	Nalidixic acid	X	X	
Glycopeptide antibacterials	Vancomycin			X
	Teicoplanin			X
Polymyxins	Colistin	X	X	
Other antibacterials	Linezolid			X
	Daptomycin			X

*(X) = only ESBL/AmpC suspected isolates tested as described in Commission implementing decision of 12. Nov 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU), data not shown in the report tables.

Appendix 4:
Sampling, microbiological methods and data processing of zoonotic and non-zoonotic enteropathogenic bacteria in NORM and NORM-VET

NORM-VET enteropathogenic bacteria
Sampling strategy - animals

Salmonella
Samples from animals were collected according to the Norwegian *Salmonella* control programme for live animals. Additional isolates were obtained from clinical submissions or necropsies at the Norwegian Veterinary Institute (NVI). One isolate of each serovar per incident was included for susceptibility testing.

Susceptibility testing animal isolates
Isolates were tested for antimicrobial susceptibility at NVI, Oslo. MIC values were obtained using plates from Sensititre® (TREK Diagnostic LTD) with different panels depending on the bacteria to be tested.
For animal isolates, epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 19.05.2015) were used, except for azithromycin for *Salmonella* spp. for which cut-off values are not defined. For additional antimicrobial agents not defined in the EUCAST recommendations, cut-off values were defined on the basis of the actual MIC distributions obtained in the NORM-VET programme (see also Appendix 6).

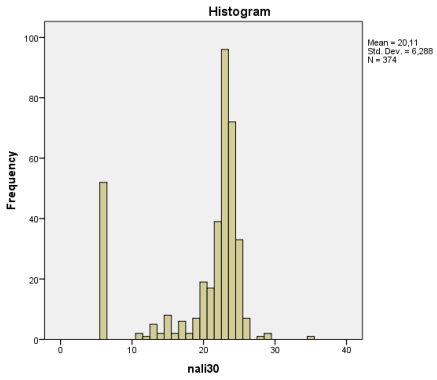
Quality assurance systems NORM-VET
NVI and the Reference Laboratory for Enteropathogenic Bacteria/NIPH have a quality assurance system according to the requirements of NS-EN ISO/IEC 17025. The participating laboratories at NVI are accredited according to the requirements of NS-EN ISO/IEC 17025 and participate in external quality assurance programmes for veterinary pathogens (Veterinary Laboratories Agency Quality Assurance Unit Loughborough, UK) and for resistance monitoring (EURL for Antimicrobial Resistance in Denmark).

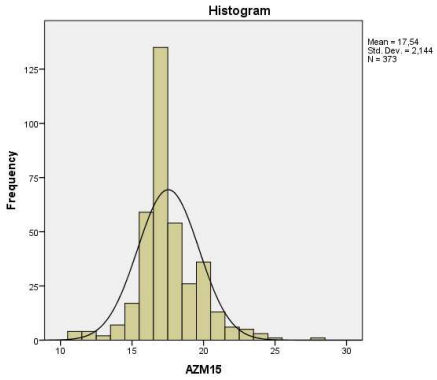
Data processing animal isolates
Susceptibility data were recorded and stored in the sample registration system at NVI. The susceptibility data were stored as discrete values (MIC). Data management and analysis was performed in SAS-PC System® v 9.1.3 for Windows (SAS Institute Inc., Cary, NC, USA). The 95% confidence intervals were calculated by the exact binomial test using R version 3.02 for Windows (R Development Core Team, 2012).

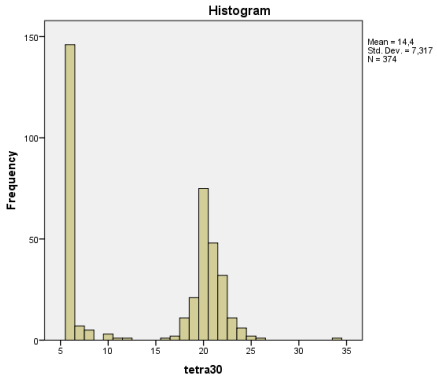
NORM – enteropathogenic bacteria
Sampling strategy - humans
All human isolates of *Salmonella, Yersinia enterocolitica* and *Shigella* were obtained from clinical specimens. One isolate per patient or one isolate per recognised outbreak was included for susceptibility testing. *Campylobacter* isolates from a selection of a little less than 10% of campylobacteriosis cases registered were submitted in the following way: Five regional laboratories submitted the first five independent isolates each month to the NRL for Enteropathogenic Bacteria at the Norwegian Institute of Public Health.

Identification of bacteria – human isolates
The reference analyses on human clinical isolates of enteropathogenic bacteria were performed according to conventional methods described in standard reference literature (e.g. Murray PR & al.: Manual of Clinical Microbiology, 8th edition ASM-Press, Washington 2003 and Ewing WH: Edwards and Ewing's Identification of *Enterobacteriaceae*, 4. edition, Elsevier, New York 1986).

Susceptibility testing human isolates
Salmonella spp., *Yersinia* spp. and *Shigella* spp. isolates from humans were susceptibility tested at the NRL for Enteropathogenic Bacteria at the NIPH by agar disk diffusion tests according to the EUCAST standardised method for AMR testing of non-fastidious bacteria. *Campylobacter* isolates from humans were tested for antimicrobial susceptibility using MIC Test Strips (Liofilchem).
For human isolates EUCAST clinical or epidemiological breakpoints for Enterobacteriaceae, version 5.0 2015 were used if established, otherwise local epidemiological cut-off values were used (nalidixic acid, azithromycin and tetracycline). Distributions of zone diameters are given below.







Isolates with reduced susceptibility to cefotaxime or ceftazidime were tested for the presence of $ESBL_A$ by a double disk approximation test (BD Sensidisc), and for the presence of $ESBL_M$ by an AmpC detection test (Liofilchem MIC-test strips). Isolates with reduced susceptibility to meropenem were forwarded to the Norwegian National Advisory Unit on Detection of Antimicobial Resistance (K-Res) for further analyses.

Quality assurance systems human isolates

The NRL for Enteropathogenic Bacteria at the NIPH is accredited according to the requirements of NS-EN ISO/IEC 17025. *E. coli* ATCC 25922 was used as quality control strain for AMR testing of non-fastidious Enterobacteriaceae. The NRL participated in the external quality assessment programme for *Salmonella* spp. and antimicrobial susceptibility testing organized by ECDC.

Data processing human isolates

The NRL at the NIPH stored susceptibility data of human isolates as either millimeter zone diameters or MIC values. Data analysis was performed in SPSS version 20 (SPSS Inc. Chicago, USA).

Appendix 5:
Sampling, microbiological methods and data processing in NORM

General considerations

NORM is based on a combination of periodic sampling and testing in primary diagnostic laboratories and annual results from national reference laboratories for specific microoganisms. Isolates are included from defined clinical conditions such as respiratory tract infections, wound infections, urinary tract infections, and septicaemiae. For enteric infections see Appendix 4. 2014 was the fifteenth year of surveillance, and all 21 diagnostic laboratories in Norway participated in the surveillance system in addition to eleven reference laboratories. All diagnostic laboratories followed the same sampling strategy and used identical criteria for the inclusion of microbial strains. Only one isolate per patient and infectious episode was included unless otherwise stated. All microbes were identified using conventional methods as described in the ASM Manual of Clinical Microbiology. Species identification of anaerobes was confirmed by MALDI-TOF or 16S-RNA sequencing. The surveillance period started in the beginning of January, and consecutive isolates were included for defined time periods for each surveillance category. The surveillance categories and sampling periods in 2014 were as follows: *E. coli* in blood cultures (6 months); *Klebsiella* spp., *Staphylococcus aureus* and *Enterococcus* spp. in blood cultures (9 months); *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Haemophilus influenzae*, *Neisseria meningitidis* and *Candida* spp. from blood cultures and cerebrospinal fluids (12 months); *S. pneumonia* and *H. Influenza* from respiratory tract specimens (3 weeks); *S. aureus* from wound specimens (1 week); *E. coli* from urinary tract infections (2 days); *Klebsiella* spp. from urinary tract infections (3 weeks); *Mycobacterium tuberculosis* and *Neisseria gonorrhoeae* from all samples (12 months). Anaerobes from blood cultures were collected by seven laboratories (12 months). *S. pneumoniae, S. pyogenes, H. influenzae* and *N. meningitidis* from blood cultures and cerebrospinal fluids were analysed at the the Norwegian Institute of Public Health in Oslo. *Candida* spp. isolates from blood cultures were analysed at Oslo University Hospital, Rikshospitalet. *N. gonorrhoeae* isolates were characterised at Oslo University Hospital, Ullevål. MRSA and *S. agalactiae* isolates were analysed at St. Olav University Hospital in Trondheim. ESBL-producing *Enterobacteriaceae* were genetically characterised at University Hospital of North Norway in Tromsø. *M. tuberculosis* isolates were analysed at the Norwegian Institute of Public Health and Oslo University Hospital (Ullevål and Rikshospitalet).

Susceptibility testing

E. coli, *Klebsiella* spp., *Enterococcus* spp. and *S. aureus* isolates were examined according to the EUCAST disk diffusion standard using antibiotic disks and Mueller Hinton II agar from either Oxoid or Beckton Dickinson. Suitable antibiotics were selected for each bacterial species, and the results were interpreted according to the breakpoints of the Norwegian Working Group on Antibiotics (NWGA). The NWGA breakpoints are harmonised with EUCAST. *S. aureus* isolates were tested for beta-lactamase production by nitrocefin disks, acidometric agar plates (3.6 mg/L penicillin G and phenol red) or clover leaf test. *Enterococcus* strains were screened for glycopeptide resistance using vancomycin 6 mg/L BHI agar. *S. pneumoniae*, *S. pyogenes*, *S. agalactiae*, *H. influenzae*, *N. meningitides, N. gonorrhoeae* and anaerobic isolates were susceptibility tested using MIC gradient tests (bioMerieux or Liofilchem) on MH II agar supplemented with 5% lysed horse blood, GC agar with 1% haemoglobin and Isovitalex (*N. gonorrhoeae*), or Brucella agar (anaerobes). Susceptibility testing of *Candida* spp. isolates was performed by MIC gradient tests using RPMI agar containing 2% glucose and MOPS. Resistance values were recorded mm inhibition zone sizes or MIC values in order to monitor trends in the occurrence of resistance.
M. tuberculosis isolates were tested using BACTEC MGIT 960 systems. All three test laboratories participate in the WHO external DST quality control programme. They also tests for mutations in the *rpoB* gene to detect rifampicin resistance.

Confirmation of resistance phenotypes

E. coli and *Klebsiella* spp. with reduced susceptibility to 3rd generation cephalosporins were examined for ESBL production using ESBL combination MIC gradient tests according to the instructions of the manufacturer (Liofilchem). ESBL positive strains from blood cultures were subjected to PCR and DNA sequencing for determination of ESBL genotype. *S. aureus* isolates with reduced susceptibility to cefoxitin were examined by *mecA* PCR for confirmation of methicillin resistance (MRSA). *Enterococcus faealis* and *E. faecium* isolates displaying growth on the vancomycin screening agar were examined by *van* PCRs. Erythromycin resistant *S. pneumoniae*, *S. pyogenes* and *S. aureus* isolates were analysed for determination of MLS phenotype using the double disk diffusion (DDD) synergy assay with erythromycin and clindamycin disks.

Quality control

The following strains were used for quality control: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 (ESBL positive), *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *S. pneumoniae* ATCC 49619, *S. pneumoniae* TIGR4, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 (heterogeneous MRSA), *S. aureus* CCUG 35600 (homogeneous MRSA), *N. gonorrhoeae* CCUG 41811, *Bacteroides fragilis* ATCC 25289, *C. albicans* ATCC 90028.

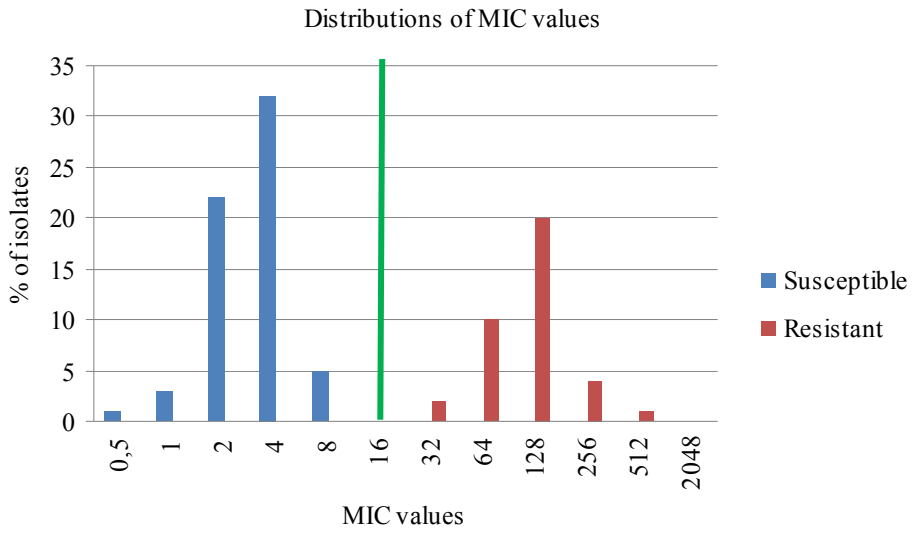
Data processing

The specially designed eNORM computer programme was used for registration and storage of patient data, sample data and resistance data. The results were further analysed by WHONET 5.6 with the aid of the BacLink programme, both developed by Dr. John Stelling. The distribution of microbial species in blood culture was based on extraction of routine data from the laboratory information systems of the participants. All isolates of the same species recovered within one month after the initial finding were considered duplicates and omitted from the survey. No attempts were made to evaluate the clinical significance of each finding.

Appendix 6:
Definitions and classification of resistances used in this report

General guidelines for the reader of this report
The results presented in the NORM and NORM-VET programme are not directly comparable. This is because the sampling between the programmes and also the classification of resistance differ between the programmes. Clinical breakpoints are used for the classification within NORM, while epidemiological cut-off values are used for the classification of resistance within NORM-VET. EUCAST definitions of clinical breakpoints and epidemiological cut-off values are presented at the web page: http://www.srga.org/Eucastwt/eucastdefinitions.htm. The terms and usage of these two ways of classification of resistance are further explained below. The epidemiological breakpoint would normally be lower for MIC values and higher for disk diameters than the clinical breakpoins. However this is not always the case.

Epidemiological cut-off values
The epidemiological cut-off values are mainly used by epidemiologists and could indicate emerging resistance in the bacterial populations. Based on the distribution of the minimum inhibitory concentration (MIC) or the inhibition zone diameter distribution, each bacterial population could (in an ideal case) be divided into two populations by a biphasic curve as shown in the example below. The curve to the left (blue) shows the susceptible or wild type distribution whereas the curve to the right (red) shows the resistant or non-wild type distribution. The green line indicates a possible epidemiological cut-off value applicable to the distributions in the example.



However, for several bacterial populations and corresponding tested antimicrobial substances these distributions may be overlapping. A part of the population within the overlapping area may carry resistance mechanisms and others not. In the area with the non-wild type distribution, new resistance mechanisms are responsible for the resistance either alone or in addition to the resistance mechanisms present at lower MIC values. In order to establish MIC values for each specific bacterial population and antimicrobial agent, large amounts of data are collected and assessed. In the NORM-VET part of this report, we have mainly used the epidemiological cut-off values recommended by EUCAST. However, for some combinations of bacteria and antimicrobial agents these were not applicable to our data. In these cases epidemiological cut-off values defined on the basis of the actual MIC distributions obtained in the NORM-VET programme were used.

Clinical breakpoints
Clinical breakpoints are defined in order to indicate if a treatment of a specific pathogen is likely to succeed or not. Other factors like dosage and formulations also affect the clinical result. The MIC values are ideally obtained for the pathogen *in vitro*, and this is compared with the predetermined clinical breakpoint to determine whether the organism is likely to respond *in vivo*.

Term used to describe antimicrobial resistance levels
In this report the level of resistance (i.e. the percentage of resistant isolates among the tested isolates) in the NORM-VET programme have been classified according to the levels presented in The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013 by EFSA Journal 2015; 13(2):4036 as follows:

Rare:	<0.1%
Very Low:	0.1% to 1%
Low:	>1% to 10%
Moderate:	>10% to 20%
High:	>20% to 50%
Very high:	>50% to 70%
Extremely high:	>70%

Appendix 7:
Cut-off values NORM-VET

Epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 19.05.2015) were used. For additional antimicrobial agents not defined in the EUCAST recommendations, cut-off values were defined on the basis of the actual MIC distributions obtained in the NORM-VET programme.

Antimicrobials	Resistant MIC (mg/L)	*Salmonella* spp.	*Escherichia coli*	*Enterococcus faecium*	*Enterococcus faecalis*
Ampicillin	> 4			■	■
	> 8	■	■		
Azithromycin*	ND	X	X		
Cefotaxime	> 0.25		■		
	> 0.5	■			
Ceftazidime	> 0.5		■		
	> 2	■			
Chloramphenicol	> 16	■	■		
	> 32			■	■
Ciprofloxacin	> 0.06	■	■		
	> 4			■	■
Colistin	> 2	■	■		
Daptomycin	> 4			■	■
Erythromycin	> 1				
	> 4			■	■

Antimicrobials	Resistant MIC (mg/L)	*Salmonella* spp.	*Escherichia coli*	*Enterococcus faecium*	*Enterococcus faecalis*
Fusidic acid	> 0.5				
Gentamicin	> 2	■	■		
	>32			■	■
Linezolid	>4			■	■
Meropenem	> 0.125	■	■		
Nalidixic acid	> 16	■	■		
Oxacillin	> 2				
Quinupristin-dalfopristin*	ND			X	X
Sulfamethoxazole	> 64		■		
	> 256	●			
Teicoplanin	> 2			■	■
Tetracycline	> 4			■	■
	> 8	■	■		
Tigecycline	> 0.25			■	■
	> 1	#	■		
Trimethoprim	> 2	■	■		
Vancomycin	>4			■	■

Squares: Cut-off values recommended by EUCAST
*Cut-off not defined (ND) by EUCAST, ●Cut-off defined by the MIC distributions obtained in NORM-VET.
Cut-off defined by EFSA

Appendix 8: Breakpoints NORM

NORM data are categorised according to the breakpoints of the Norwegian Reference Group on Antibiotic Susceptibility Testing (NWGA) which are harmonised with EUCAST breakpoints. NWGA breakpoints are available at www.antibiotikaresistens.no.

Antimicrobials	MIC (mg/L) S	MIC (mg/L) R	*Escherichia coli*	*Klebsiella* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp	*Haemophilus influenzae*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
Amphotericin B	≤ 1	> 1															■	■	■	■
Ampicillin	≤ 1	> 1							■											
	≤ 4	> 8											■							
	≤ 8	> 8	■		■	■	■													
Amoxi-Clav*	≤ 2	> 2							■											
	≤ 32	> 32	■	■																
Anidulafungin	≤ 0.002	> 4																		■
	≤ 0.03	> 0.03															■			
	≤ 0.06	> 0.06																■	■	
Azithromycin	≤ 0.25	> 0.5									■									
					■[#]	■[#]	■[#]													
Cefaclor									■[#]											
Cefepime	≤ 1	> 4	■	■																
Cefixime	≤ 0.125	> 0.125									■									
Cefoxitin												■[#]								
Cefotaxime	≤ 0.125	> 0.125							■											
	≤ 0.5	> 0.5														■				
	≤ 0.5	> 2												■						
	≤ 1	> 2	■	■	■	■	■													
Ceftazidime	≤ 1	> 4	■	■	■	■	■													
Ceftriaxone	≤ 0.125	> 0.125							■	■	■									
	≤ 0.5	> 2												■						
Cefuroxime	≤ 1	> 2							■											
	≤ 8	> 8	■	■																
Chloramphenicol	≤ 2	> 2							■											
	≤ 2	> 4								■										
	≤ 8	> 8			■	■	■							■						
Ciprofloxacin	≤ 0.03	> 0.03								■										
	≤ 0.03	> 0.06									■									
	≤ 0.5	> 0.5						■	■											
	≤ 0.5	> 1	■	■	■	■	■													
	≤ 1	> 1										■								
Clindamycin	≤ 0.25	> 0.5										■								
	≤ 0.5	> 0.5												■	■	■				
Erythromycin	≤ 0.25	> 0.5												■	■	■				
	≤ 1	> 2										■								
	≤ 4	> 4						■												
Fluconazole	≤ 0.002	> 32																■		
	≤ 2	> 4															■		■	■

Antimicrobials	MIC (mg/L)		*Escherichia coli*	*Klebsiella* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp.	*Haemophilus influenzae*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
	R	R																		
Fusidic acid	≤ 1	> 1										■								
Gentamicin	≤ 1	> 1										■								
	≤ 2	> 2						■#												
	≤ 2	> 4	■	■	■	■	■													
	≤ 128	> 128											■							
Imipenem	≤ 4	> 8											■							
Linezolid	≤ 4	> 4										■	■							
Mecillinam	≤ 8	> 8	■	■																
Meropenem	≤ 2	> 8	■	■	■	■	■													
Micafungin	≤ 0.002	> 2																		■
	≤ 0.016	> 0.016															■			
	≤ 0.03	> 0.03																■		
Mupirocin	≤ 1	> 256										■#								
Nalidixic acid	≤ 16	> 16						■#												
					■#	■#	■#													
Nitrofurantoin	≤ 64	> 64	■																	
Norfloxacin	≤ 4	> 4										■#								
Oxacillin														■#						
Penicillin G	≤ 0.06	> 0.25								■										
	≤ 0.06	> 1									■									
	≤ 0.06	> 2												■						
	≤ 0.25	> 0.25													■	■				
									■#											
Pip-Tazo**	≤ 8	> 16	■	■																
Rifampicin	≤ 0.06	> 0.5										■								
	≤ 0.25	> 0.25								■										
Spectinomycin	≤ 64	> 64									■									
Tetracycline	≤ 0.5	> 1									■									
	≤ 1	> 2							■			■		■	■	■				
	≤ 2	> 2						■												
					■#	■#	■#													
Tigecycline	≤ 0.25	> 0.5											■							
	≤ 0.5	> 0.5										■								
	≤ 1	> 2	■																	
Trimethoprim	≤ 2	> 4	■	■																
TMS***	≤ 0.5	> 1							■											
	≤ 1	> 2												■	■					
	≤ 2	> 4	■	■	■	■	■					■								
Vancomycin	≤ 2	> 2														■				
	≤ 4	> 4											■							
Voriconazole	≤ 0.12	> 0.12															■		■	■

Epidemiological cut-off value based on the wild type distribution by EUCAST. * Amoxi-Clav= Amoxicillin-Clavulanic acid. ** Pip-Tazo=Piperacillin-Tazobactam. *** TMS Trimethoprim-sulfamethoxazole. Breakpoints for the e combination are given for the trimethoprim component only.

Appendix 9:
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