2012

NORM
NORM-VET

Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway

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CONTRIBUTORS AND PARTICIPANTS

Editors:

Gunnar Skov Simonsen	NORM, Univ. Hosp. of North Norway
Anne Margrete Urdahl	NORM-VET, Norwegian Veterinary Institute

Authors:

Cecilie Torp Andersen	Yeasts from humans	ceanders@ous-hf.no	Oslo Univ. Hosp.
Hege Salvesen Blix	Antibiotic usage in humans	hege.salvesen.blix@fhi.no	Norw. Inst. of Pub. Health
Ulf R. Dahle	Enteropathogenic bacteria from humans	ulf.dahle@fhi.no	Norw. Inst. of Pub. Health
Susanne Dudman	Influenza virus from humans	sudu@fhi.no	Norw. Inst. of Public Health
Petter Elstrøm	MRSA from humans	petter.elstrom@fhi.no	Norw. Inst. of Pub. Health
Frode Width-Gran	MRSA from humans	frode.gran@stolav.no	St. Olav Univ. Hosp.
Kari Grave	Antibiotic usage in animals	kari.grave@vetinst.no	Norw. School of Vet. Sc. / Vet. Inst.
Olav Hungnes	Influenza virus from humans	olav.hungnes@fhi.no	Norw. Inst. of Pub. Health
Kjersti Wik Larssen	MRSA from humans	kjersti.wik.larssen@stolav.no	St. Olav Univ. Hosp.
Arve Lund	Bacteria from animals	arve.lund@vetinst.no	Norw. Vet. Inst.
Madelaine Norström	Bacteria from animals	madelaine.norstrom@vetinst.no	NORM-VET, Norw. Vet. Inst.
Andreas Radtke	Group B streptococci from humans	andreas.radtke@stolav.no	St. Olav Univ. Hosp.
Karin Rønning	Tuberculosis	karin.ronning@fhi.no	Norw. Inst. of Pub. Health
Gunnar Skov Simonsen	Bacteria from humans	gunnar.skov.simonsen@unn.no	NORM, Univ. Hosp. of North Norw.
Jannice Schau Slettemeås	Bacteria from animals	jannice.schau-slettemeas@vetinst.no	Norw. Vet. Inst.
Martin Steinbakk	Bacteria from humans	martin.steinbakk@fhi.no	Norw. Inst. of Pub. Health
Marianne Sunde	Bacteria from animals	marianne.sunde@vetinst.no	Norw. Vet. Inst.
Anne Margrete Urdahl	Bacteria from animals	anne-margrete.urdahl@vetinst.no	NORM-VET, Norw. Vet. Inst.
Didrik Vestrheim	Pneumococci from humans	didrik.vestrheim@fhi.no	Norw. Inst. of Pub. Health
Astrid Louise Wester	Enteropathogenic baceria from humans	astrid.louise.wester@fhi.no	Norw. Inst. of Pub. Health

Institutions participating in NORM-VET:

Norwegian Food Safety Authority	Kjell Hauge
Norwegian Veterinary Institute, Dep. of Health Surveillance	Anne Margrete Urdahl / Arve Lund / Merete Hofshagen / Madelaine Norström / Kari Grave
Norwegian Veterinary Institute, Dep. of Diagnostics	Marianne Sunde / Jannice Schau Slettemeås

Institutions participating in NORM:

Akershus University Hospital, Lørenskog, Department of Microbiology	Trond Egil Ranheim / Marit Vattøy
Bærum Hospital, Department of Medical Microbiology	Annette Onken / Merriam Sundberg
Drammen Hospital, Department of Medical Microbiology	Carina Merethe Thilesen / Ellen-Margrete Grimstad
Førde Hospital, Department of Microbiology	Reidar Hjetland / Astrid Vedde
Haugesund Hospital, Department of Microbiology	Liv Jorunn Sønsteby / Pirrko-Liisa Kellokumpu
Haukeland Univ. Hospital, Bergen, Dep. of Microbiology	Haima Mylvaganam / Torunn Sneide Haukeland
Innlandet Hospital, Lillehammer, Department of Microbiology	Carola Grub / Kari Ødegaard
Levanger Hospital, Department of Microbiology	Angela Kümmel / Anne-Kristine Lorås
Molde Hospital, Department of Microbiology	Einar Vik / Per Gerhard Skotgård
Norwegian Insititute of Public Health, Ref. Lab. for Enteropathogenic Bacteria	Astrid Louise Wester / Trine-Lise Stavnes
Norwegian Insititute of Public Health, Ref. Lab. for Pneumococci	Didrik Vestrheim / Anne Ramstad Alme
Norwegian Insititute of Public Health, Ref. Lab. for Tuberculosis	Turid Mannsåker / Kari Nilsen
Norwegian Insititute of Public Health, Ref. Lab. for Influenza	Susanne Dudman / Olav Hungnes
Nordland Hospital, Bodø, Department of Microbiology	Liisa Mortensen / Hege Elisabeth Larsen
Oslo University Hospital, Radiumhospitalet, Laboratory of Microbiology	Peter Gaustad / Sunniva Fagerås Røst
Oslo University Hospital, Rikshospitalet, Institute of Medical Microbiology	Peter Gaustad / Pia Langseth
Oslo University Hospital, Ullevål, Department of Microbiology	Gaute Syversen / Thea Bergheim
Oslo University Hospital, Rikshospitalet, Ref. Lab. for Mycology	Cecilie Torp Andersen / Lonny Margrethe Kløvfjell
Stavanger University Hospital, Department of Microbiology	Paul Naaber / Anita Løvås Brekken
St. Olav University Hospital, Trondheim, Department of Microbiology	Jan Egil Afset / Toril Nordtømme
St. Olav University Hospital, Trondheim, Ref. Lab. for GBS	Andreas Radtke / Randi Valsø Lyng
St. Olav University Hospital, Trondheim, Ref. Lab. for MRSA	Lillian Marstein / Hege Snøsen
Sørlandet Hospital, Kristiansand, Department of Microbiology	Ståle Tofteland / Torill Sofie Larsen
Unilabs Telelab A/S, Skien	Andreas Emmert / Anne Ragnhild Oseid
University Hospital of North Norway, Tromsø, Department of Microbiology	Gunnar Skov Simonsen / Ellen Haldis Josefsen
University Hospital of North Norway, Ref. Lab. for Detection of AMR	Ørjan Samuelsen / Bjørg C. Haldorsen
Vestfold Hospital, Tønsberg, Department of Microbiology	Dagfinn Skaare / Anja Hannisdal
Østfold Hospital, Fredrikstad, Department of Microbiology	Anita Kanestrøm / Anne Cathrine Hollekim
Ålesund Hospital, Department of Microbiology	Reidar Hide / Kristin Ulla

NORM reference group in 2012:

Martin Steinbakk	Norwegian Institute of Public Health, Oslo
Anita Kanestrøm	Østfold Hospital Trust, Fredrikstad
Thea Bergheim	Norwegian Society of Engineers and Technologists
Dag Berild	Norwegian Society for Infectious Diseases
Knut Eirik Eliassen	Norwegian College of General Practitioners
Dag Harald Skutlaberg	Haukeland University Hospital, Bergen
Ståle Tofteland	Norwegian Society for Medical Microbiology

The NORM and NORM-VET programmes are part of the Norwegian Government's Action Plan against Antimicrobial Resistance (2000 – 2004) and the National Strategy for Prevention of Infections in the Health Service and Antibiotic Resistance (2008 – 2012).

CONTENTS

I. 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 sources. 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 monitoring antimicrobial drug usage and resistance in both human and veterinary medicine and published several reports and recommendations in this regard.

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, a conference organized in September 2004 by the Norwegian Institute of Public Health and supported by the Norwegian government, issued a report, which forms the basis for containment of antimicrobial resistance in the years to come. The need for continued surveillance of both resistance and drug usage was emphasised. An integrated national strategy for prevention of infections in the health service and antibiotic resistance (2008 – 2012) was issued in the summer of 2008.

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 thirteenth annual joint report from NORM and NORM-VET, presents data for 2012. 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 2013

II. SAMMENDRAG

Dette er den trettende 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 over forekomst av antibiotikaresistens og forbruk av antibiotika til mennesker og dyr i 2012. Data fra relevante prosjekter som ikke er med i de kontinuerlige overvåkingsprogrammene, presenteres også.

Både NORM og NORM-VET programmene er 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 2012 var 6161 kg. Fra 1995 til 2012 er salget av veterinære antibiotika til landdyr redusert med 36 %. For preparater som nesten utelukkende benyttes til produksjonsdyr (landdyr) er reduksjonen i denne perioden på 38 %, mens salget av veterinære antibakterielle preparater som kun brukes til kjæledyr, har økt med 17 % (fra 467 til 545 kg).

Forbruksmønsteret til produksjonsdyr har utviklet seg i gunstig retning siden 1995 idet andelen av rene penicillinpreparater har økt betraktelig parallelt med at bruk av kombinasjonspreparater med penicillin og dihydro-streptomycin har gått ned. Siden det første penicillin-preparatet til smådyr kom på markedet i Norge i 1994 har bruk av veterinære penicillinpreparater, i kg, til smådyr økt fra 1 til 66 % 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 2012 på 1591 kg aktiv substans, hvorav 88 % var kinoloner. 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 fordoblet siden forbudet mot bruk av antibakterielle vekstfremmere, 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 2012 var humant forbruk av antibiotika til systemisk bruk 21,0 DDD/1000 innbyggere/dag. Det samlede forbruket har vært forholdsvis stabilt gjennom mange år, men det har skjedd en gradvis økning og en forskyvning mellom de ulike antibakterielle undergrupper.

Salget av penicilliner med utvidet spektrum øker jevnt, mens salg av smalspektret penicillin ble redusert i 2012. Det urinveisantiseptiske middelet metenamin har de seneste årene økt kraftig og i 2012 utgjorde metenamin 17 % av totalt salg målt i DDD. I 2012 utgjorde penicillinene 41 % av det totale antibiotikaforbruket i Norge målt i DDD. Bredspektrede og penicillinase-stabile penicilliner har økt hvert år siden 2005. Tetracykliner utgjorde 18 %, mens makrolider og linkosamider utgjorde 11 % av totalt salg i 2012. Salget av cefalosporiner, monobaktamer og karbapenemer utgjør kun 3 % av totalsalget. Over år har forbruket av fluorokinoloner økt; det utgjør kun 4 % av totalforbruket i 2012, men bruken er mer enn doblet på 10 år. Rundt 85 % av all DDD selges på resept i allmennpraksis. Bruken av antibakterielle midler avhenger av kjønn, alder og bosted. I 2012 utgjorde salget til sykehus 7 % av totalt antibiotikasalg. I sykehus utgjorde penicilliner 45 % av antibiotikasalget målt i DDD, fulgt av cefalosporiner med 18 %. I allmennpraksis utgjorde penicilliner 40 % av salget, mens 20 % var tetracykliner.

Resistens hos kliniske isolater fra dyr

Kliniske isolater av *Staphylococcus schleiferi* (n=53) fra hund ble undersøkt. Forekomsten av antibiotikaresistens var moderat og 67,9 % av isolatene var følsomme for alle undersøkte antibiotika. Til sammen var 24,5 % av isolatene resistente mot ett antibiotikum (hovedsakelig fusidinsyre), mens vel 3,8 % var samtidig resistente mot to, 1,9 % mot tre og 1,9 % mot fire antibiotika. Ett isolat var meticillinresistent.

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 2012 ble det resistenstestet *E. coli* isolert fra avføringsprøver fra reinsdyr (107 isolater) og fra fjørfe foreldredyr (113 isolater), samt fra kyllingfilet (196 isolater). Det ble påvist en lav forekomst av resistente bakterier fra reinsdyr, med 89,7 % av isolatene var følsomme for alle de testede antibiotika. Ett isolat fra reinsdyr var resistent mot cefalosporiner som følge av en kromosomal mutasjon som gir oppregulert AmpC-produksjon. Blant isolatene fra fjørfe foreldredyr og fra kyllingfilet var hhv. 74,3 % og 82,6 % av isolatene følsomme for alle antibiotika det ble testet for.

Fra fjørfe foreldredyr ble resistens mot ett antibiotikum påvist hos 16,8 % av isolatene, hyppigst mot ampicillin. Fra kyllingfilet var 9,7 % av isolatene resistente mot ett antibiotikum og da hovedsaklig sulfametoxazole. Ved bruk av selektiv metode hadde imidlertid 7,3 % av prøvene fra fjørfe foreldredyr og 32,2 % av prøvene fra kyllingfilet ESBL-positive *E. coli* isolater, og samtlige av disse hadde bla_{CMY-2} genet. Forekomsten i kyllingfilet er ikke overraskende og korresponderer med funnene i

broilere i NORM-VET 2011. Sannsynligvis avspeiler resultatene situasjonen internasjonalt da Norge er avhengig av import av avlsdyr. Forekomst av bakterier som er resistente mot cefalosporiner i produksjonsdyr og i matkjeden, er bekymringsfullt da dette muligens kan ha betydning for resistensutvikling i bakteriepopulasjonen hos mennesker.

Resistens hos zoonotiske bakterier og andre enteropatogene bakterier

I 2012 ble det resistenstestet 25 isolater av *Salmonella* spp. fra norske dyr. To isolater viste resistens mot fluorokinoloner. Multiresistent *S. enterica* subsp. *enterica* serovar 4,[5],12:i ble isolert fra fire dyr. Tre av disse var resistente mot tetracykliner, ampicillin, streptomycin og sulfametoxazole. Det siste isolatet var resistent mot streptomycin, trimetoprim, sulfametoxazole, nalidixinsyre og ciprofloxacin. I tillegg ble det undersøkt 35 isolater av *Salmonella* spp. isolert fra reptiler i perioden 2010-2012. Ti av isolatene var resistente mot fluoroquinoloner, åtte mot streptomycin, og ett mot kloramfenikol og florfenikol. Kun ett isolat var resistent mot tre midler og kan således defineres som multiresistent; mot fluorokinoloner, kloramfenikol og florfenikol.

Samleprøver fra 175 svinebestninger ble undersøkt for meticillinreistente *Staphylococcus aureus* (MRSA). Kun én positiv prøve ble identifisert og isolatet var dyreassosiert MRSA (CC398, s*pa*-type t034), dvs. at 0,6 % av svinebesetningene var positive for MRSA CC398, s*pa*-type t034. Dette er første gang denne blir påvist i en svinebesetning i Norge. Imidlertid ble MRSA CC398, s*pa*-type t034 påvist i prøver fra svin tatt på ett slakteri i 2011. Det ble da forsøkt å spore tilbake til besetninger, men uten å lykkes. Senere viste det seg at MRSA CC398, s*pa*-type t034 var til stede i miljøet på slakteriet og slik kan ha forurenset prøvene som inngikk i NORM-VET 2011. *Staphylococcus aureus* (117 isolater) fra kliniske tilfeller av mastitt hos storfe ble også testet for meticillin-resistens. Ingen MRSA ble påvist.

For kliniske *Salmonella*-isolater fra mennesker er det gjennomgående at forekomsten av resistens i *S.* Typhimurium-gruppen (som inkluderer *S. enterica* serovar 4,[5],12:i:-) er høyere for flere antibiotika enn for andre *Salmonella* serovarer, samt at resistensforekomsten er økende for tetracyklin og ampicillin. Dette gjelder både for innenlandssmittede og pasienter som er smittet i utlandet.

De fleste tilfeller av *Shigella*-infeksjoner i Norge kan knyttes til smittekilder i utlandet. Antibiotikaresistens var utbredt hos *Shigella* isolater, spesielt hos *Shigella flexneri,* i likhet med det som rapporteres fra andre land. Forekomsten synes imidlertid rimelig stabil, kanskje bortsett fra resistens mot fluorokinoloner, som synes å ha økt hos *S. sonnei.*

Forekomsten av ESBL-produserende tarmpatogene bakterier fra mennesker er fortsatt lav. I 2012 ble det påvist 11 *Salmonella* med $ESBL_A$ samt 7 med $ESBL_M$. Tre *Shigella*-isolater hadde ESBL (alle $ESBL_A$).

Resistens hos kliniske isolater fra mennesker

Forekomsten av antibiotikaresistente kliniske bakterieisolater fra mennesker var, som i de foregående år, meget lav i 2012. Det ble påvist 11 tilfeller av meticillin-resistente *Staphylococcus aureus* (MRSA) blant de 1141 blodkulturisolater (1,0 %) som ble inkludert i NORM-protokollen. Dette samsvarer med at 16 av 1556 (1,0 %) *S. aureus* blodkulturisolater i laboratorienes datasystemer ble rapportert som MRSA. I 2012 var dermed 16 av 1576 (1,0 %) *S. aureus* fra blodkultur og spinalvæske MRSA. Andelen er på samme nivå som i 2010 (1,0 %) og 2011 (0,5 %). Meldesystemet for infeksjonssykdommer (MSIS) registrerte 575 tilfeller av MRSA-infeksjon i 2012 mot 563 i 2011. De fleste tilfellene var pasienter med sår-infeksjoner og abscesser. MRSA utgjør fortsatt en svært liten andel av *S. aureus* isolater fra sårprøver (7 av 956, 0,7 %) hvilket er på samme nivå som 0,6 % in 2010 og 1,3 % i 2011. MSIS registrerte videre 635 tilfeller av MRSA-kolonisering i 2012 mot 481 tilfeller i 2010 og 496 i 2011. Det totale antallet MRSA-meldinger økte dermed fra 1059 i 2011 til 1210 i 2012 (+14,2 %). Resultatene fra 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. Nedgangen i forekomst av fusidinresistens blant *S. aureus* isolater fra sårprøver holder seg stabil og utgjør 9,5 % sammenliknet med 9,9 % i 2011.

Blodkulturisolater av *E. coli* og *Klebsiella* spp. var som tidligere stort sett følsomme for bredspektrede antibiotika. Forekomsten av resistens og nedsatt følsomhet for gentamicin hos *E. coli* var 5,9 % sammenliknet med 5,2 % i 2010 og 4,7 % i 2011. Forekomsten av resistens og nedsatt følsomhet for ciprofloxacin i *E. coli* fortsatte å øke til 11,7 % sammenliknet med 9,1 % i 2011. Det er en klar samvariasjon mellom forbruket av fluorokinoloner og nedsatt følsomhet for denne antibiotikagruppen. *Klebsiella* spp. hadde lavere forekomst av resistens mot fluorokinoloner 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 90 av 1646 *E. coli* (5,5 %) og 16 av 681 *Klebsiella* spp. (2,3 %) isolater fra blodkultur ble rapportert som ESBL-positive. For *E. coli* er dette en betydelig økning fra 3,3 % i 2011. Forekomsten av ESBL blant *Klebsiella* spp. er også økende. De fleste isolatene kunne verifiseres som ESBL positive ved molekylære analyser, og det er derfor grunn til å følge utviklingen med spesiell oppmerksomhet. Andelen av ESBL positive isolater var fortsatt høyere blant *E. coli* fra blodkulturer (5,5 %) enn fra urinprøver (2,2 %). Et enkelt *K. pneumoniae* blodkulturisolat hadde nedsatt følsomhet for meropenem og inneholdt en KPC gensekvens forenlig med karbapenemaseproduksjon. Fra juli 2012 er karbapenemasproduserende *Enterobacteriaceae*, *Pseudomonas aeruginosa* og *Acinetobacter* spp. underlagt meldeplikt til MSIS.

Det ble bare påvist et enkelt isolat av *Enterococcus faecium* med klinisk signifikant VanA vankomycin-resistens i 2012. Forekomsten av nedsatt følsomhet for ampicillin i *E. faecium* ligger fortsatt på rundt 80 %, og høygradig gentamicinresistens ble påvist i 25,6 % av *E. faecalis* og 47,2 % av *E. faecium*. Nesten alle *E. faecium* isolater med høygradig gentamicinresistens hadde samtidig nedsatt følsomhet for ampicillin. Det ble for første gang påvist linezolidresistente enterokokker i NORM med tre tilfeller på to ulike sykehus. Påvisningen av linezolidresistens gir grunn til stor bekymring da linezolid ofte brukes som en siste behandlingsmulighet mot multiresistente stammer.

Streptococcus pneumoniae fra blodkultur og spinalvæske var generelt følsomme for alle relevante antibiotika, men 6,3 % (39/615) av isolatene hadde nedsatt følsomhet for penicillin G. Dette er en økning fra 3,0 % i 2010 og 4,3 % i 2011. Seks av isolatene hadde samtidig nedsatt følsomhet for cefalosporiner. Ingen isolater var høygradig resistente mot betalaktamer. Forekomsten av makrolidresistens blant systemiske pneumokokkisolater økte svakt fra 4,0 % i 2011 til 6,0 % i 2012, men er fortsatt lavere enn toppåret 2006 (12,4 %). Resultatene fra luftveisisolater av *S. pneumoniae* var stort sett samsvarende med de systemiske isolatene.

Analyse av *Streptococcus agalactiae* (beta-hemolytiske streptokokker gruppe B) fra sterile områder viste økende forekomst av erytromycinresistens fra 7,7 % i 2009 til 12,0 % i 2012. Alle isolatene var følsomme for betalaktamantibiotika.

I alt 378 tilfeller av tuberkulose ble meldt til MSIS i 2012. Det ble utført resistensbestemmelse av 280 isolater av *Mycobacterium tuberculosis*. Syv isolater fra pasienter smittet i henholdsvis Afrika (n=3), Asia (n=1) og Europa utenfor Norge (n=3) ble klassifisert som multiresistente.

Det ble utført resistensbestemmelse av 186 *Candida* blodkulturisolater av ti ulike species. De vanligste artene var *C. albicans* (n=127), *C. glabrata* (n=23), *C. tropicals* (n=10) og *C. parapsilosis* (n=7). Alle *C. albicans* og *C. tropicalis* var følsomme for amphotericin B, fluconazol, voriconazol og anidulafungin. Som forventet ble det påvist høy forekomst av resistens mot fluconazol og voriconazol blant *C. glabrata*. Amfotericin B var aktivt mot alle gjærsopp bortsett fra et enkelt isolat av *C. kruzei*. Resultatene er i samsvar med tidligere studier fra Norge.

Overvåking av resistens mot antivirale midler omfattet i 2011 både influensavirus og HIV, men resultatene for HIV ble publisert nylig i RAVN årsrapport 2011-12. Influensasesongen 2012/2013 var en utpreget blandingssesong, der det var omfattende utbrudd med influensavirus A(H1N1)pdm09, B (genotype Yamagata) og A(H3N2). Alle isolater av influensa A(H3N2) og A(H1N1)pdm var resistente mot M2-blokkere men følsomme for neuraminidasehemmerne oseltamivir og zanamivir. Alle isolater av influensa B var følsomme for neuraminidasehemmere.

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.

III. SUMMARY

This is the 13th 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 2012. The NORM and NORM-VET programmes are 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 2012, the total sales of antimicrobial VMPs for terrestrial animals were 6,161 kg. The annual sales, in kg active substance, of antimicrobial VMPs for use in terrestrial animals decreased by approximately 36% from 1995 to 2012. The reduction in use is accounted for by a reduction in the use in food producing animals (38% reduction) while for antimicrobial VMPs marketed for companion animals an increase of 17% in the sales is observed. The sales patterns of antimicrobial VMPs for terrestrial 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 48% in 2012. In this period the sales of aminoglycosides decreased from 27% to 11% 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 is mainly explained by a campaign for 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 2012, the total sales of antimicrobial agents for therapeutic use in farmed fish were 1,591 kg of active substance of which quinolones accounted for 88%. The sales of antimicrobial VMPs in Norwegian aquaculture declined by approximately 99% from 1987 to 1996 and has thereafter remained relatively constant. This reduction is mainly attributed to the introduction of effective vaccines in salmonids. In 2012, the total sales of ionophore coccidiostat feed additives, in kilograms of active substance, was more than twice the amounts used prior to the withdrawal of the 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 2012, the overall sales of antibacterials for systemic use in humans were 21.0 DDD/1,000 inhabitants/day. 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 penicillins with extended spectrum are steadily increasing, while sales of narrow spectrum penicillin have dropped. The use of the urinary antiseptic agent methenamine still increases and, in 2012, it accounted for 17% of total sales, measured in DDDs. In 2012, 41% of the total antibiotic use in human, measured in DDDs, was penicillins. Since 2005, an annual increase in the use of penicillins with extended spectrum and beta-lactamase resistant penicillins has been observed. Tetracyclines accounted for 18% of total consumption in 2012 while the consumption of macrolides and lincosamides accounted for 11%. Sales of cephalosporins, monobactams and carbapenems constitute 3% of total sales. Over years, there has been a marked increase in quinolone use. This group accounted for only 4% of total consumption in 2012, but sales have more than doubled in 10 years. Around 85% of all DDDs are sold through prescriptions in ambulatory care. The use of antibacterials varies according to gender, age and residence. In 2012, sales to hospitals accounted for 7% of total antibiotic sales. Penicillins accounted for around 45% of the sales to hospital and 40% in ambulatory care. The other main group in hospitals was cephalosporins (18%), and in ambulatory care tetracyclines (20%).

Resistance in animal clinical isolates

Clinical isolates of *Staphylococcus schleiferi* (of both ssp. *coagulans*/*schleiferi*) (n=53) from dogs were included in the survey. The prevalence of antimicrobial resistance was moderate. In total, 67.9% of the isolates were susceptible to all antimicrobial agents included. Altogether, 24.5% of the isolates were resistant to one antimicrobial agent (predominantly fusidic acid), 3.8% to two, 1.9% to three and 1.9% to four antimicrobial agents. One isolate was confirmed to be methicillin resistant.

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 2012, *E. coli* isolated from faecal samples from reindeer (107 isolates), poultry breeders (113 isolates), and broiler meat samples (196 isolates) were included. The prevalence of resistant *E. coli* was low in reindeer and moderate in poultry breeders and in broiler meat. Of the reindeer isolates, 89.7% were susceptible to all antimicrobial agents included, while of the poultry breeder and broiler meat isolates, 74.3% and 82.6% of the isolates were susceptible to all antimicrobials included in the test panel, respectively.

In reindeer, 4.7% of the isolates were resistant to one antimicrobial agent and this was predominantly streptomycin or ceftazidime. One reindeer isolate expressed high MIC values to cephalosprines, and upregulated AmpC production due to chromosomal

mutation in the promotor area was found to be the mechanism behind this cephalosporin resistance.
In poultry breeders, resistance to one of the antimicrobial agents was identified in 16.8% of the isolates and most prevalent was resistance to ampicillin. In contrast, 9.7% of the broiler meat isolates were resistant to one antimicrobial agent, predominantly sulfamethoxazole. However, by using a selective method for detection of ESBL producing *E. coli, E. coli* resistant to third generation cephalosporins were found in 7.3% of the poultry breeder flocks and in 32.2% of the broiler meat samples. The bla_{CMY-2} gene was identified in all the isolates. The relatively high prevalence found in broiler meat is not surprising and corresponds to the prevalence found in broilers in NORM-VET 2011. The results probably mirror the international situation as Norway is dependent on import of poultry breeders. However, the presence of bacteria resistant to cephalosporins in food production animals and in the food chain is of 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.

Resistance in zoonotic and non-zoonotic enteropathogenic bacteria

In 2012, a total of 25 isolates of *Salmonella* spp. from animals were susceptibility tested. Two isolates showed resistance to fluoroquinolones. The emerging multiresistant *S. enterica* subsp. *enterica* serovar 4,[5],12:i- was isolated from four different animals. Three of these isolates were resistant to tetracyclines, ampicillin, streptomycin and sulfamethoxazole, whereas the last isolate was resistant to streptomycin, trimethoprim, sulfamethoxazole, nalidixic acid and ciprofloxacin.
In addition, *Salmonella* spp. from reptiles collected during the years 2010-2012 (35 isolates) were included. Ten isolates were resistant to fluoroquinolones, eight to streptomycin and one to chloramphenicol and florfenicol. Only one of the isolates was resistant to three agents and may be described as multiresistant; to fluoroquinolones, chloramphenicol and florfenicol.
In 2012, screening for methicillin resistant *Staphylococcus aureus* (MRSA) was performed on samples from 175 swine holdings. One positive sample was identified, giving a prevalence of 0.6% MRSA in Norwegian swine holdings. The isolate belonged to clonal complex 398 (CC398), *spa*-type t034. This is the first detection of MRSA CC398 in a Norwegian swine holding. However, MRSA CC398 (*spa*-type t034) was detected from samples from one slaughterhouse in 2011. An attempt to identify positive swine holdings was performed unsuccessfully, but follow-up sampling at the slaughterhouse showed that MRSA CC398, *spa*-type t034 was present in the environment and could have contaminated the slaughtered pigs that were included in the screening. *Staphylococcus aureus* (117 isolates) from bovine mastitis were also susceptibility tested for methicillin. No methicillin resistant *S. aureus* (MRSA) was detected.

Antimicrobial resistance in human clinical isolates of the *S.* Typhimurium-group (including *S. enterica* serovar 4,[5],12:i-) seems to be on a higher level than for other serovars, and the resistance is increasing. This applies to domestically acquired strains as well as to strains acquired abroad.
The absolute numbers of tested *Shigella* species and *Yersinia enterocolitica* are low. The results are thus sensitive to individual cases. However, the levels of resistance in *Shigella* were higher in 2012 than the previous years, with a few exceptions. The resistance rates in *Yersinia enterocolitica* remain low, except for resistance to ampicillin which is due to intrinsic resistance.
The number of ESBL-carrying non-fastidious enteropathogenic bacteria remains low. In 2012, 11 *Salmonella* isolates carried $ESBL_A$ and 7 $ESBL_M$. Three *Shigella*-isolates carried $ESBL_A$.

Resistance in human clinical isolates

The prevalence of resistance in human clinical isolates was still very low in Norway in 2012. Only 11 methicillin resistant *Staphylococcus aureus* (MRSA) blood culture isolates were detected among the 1,141 strains included in the NORM protocol (1.0%), and 16 out of 1,556 (1.0%) *S. aureus* isolates were reported as MRSA from the laboratories´ information systems. The total number of systemic *S. aureus* isolates from blood cultures and cerebrospinal fluids was 1,576 including 16 MRSA strains (1.0%). This prevalence is at the same level as in 2010 (1.0%) and 2011 (0.5%). The Norwegian Surveillance System for Communicable Diseases (MSIS) registered 575 cases of MRSA infections in 2012 compared to 432 in 2010 and 563 in 2011. The majority of MRSA cases were reported to be wound infections and/or abscesses. Conversely, the prevalence of MRSA among non-invasive *S. aureus* isolates is still very low at 0.7% (7/956) which is at the same level as 0.6% in 2010 and 1.3% in 2011. Furthermore, MSIS registered 635 cases of MRSA colonisation compared to 481 in 2010 and 496 in 2011. The total number of MRSA notifications thus increased from 1,059 in 2011 to 1,210 in 2012 (+14.2%). 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 seems to have stabilised around 9-10%.
E. coli and *Klebsiella* spp. blood culture isolates were generally susceptible to broad-spectrum antimicrobials. The prevalence of gentamicin non-susceptibility in *E. coli* was 5.9% compared to 5.2% in 2010 and 4.7% in 2011. The increase in the prevalence of *E. coli* non-susceptibility to fluoroquinolones continued and reached 11.7% in 2012 compared to 9.1% in 2011. There is a clear correlation between the total usage of fluoroquinolones and non-susceptibility to these agents. The prevalence of resistance to 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 90/1,646 (5.5%) *E. coli* and 16/681 (2.3%) *Klebsiella* spp. blood culture isolates were reported with this phenotype in 2012. For *E. coli*, this is a significant increase from 3.3% in 2011. The prevalence of ESBL production in *Klebsiella* spp. is also generally increasing. As most of these isolates were verified by molecular methods, the trend should be closely monitored. The proportion of ESBL positive isolates is still higher among *E. coli* from blood cultures (5.5%) than among urinary tract isolates (2.2%). A single *K. pneumoniae* blood culture isolate (0.1%) displayed reduced

susceptibility to meropenem and contained a KPC determinant compatible with carbapenemase production. From July 2012 it is mandatory to report all cases of carbapenemase producing *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. to MSIS.

Only a single *Enterococcus faecium* isolate with clinically significant VanA vancomycin resistance was detected in 2012. The prevalence of non-susceptibility to ampicillin in *E. faecium* has stabilised around 80%, and high-level gentamicin resistance (HLGR) was detected in 25.6% of *E. faecalis* and 47.2% of *E. faecium*. Almost all HLGR *E. faecium* (65/68) isolates were also non-susceptible to ampicillin. Enterococcal resistance to linezolid was detected for the first time in NORM in 2012 with three positive isolates at two different hospitals. The emergence of linezolid resistant enterococci is cause for great concern as linezolid is often used as an antimicrobial of last resort against multiresistant strains.

Streptococcus pneumoniae from blood cultures and cerebrospinal fluids were generally susceptible to relevant antimicrobials, but 6.3% (39/615) of the isolates displayed reduced susceptibility to penicillin G which is an increase from 3.0% in 2010 and 4.3% in 2011. Furthermore, six isolates were non-susceptible to cephalosporins. No isolates displayed high-level resistance to beta-lactam antibiotics. The prevalence of macrolide resistance among pneumococcal blood culture isolates increased slightly from 4.0% in 2011 to 6.0% in 2012, but it is still well below the previous peak of 12.4% in 2006. The results from *S. pneumoniae* respiratory tract isolates generally corresponded to the findings among systemic isolates.

Analysis of *Streptococcus agalactiae* (beta-haemolytic streptococci group B) isolates from sterile sites showed increasing prevalence of erythromycin resistance from 7.7% in 2009 to 12.0% in 2012. All isolates were susceptible to beta-lactams.

A total of 378 cases of tuberculosis were reported to MSIS in 2012. Susceptibility testing was performed on 280 *Mycobacterium tuberculosis* isolates. Seven isolates (2.5%) originating from Africa (n=3), Asia (n=1) and Europe outside Norway (n=3) were classified as multidrug-resistant (MDR).

Susceptibility testing was performed on 186 *Candida* blood culture isolates of ten different species. The most common species were *C. albicans* (n=127), *C. glabrata* (n=23), *C. tropicals* (n=10) and *C. parapsilosis* (n=7). All *C. albicans* and *C. tropicalis* isolates were susceptible to amphotericin B, fluconazole, voriconazole and anidulafungin. As expected, high prevalences of resistance to fluconazole and voriconazole were detected in *C. glabrata*. Amphotericin B was active against all yeasts except for a single *C. kruzei* isolate. The results are in accordance with previous studies from Norway.

Surveillance of resistance to antiviral agents in 2011 included both influenza virus and HIV, but the HIV data were recently published in the 2011-2012 RAVN yearly report. The 2012/2013 influenza season was a typical mixed season, with widespread outbreaks of influenza A(H1N1)pdm09, B (genotype Yamagata) and A(H3N2). All isolates of influenza A(H3N2) and A(H1N1)pdm were resistant to M2 blockers but fully susceptible to the neuraminidase inhibitors oseltamivir and zanamivir. All influenza B isolates were susceptible to neuraminidase inhibitors.

Conclusion

Antimicrobial resistance is still a limited problem in Norway. The relatively low usage of antimicrobial agents as well as the appropriate patterns of use must be maintained to preserve this 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.

IV. 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, 2013.
Data provided by Statistics Norway.

Age group	All	Males	Females
0 to 4 years	313,215	160,859	152,356
5 to 14 years	641,169	341,045	300,124
15 to 24 years	665,116	341,527	323,589
25 to 44 years	1,389,021	712,954	676,067
45 to 64 years	1,279,140	653,196	625,944
65 years and older	790,614	353,327	437,287
All age groups	5,051,275	2,535,908	2,515,367

TABLE 2. Livestock population in Norway in 2012.
Data provided by the Register of Production Subsidies as of 31 July, 2012.

	Number* of	
Animal category	Herds	Animals
Cattle	15,500	861,000
Dairy cows only**	9,100	209,000
Suckling cow only**	4,200	64,700
Combined production (cow)**	870	33,100
Goat	1,300	65,400
Dairy goat**	350	35,000
Sheep	14,400	2,215,000
Breeding sheep > 1 year**	14,300	867,000
Swine	2,200	839,000
Breeding animal > 6 months**	1,200	56,000
Fattening pigs for slaughter**	2,000	464,000
Poultry		
Egg laying hen (> 20 weeks of age)	1,800	3,906,000
Flocks > 250 birds**	540	3,884,000
Broiler	588#	-
Turkey, ducks and geese for slaughter	150	497,000
Flocks > 25 birds**	57	496,000

* Numbers > 100 rounded to the nearest ten, numbers > 1000 rounded to the nearest hundred.
** Included in above total.
#Number tested in the surveillance for *Campylobacter*.

TABLE 3. Production volume of the most important species in Norwegian aquaculture during the time period 1992-2012. *Data provided by the Norwegian Directorate of Fisheries updated by 06.06.2013.*

Year	Atlantic salmon (ton)	Rainbow trout (ton)	Cod (ton)	Arctic char (ton*)	Halibut (ton*)	Blue mussels (ton)	Scallops[1] (ton)	Oysters (ton)
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	10,016	343	1,741	1,967	21	6.3

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

Import of live animals

Import of live animals (excluding fish and companion animals) to Norway in 2012 was limited to 24 swine, 17 sheep and 21,596 day old chicken.

V. USAGE OF ANTIMICROBIAL AGENTS

USAGE IN ANIMALS

Kari Grave

Therapeutic usage of veterinary antimicrobial agents

Total sales (6,161kg active substance) in Norway of antimicrobial veterinary medicinal products (VMPs) for therapeutic use split into sales for use in food producing animals and companion animals in the period 1995-2012 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 includes 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).

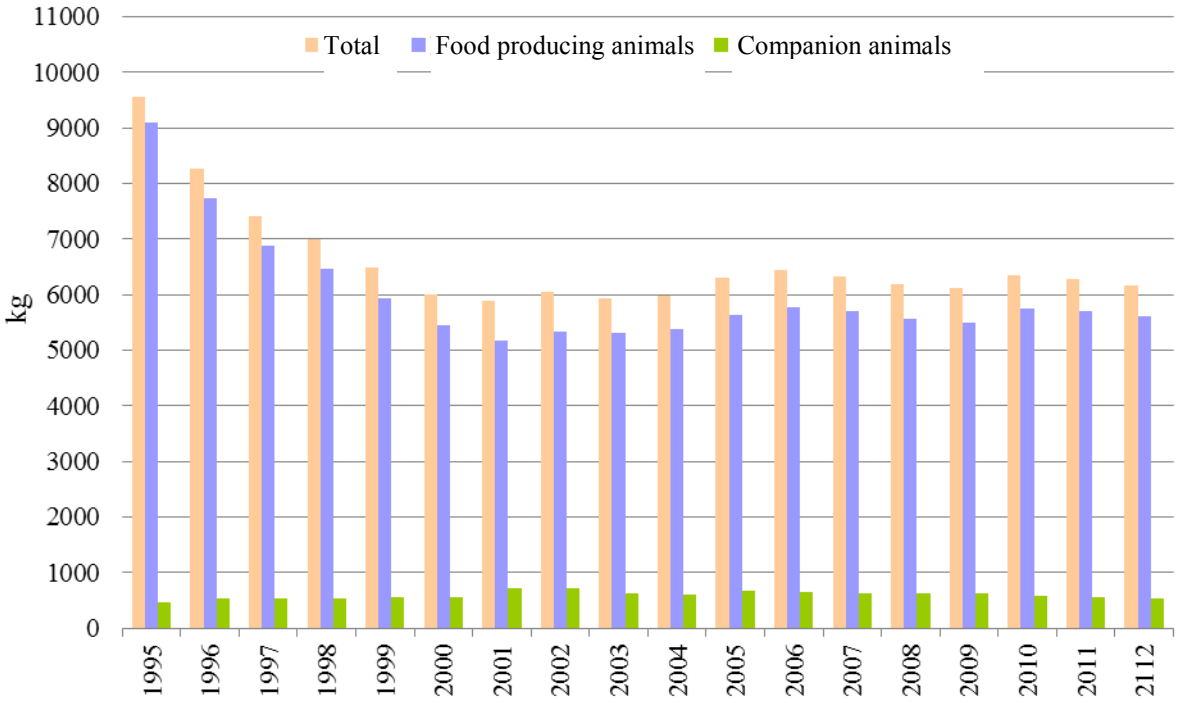

FIGURE 1. Total sales, in kilograms active substance, and estimated sales for food producing animals (terrestrial animals) and companion animals of antimicrobial veterinary medicinal products (VMP) for therapeutic use in Norway for the years 1995-2012 (farmed fish not included).

In the period 1995-2012 the total sales of antimicrobial VMPs for use in terrestrial animals decreased by 36%. Of antimicrobial VMPs used almost solely for food production animals the reduction was 38%, while for products used in companion animal only an increase of 17% was observed (Figure 1).

An increase in the sales of penicillin VMPs is observed for the period 1995-2012 from 25% to 48% of total sales and this is accounted for by products used in food producing and in companion animals (Figures 2-3). In this period the sales of aminoglycosides decreased from 27% to 11% 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) as critically important in human medicine are negligible, i.e. 3rd and 4th generation cephalosporins, fluoroquinolones and macrolides (Figures 2-3). There are no cephalosporin VMPs marketed in Norway for food producing animals and only one such product for companion animals (3rd generation cephalosporin).

The reduced sales of antimicrobial VMPs in food producing animals as well as the favourable prescribing patterns are mainly explained by a campaign for 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.

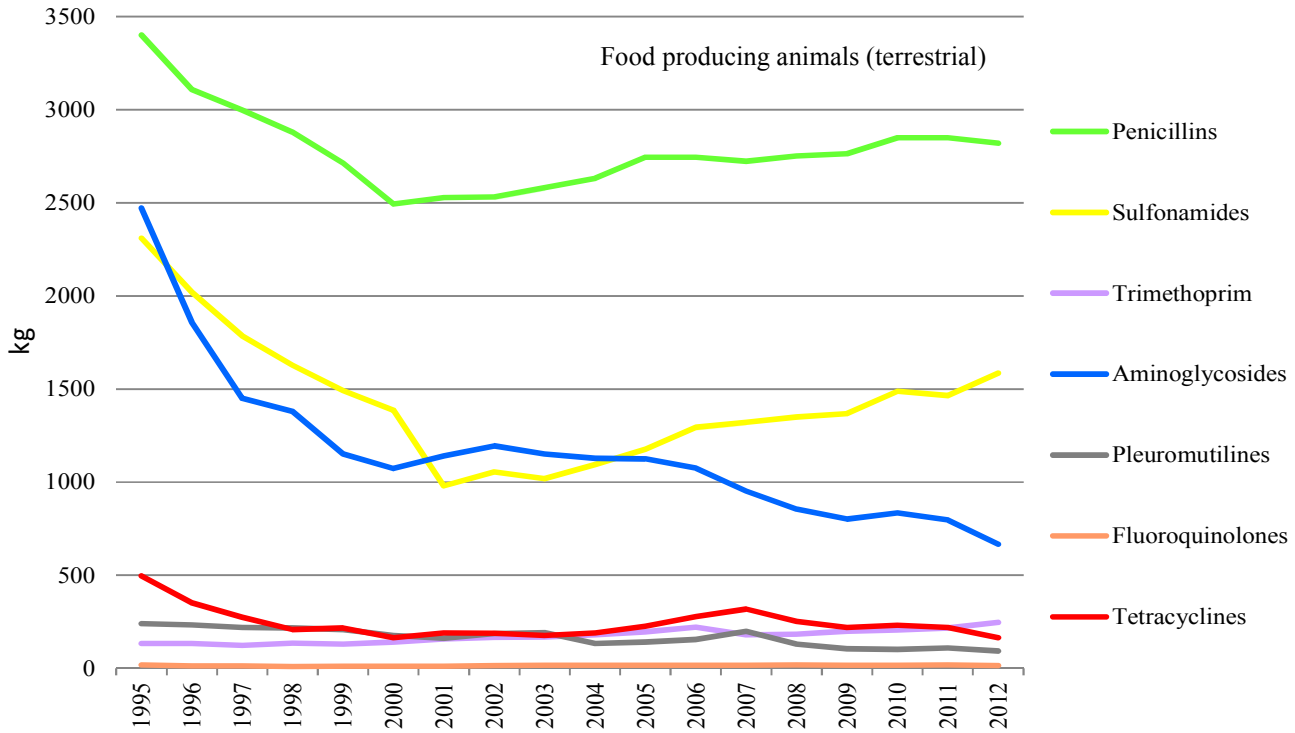

FIGURE 2. Sales in Norway (kilograms active substance) of antimicrobial veterinary medicinal products (VMP) mainly for therapeutic used in food producing animals for the years 1995-2012 (farmed fish not included). In addition, minor amounts of amphenicols (range 19-27 kg) were sold in 2008-2012 and of macrolides (range 0.2-18 kg) during 1995-2012.

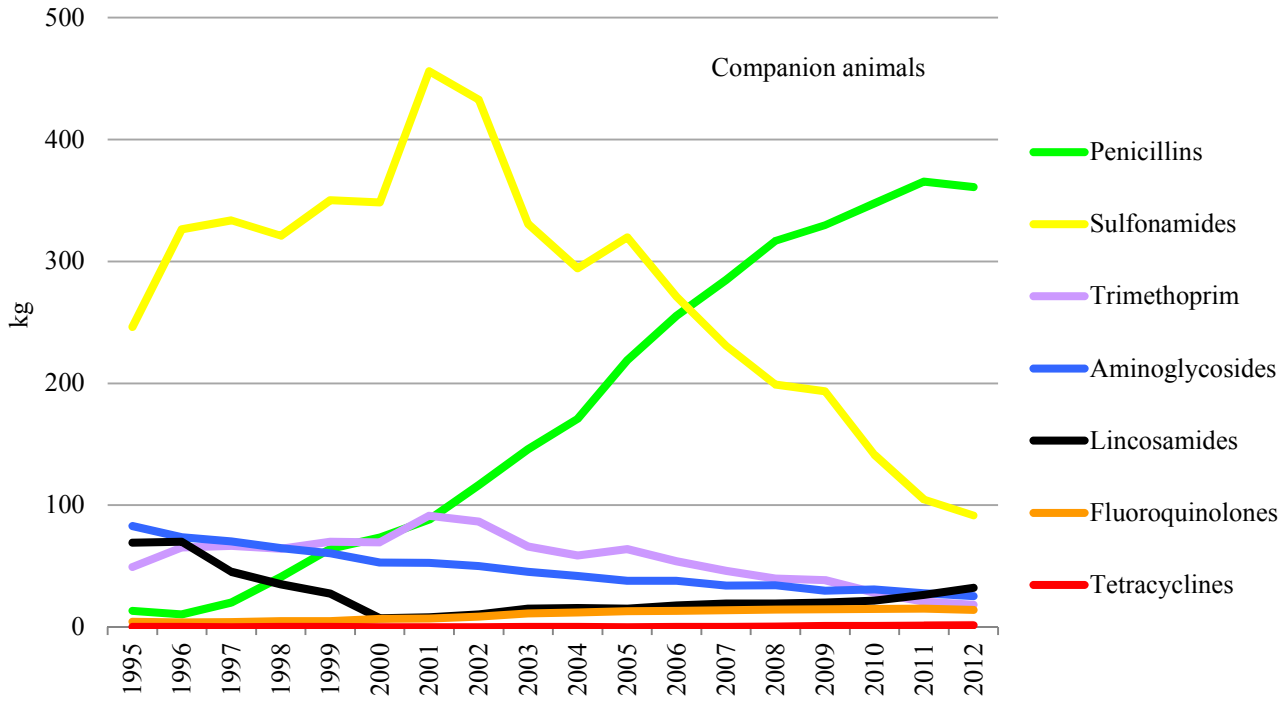

FIGURE 3. Sales in Norway, in kilograms active substance, of antimicrobial veterinary medicinal products (VMP) marketed for therapeutic use in companion animals for the years 1995-2012. In addition, minor amounts of a 3rd generation cephalosporin (0.9-1.1 kg) were sold annually during 2008-2012 and of macrolides (0.4-5kg) from 1998-2005.

An increase of 17% in the sales, in kg active substance, from 465 to 544 kg of antimicrobial VMPs marketed for companion animals from 1995-2012 is observed (Figure 3). This increase is mainly accounted for by penicillins, and in 2012 approximately 88% of the penicillins sold for companion animals were as a combination of amoxicillin and the beta-lactamase inhibitor clavulanic acid (Figure 4).

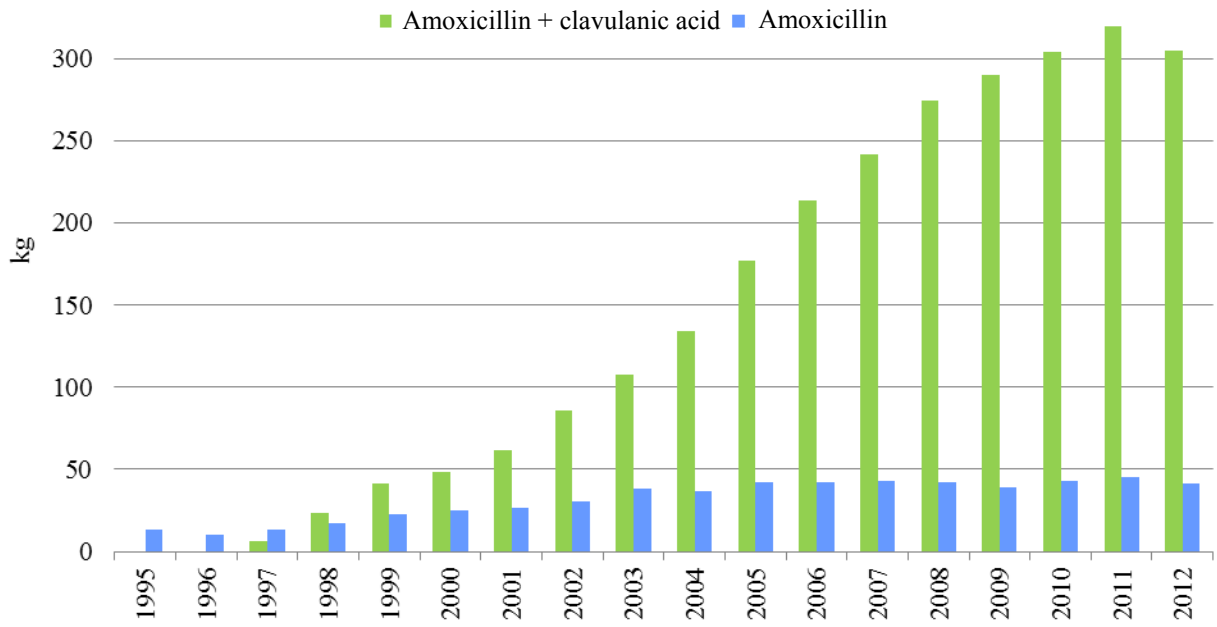

FIGURE 4. Sales, in kg active substance, of penicillin veterinary medicinal products for companion animals 1995-2012.

The annual sales of antimicrobial VMPs for use in farmed fish peaked in 1987 when the sales amounted to 48 tonnes (Figure 5). In 2012, the sales of antimicrobial VMPs for use in farmed fish were 1,591 kg active substance, of which 88% were quinolones (Table 4); this implies that the sales have declined by approximately 99% from 1987.

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.

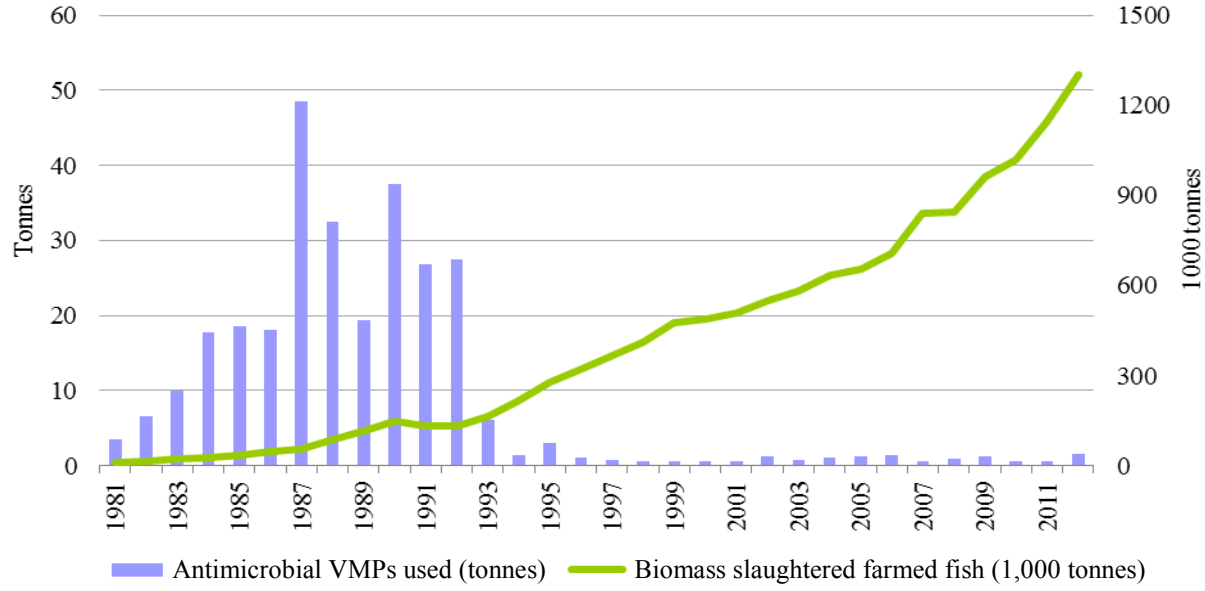

FIGURE 5. Total sales, in kilograms of active substance, of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in farmed fish in Norway in the period 1981-2012 versus produced biomass (slaughtered) farmed fish.

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 2000-2012.

Group of substances/active substance		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Tetracyclines:	Oxytetracycline	15	12	11	45	9	8	0	19	23	40	10	1	1
Amphenicols:	Florfenicol	148	109	205	154	111	202	302	139	166	303	275	336	191
Quinolones:	Flumequine	52	7	5	60	4	28	7	18	1	1	0	0	0
	Oxolinic acid	470	517	998	546	1,035	977	1,119	406	681	926	308	212	1,399
Combinations:	Spectinomycin + lincomycin (2+1)	0	0	0	0	0	0	50	66	70	43	57	0	0
Total		**685**	**645**	**1,219**	**805**	**1,159**	**1,215**	**1,478**	**648**	**941**	**1,313**	**649**	**549**	**1,591**

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 Norwegian Agricultural Inspection Service (2000-2002) and the Norwegian Food Safety Authority (2003-2012).
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, including avoparcin. These measures resulted in an immediate reduction in the usage of these substances (Figur 6). No antimicrobial growth promoters have been used in food producing animals in Norway since 1997.

TABLE 5. Total sales, in kilograms of active substance, of coccidiostats as feed additives in Norway 2000-2012. Data were obtained through annual reports from the Norwegian Agricultural Inspection Service (2000-2002) and the Norwegian Food Safety Authority (2003-2012).

Active substance	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Lasalocid	80	96	514	108	173	37	13	17	16	63	0	0	0
Monensin	776	629	521	717	817	852	889	919	897	885	805	1,060	1,204
Salinomycin	233	12	0	0	0	0	0	0	0	0	0	0	0
Narasin	4,486	4,195	4,470	5,067	5,270	5,318	5,615	7,065	9,212	8,621	9,080	9,394	10,686
Total ionophore coccidiostats	**5,575**	**4,932**	**5,505**	**5,892**	**6,260**	**6,207**	**6,517**	**8,001**	**10,125**	**9,569**	**9,885**	**10,454**	**11,890**
Amprolium/ etopabat	135	159	74	42	0.8	0	0	0	0	0	0	0	0
Total others	**135**	**159**	**74**	**42**	**0.8**	**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 highly correlated to the number of slaughtered chicken produced in this period.

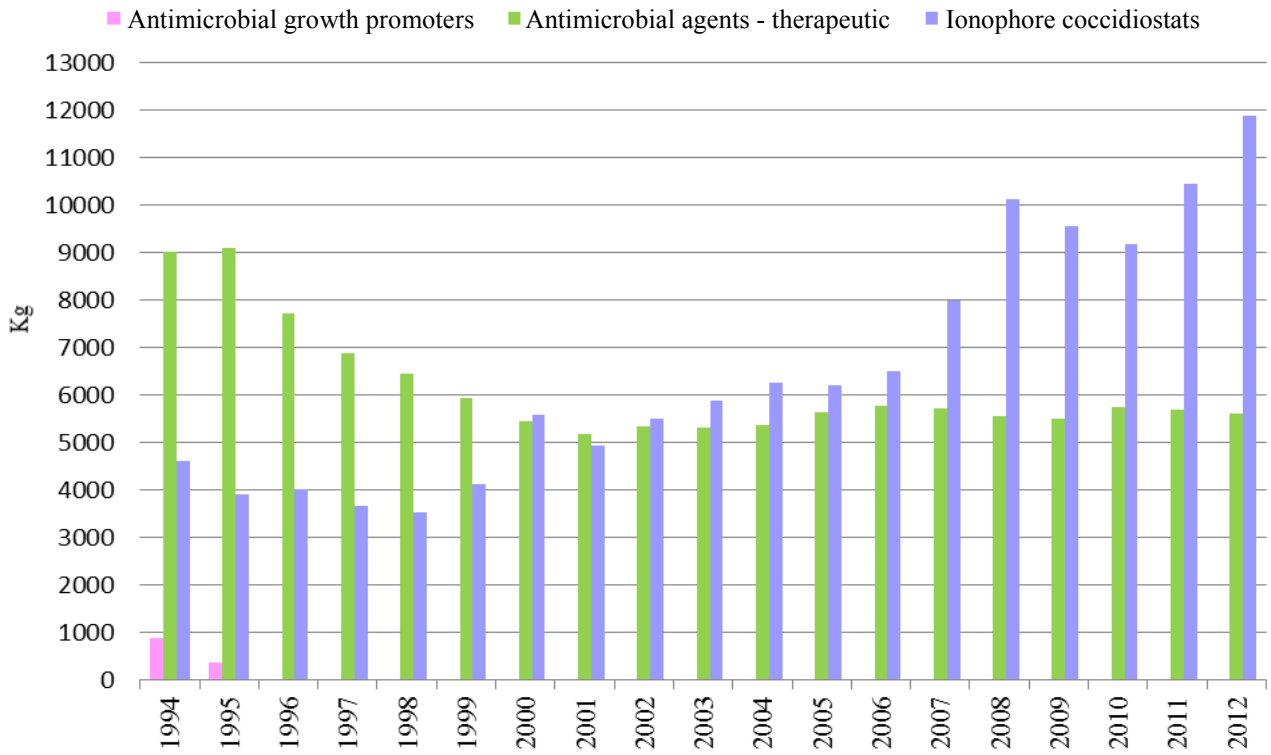

FIGURE 6. Sales, in kg active substance, of antimicrobial veterinary medicinal products for food producing animals (terrestrial) and of antimicrobial growth promoters and ionophore coccidiostats in Norway during 1994-2012.

Sales of veterinary antimicrobial agents in 19 EU/EEA countries in 2010

For 2010, sales of veterinary antimicrobial agents at package level were collected in harmonised manner from 19 EU/EEEA countries for the first time by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) at the European Medicines Agency. The sales data were normalised for the animal population by use of a population correction unit (PCU) introduced by ESVAC as a proxy for the size of the animal population. The sales data are expressed as mg active ingredient/PCU (1 PCU=1 kg).

The differences in the sales between the countries are in part likely to be due to differences in the composition of the animal population (e.g. more pigs than cattle; a high proportion of veal calves within the cattle population) in the various countries. There may also be considerable variation in terms of dosage used for the various antimicrobial agents, length of treatment period or formulation of the various antimicrobial agents used; this may also in part explain some of the differences between the countries. The prescribing patterns of the various antimicrobial classes, expressed as mg/PCU, varied substantially between the countries (Figure 7).

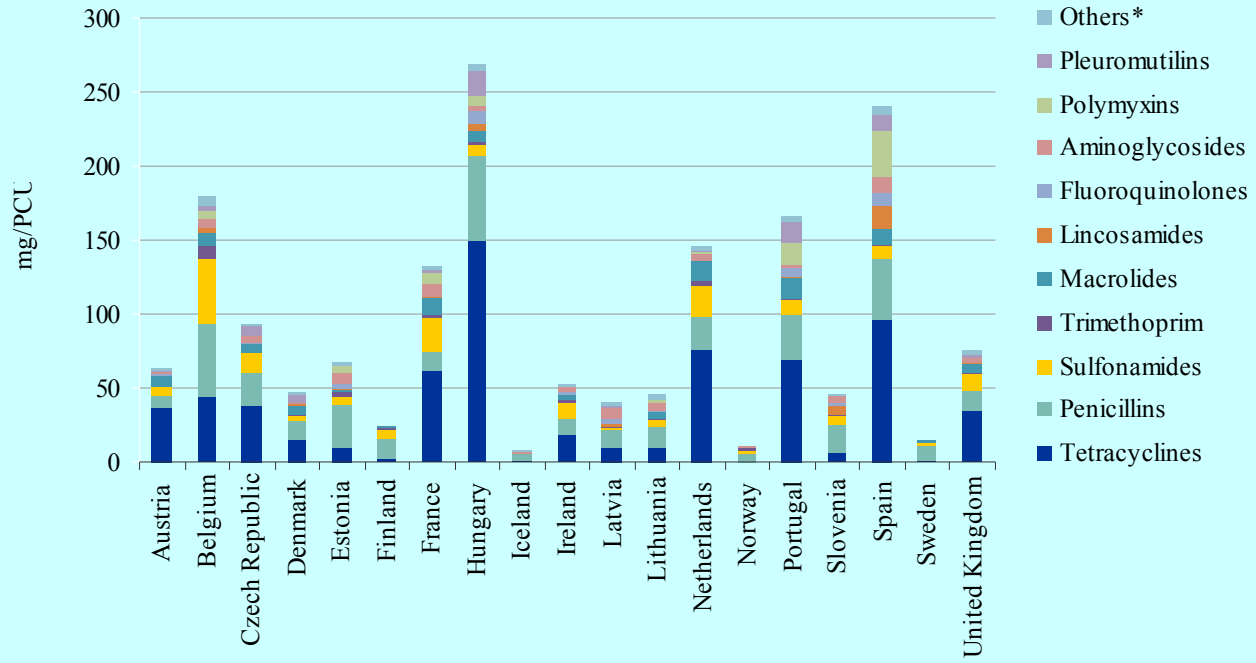

FIGURE 7. Sales for food-producing species, including horses, in mg/PCU, of the various veterinary antimicrobial classes, by country, for 2010. Differences between countries can partly be explained by differences in animal demographics, in the selection of antimicrobial agents and in dosage regimes, among other factors. *Amphenicols, cephalosporins, other quinolones and other antibacterials (classified as such in the ATCvet system).

Of the total sales in the 19 countries, the major proportion, expressed as mg/PCU, was accounted for by tetracyclines (39%), penicillins (23%) and sulfonamides (11%) (Figure 8). The sales of the critically important antimicrobial agents (CIA) with highest priority in human medicine as defined by the World Health Organization — namely 3rd and 4th generation cephalosporins, fluoroquinolones and macrolides — accounted for 0.2%, 2.2% and 5.7%, respectively, of the total sales (mg/PCU) of veterinary antimicrobial agents.

The amount accounted for by the pharmaceutical forms applied for mass treatment (premixes) and group treatment (oral powders and oral solutions) varied considerably between the countries. An important finding is that mass and group treatment is the most frequently used method for administration of veterinary antimicrobial agents for use in food-producing animals, except in Iceland, Finland, Norway and Sweden. Overall in the 19 countries, approximately 49% of the sales of veterinary antimicrobial agents, in tonnes of active ingredient, were for products applicable for mass treatment and 42% were for group treatment (Figure 9).

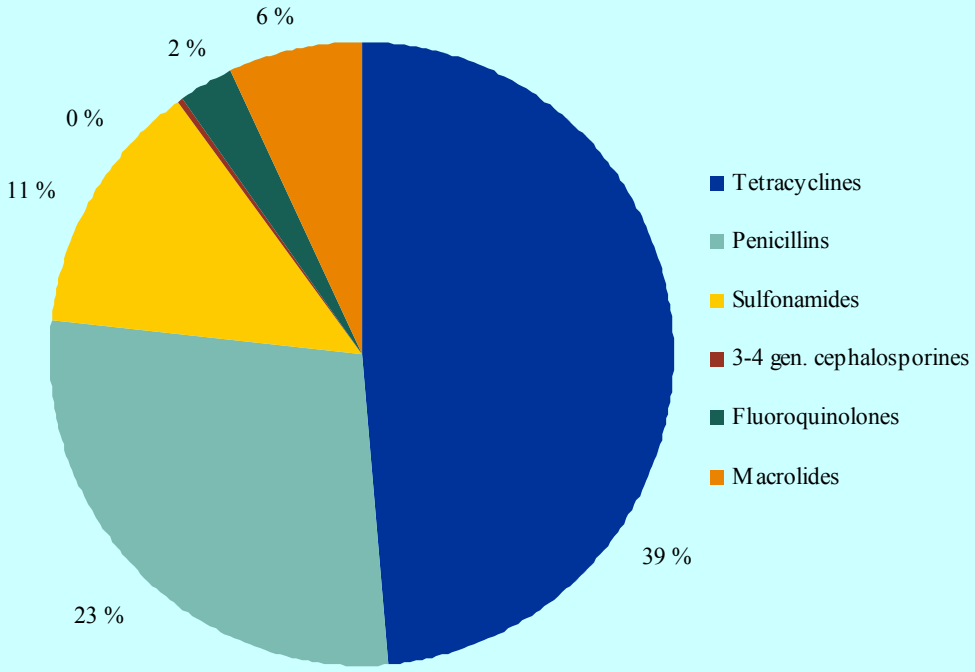

FIGURE 8. Proportion of total sales for food-producing species, including horses, in mg/PCU, in 19 EU/EEA countries in 2010[1]of the most selling veterinary antimicrobial agents and the critical important antimicrobial agents with highest priority in human medicine.

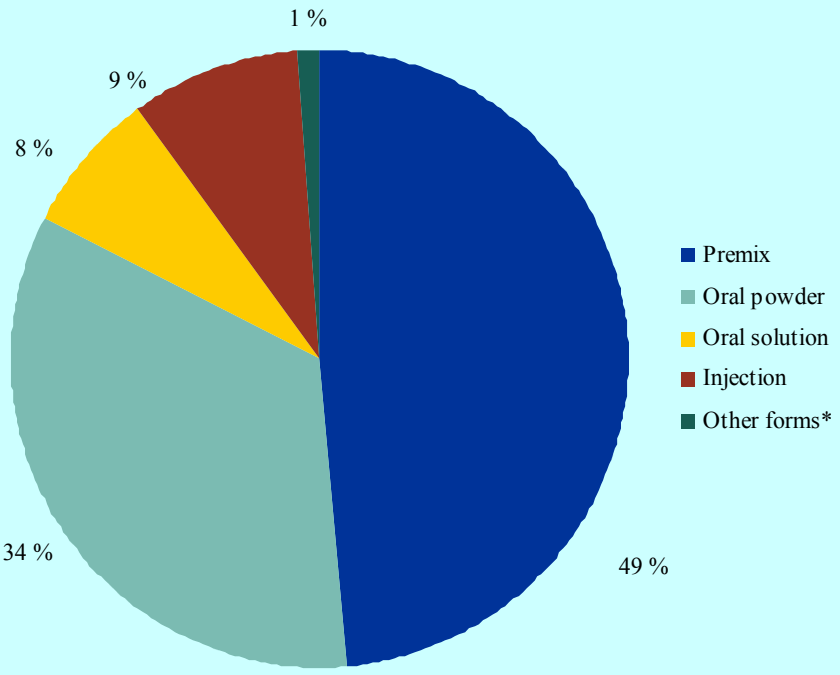

FIGURE 9. Sales of veterinary antimicrobial agents for food-producing species, including horses, in tonnes active ingredients, by formulation, in 19 EU/EEA countries, for 2010[1]. *Intramammaries and intrauterine preparations.

References :

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Kari Grave, Norwegian Veterinary Institute and Norwegian School of Veterinary Science, Oslo.

USAGE IN HUMANS

Hege Salvesen Blix

In 2012, the overall sales of antibacterials for systemic use in humans were 21.0 DDD/1,000 inhabitants/day. Since 2004, total sales of antibacterials have been increasing, mainly due to the penicillin group and to increased use of methenamine. When methenamine is excluded, the level of antibiotic use in 2012 was 17.4 DDD/1,000 inhabitants/day (Table 6).

TABLE 6. Human usage of antibacterial agents in Norway 2005-2012 by ATC groups. The usage is presented as DDD (Defined Daily Doses)/1,000 inhabitants/day and in % change 2011-2012. Collection methodology of data on human usage of antimicrobial agents is presented in Appendix 2.

ATC	Groups of substances	2005	2006	2007	2008	2009	2010	2011	2012	Change (%) 2011-2012
J01A	Tetracyclines	3.11	3.24	3.32	3.22	3.09	3.12	3.47	3.87	+ 11
J01B	Amphenicols	0.001	0.002	0.001	0.001	0.002	0.001	0.0005	0.0002	-
J01CA	Penicillins with extended spectrum	2.53	2.74	2.93	3.09	3.15	3.19	3.21	3.34	+ 4
J01CE	Beta-lactamase sensitive penicillins	4.55	4.63	4.70	4.71	4.47	4.44	4.47	4.3	- 4
J01CF	Beta-lactamase resistant penicillins	0.56	0.66	0.72	0.77	0.80	0.82	0.88	0.90	+ 2
J01CR	Combination of penicillins	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.04	+ 23
J01D	Cephalosporins, monobactams, carbapenems	0.57	0.60	0.60	0.60	0.58	0.55	0.56	0.55	- 2
J01E	Sulfonamides and trimethoprim	1.06	1.04	1.02	0.98	0.94	0.87	0.87	0.87	-
J01F	Macrolides, lincosamides and streptogramins	2.12	2.24	2.30	2.13	1.89	2.01	2.31	2.26	- 2
J01G	Aminoglycosides	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	-
J01M	Quinolones	0.57	0.62	0.67	0.70	0.71	0.73	0.75	0.75	-
J01X*	Other antibacterials	3.05	3.18	3.30	3.48	3.65	3.84	3.93	4.04	+ 3
	Total exclusive of methenamine	**15.6**	**16.3**	**16.9**	**16.8**	**16.2**	**16.3**	**17.2**	**17.4**	**+ 1**
	Total all antimicrobial agents	**18.2**	**19.0**	**19.7**	**19.8**	**19.4**	**19.7**	**20.6**	**21.0**	**+ 2**

[1] J01X includes glycopeptides, colistin, fusidic acid, metronidazol (i.v.), nitrofurantoin, linezolid and methenamine. Of total J01X, methenamine constitutes 3.57 DDD/1,000 inhabitants/day.

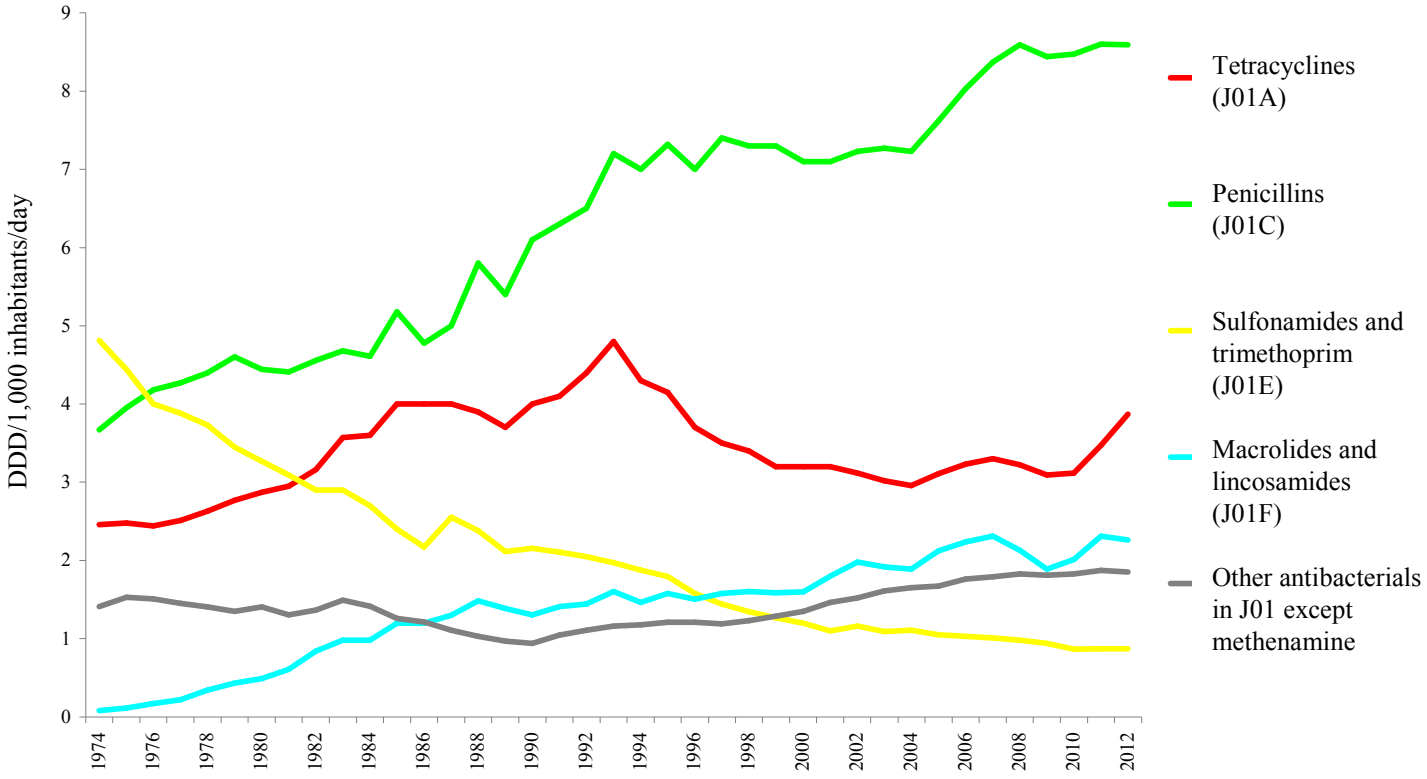

FIGURE 10. Sales of penicillins (J01C), tetracyclines (J01A), macrolides, lincosamides and streptogramines (J01F), sulfonamides and trimethoprim (J01E) and other antibacterials in Norway 1974-2012. Other types of antibacterials include all other antibacterials in ATC group J01, except methenamine (J01XX05).

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

In 2012, the overall sales in Norway of antibacterials for use in humans, terrestrial animals and farmed fish measured in weight of active substance were 59.6 tonnes (Figure 11). Humans accounted for 87% of the total use, terrestrial animals for 10% of the total use and the use in aquaculture only for 3% of the total use. The increase of 19% (in tonnes) from 2005 is mainly caused by increased use in humans. When excluding methenamine, the increase was 13% (from 41.0 tonnes in 2005 to 46.3 tonnes in 2012). During these years the use in terrestrial animals has been stable.

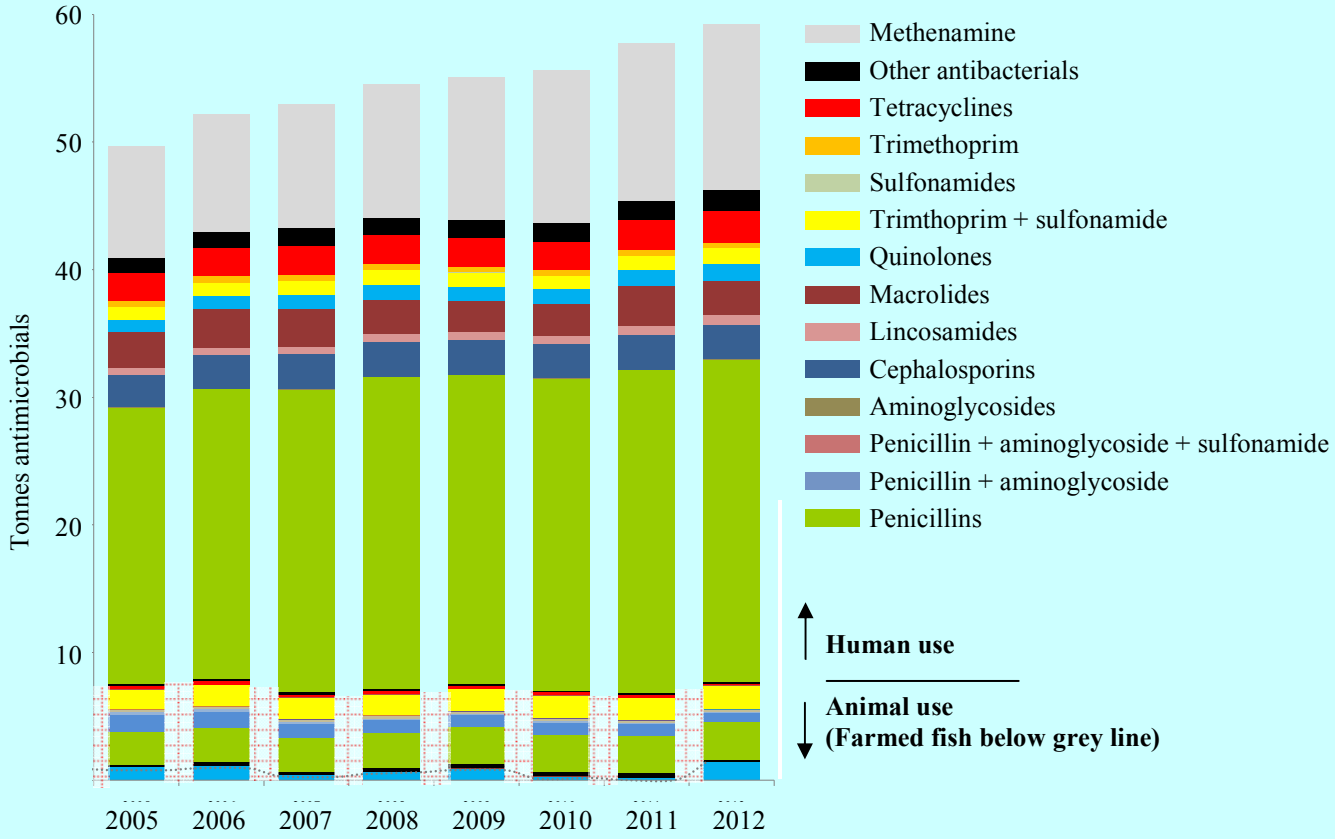

FIGURE 11. Sales, in tonnes, of active substance, of human and veterinary antibacterials, for the years 2005-2012. Use in farmed fish is included and appears below the dotted line.

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 of human antibacterials represent 77 % of the total weight. The other dominant formulations are parenteral formulations (human and animals) and oral formulations in animals. Use of other formulations e.g. for eye, ear and skin is limited.

TABLE 7. Sales in 2012, in kg of active substance, of human and veterinary antibacterials according to formulation.

Formulation	Humans	Terrestrial animals	Aquaculture
Dermal	106	3	
Oral	45,930	2,433	1,591
Parenteral	5,748	3,203	
Eye / ear	37	11	
Intramammary		404	
Others	58	121	
Total	51,880	6,175	1,591

Irene Litleskare and Hege Salvesen Blix, Department of Pharmacoepidemiology, Norwegian Institute of Public Health, Kari Grave, Norwegian School of Veterinary Science, Oslo.

Delayed antibiotic prescribing for respiratory tract infections in primary care

Delayed prescribing refers to the practice of prescribing an antibiotic but advising the patient not to start taking the medication unless he or she deteriorates or fails to improve after a certain amount of time. In addition to reducing antibiotic consumption, the strategy is said to provide a safety net for patients, and to preserve their autonomy. Randomised controlled trials in primary care have proven the strategy to be effective in lowering antibiotic consumption for conditions such as sore throat, acute otitis, acute bronchitis and acute conjunctivitis. Between 20% (1) and 45% (2) of patients receiving a delayed prescription for respiratory infections in a trial setting report to consume the medication, while between 87% (3) and 100% (2) of patients assigned to immediate antibiotics report to consume them. Delayed prescribing seems to be safe, with acceptable patient satisfaction and symptom control. On this background, the strategy is recommended in several countries' antibiotic treatment guidelines in primary care, including the Norwegian guidelines.

A qualitative study from Norwegian primary care (4) reveals that GPs endorse the strategy of delayed prescribing, especially among patients with acute otitis and acute sinusitis. However, they look upon the strategy more as a practical tool than as a tool to decrease antibiotic consumption. Overall, the GPs reported to use delayed prescribing infrequently, due to strict requirements as to which patients, for which diagnoses and in which situations they would issue delayed prescriptions.

A questionnaire study among Norwegian GPs and their patients (5) confirms that acute otitis and acute sinusitis are the preferred diagnoses for delayed prescribing. 46% of the patients reported to consume the antibiotics, varying from 35% in cases of acute otitis to 57% in cases of upper respiratory tract infection. Both GPs and patients were generally satisfied with the strategy. GPs were less satisfied when they issued delayed prescriptions due to disagreement with the patient regarding the indication for antibiotics.

Both GPs' antibiotic prescribing practices and patients' views on antibiotics and infections vary greatly between different cultures and countries. This also seems to be the case with the use of delayed prescribing. Francis *et al*. studied the practice of delayed prescribing for acute cough in 14 general practice networks all over Europe (6). Overall, delayed prescriptions were issued in 6.3% of the consultations, corresponding to 11.8% of the antibiotic prescriptions. In the Norwegian network, delayed prescribing was used only in 2% of the consultations, constituting 6.6% of the prescribed antibiotics. The antibiotic prescription rate in the Norwegian network was 30%, compared with 52.4% for all networks.

Delayed prescribing seems to be used relatively infrequently in Norwegian general practice, probably due to a relatively low antibiotic prescription rate and low expectations for antibiotics among the patients. The use of delayed prescribing should be encouraged, especially in cases of acute otitis and acute sinusitis. However, when promoting delayed prescribing, this should always be accompanied by a firm message stating that no antibiotics is the preferred option for respiratory infections which do not fulfil the requirements of immediate antibiotics at the time of consultation.

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Sigurd Høye, Antibiotic Centre for Primary Care, and Department of General Practice/Family Medicine, Institute of Health and Society, University of Oslo.

In 2012, the penicillins (ATC group J01C) accounted for 41% of the total antibacterial use in Norway (Figure 10). Within the penicillins the beta-lactamase sensitive penicillins (J01CE) is the largest subgroup. Over the years there has been a shift towards use of more broad-spectered penicillins. Penicillins with extended spectrum (J01CA) now represent 39% of the penicillin group compared to 31% a decade ago (2002) (Figures 12-13). This is mainly due to increasing use of pivmecillinam for urinary tract infections, at the expense of the subgroup of sulfonamides and trimethoprim, which has decreased over the years (Figure 10).
An epidemic of *Mycoplasma pneumoniae* was noted in Norway in the winter season 2011-12 causing increased use of macrolides. The use of of the group J01F macrolides, lincosamides and streptogramins has followed a wavy pattern over the years although the internal pattern within the group has remained relatively unchanged over the years (Figures 10 and 14). The shifts in use could be explained to some degree by the recurrent epidemics of *M. pneumoniae* in Norway, occurring with five- to seven-year intervals.
In the latest years, sales of cephalosporins, monobactams and carbapenems have been stable and this group represents 3% of the total sales of antibacterials (Figure 12). The internal subgroup pattern has changed over time (Figure 15). Today, 1st and 3rd generation cephalosporins hold 47% and 29% of ATC group J01D, respectively.
The use of quinolones is increasing. It represents only a small fraction (4%) of total antibacterial sales, but the sales have more than doubled since 2000 (Figure 12). Ciprofloxacin is the main substance accounting for 96% of the quinolone group in 2012.
The increase of ATC group J01X is mainly due to the urinary prophylactic agent methenamine, accounting for 17% of total antibacterial use (Figure 12).
The increased use of rifampicin (J04A) over the last five years is mainly due to the use for other indications than tuberculosis (Table 8).

The usage of antibacterials varies among the 19 Norwegian counties, the county using the least is using around 70% (in DDDs/1,000 inhabitants/day) of the county using the most. The use has increased in all counties (Figure 16). There is a trend of the same counties being high-use and low-use counties over the years.

Antibacterials are prescription-only drugs in Norway. Around 85% of the total human sales of antibacterials are used outside institutions (hospitals and nursing homes). Physicians are the main prescribers to humans, but dentists prescribe 5% (measured in DDDs) of antibiotics (J01) to humans in ambulatory care. Dentists most often prescribe phenoxymethylpenicillin (77% of all antibiotic-DDDs prescribed by dentists) followed by amoxicillin (10%) and clindamycin (5%).

For ambulatory care, the most important antibiotic groups in 2012 were penicillins (J01C, 40% of DDDs), tetra-cyclins (J01A, 20%) and macrolides and lincosamides (J01F, 12%). Females used more antibiotics than males; 30% of the females purchased at least one antibiotic course in 2012 compared to 21% of the males. The gender pattern was similar in all regions in the country (Figure 17). The highest use was found among young children, young women and the elderly (Figure 18). Among those who used antibacterials, the elderly use more, both with regard to amount (measured in DDDs) and to number of prescriptions. For those above 65 years 2-3 prescriptions were dispensed every year compared to 1-2 for younger persons. The 0-4 year olds on average received antimicrobial prescriptions two times per year. Since the dosages for young children are much less than in adults, the amount DDD per user will be lower than in adults (Figure 19).

In 2012, the antibacterial sales (in DDDs) to hospitals represented around 7% 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 20).
Penicillins (J01C) represent around 45% of the use measured in DDDs in hospitals (J01CE 18%, J01CA 15% and J01CF 10%). The second largest group is the cephalosporins; 18% of all DDDs, the dominant subgroup being 3rd generation cephalosporins (J01DD) (9%). In 2012, seven substances accounted for 50% of DDDs used (benzylpenicillin, cefotaxime, cloxacillin, ciprofloxacin, ampicillin, cefalotin and pivmecillinam). Three single substances accounted for 30% of all antibacterial use in hospitals; benzylpenicillin (15%), cefotaxime (8%) and cloxacillin (7%). Six selected groups mainly used in hospitals are shown in Figure 21. Since 2006, there has been a stable increase in the use of 3rd generation cephalosporins, carbapenems and piperacillin and enzyme inhibitor, while the use of 2nd generation cephalosporins has decreased over the years.

The national guidelines for antibiotic use in ambulatory care and nursing homes have been updated in 2013, and a new national guideline for hospital use was also published in 2013. The Antibiotics Centre for Primary Health Care (ASP) was established in 2006 and a National Centre for Antibiotic Use in Hospitals was established in 2011. These centres have the responsibility for the continous updating of national treatment guidelines and this will hopefully have a positive impact on therapy traditions and antibacterial prescribing patterns in Norway.

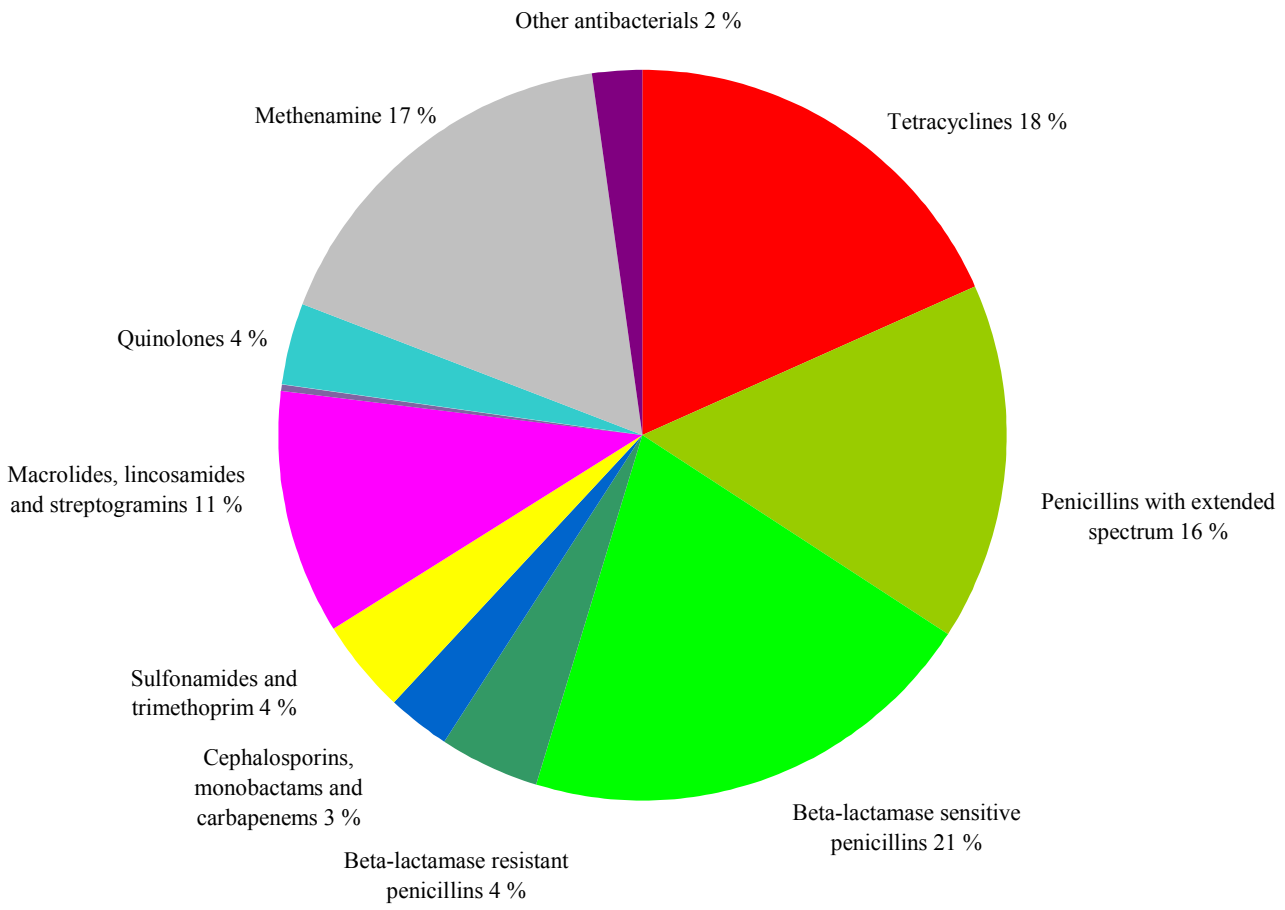

FIGURE 12. Relative amount of antibacterial agents for systemic use in 2012 in Defined Daily Doses (DDD) (total sales).

TABLE 8. Human usage of single antimicrobial agents for systemic use in Norway. 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	Substance	2004	2005	2006	2007	2008	2009	2010	2011	2012
J01A A02	Doxycycline	1.80	1.89	1.97	2.0	1.9	1.78	1.83	2.09	2.36
J01A A04	Lymecycline	0.34	0.39	0.45	0.51	0.52	0.54	0.59	0.76	0.90
J01A A06	Oxytetracycline	0.20	0.20	0.19	0.18	0.17	0.16	0.15	0.03	
J01A A07	Tetracycline	0.62	0.64	0.63	0.63	0.62	0.60	0.54	0.58	0.62
J01AA07*	Minocycline		0.0003	0.0003	0.0001	0.0002	0.0003	0.001	0.002	0.006
J01AA12	Tigecycline			0.0001	0.0002	0.0004	0.0005	0.0004	0.0002	0.0002
J01B A01	Chloramphenicol	0.001	0.002	0.002	0.001	0.001	0.002	0.0007	0.0005	0.0002
J01C A01	Ampicillin	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.09
J01C A02*	Pivampicillin	0.08	0.07	0.06	0.01					
J01C A04	Amoxicillin	0.94	1.06	1.11	1.26	1.34	1.31	1.34	1.39	1.45
J01C A08	Pivmecillinam	1.25	1.29	1.46	1.55	1.65	1.72	1.75	1.73	1.78
J01C A11*	Mecillinam	0.005	0.006	0.006	0.006	0.008	0.008	0.008	0.008	0.008
J01C E01	Benzylpenicillin	0.24	0.26	0.26	0.25	0.24	0.28	0.22	0.24	0.24
J01C E02	Phenoxymethylpenicillin	3.99	4.29	4.37	4.45	4.46	4.19	4.22	4.23	4.07
J01C E08*	Benzathine benzylpenicillin	0.0002	0.0001	0.0002	0.0001	0.0001	0.0002	0.0002	0.0001	0.0002
J01C F01	Dicloxacillin	0.51	0.41	0.54	0.61	0.64	0.67	0.70	0.74	0.76
J01C F02	Cloxacillin	0.11	0.15	0.12	0.12	0.13	0.13	0.12	0.14	0.14
J01C F05*	Flucloxacillin	0.0002	0.0001	0.0001	0.0003	0.0005	0.0007	0.0005	0.0003	0.0005
J01C R02*	Amoxicillin and enzyme inhibitor	0.0003	0.0000	0.0001	0.0001	0.0012	0.003	0.003	0.002	0.004
J01C R05	Piperacillin and enzyme inhibitor	0.005	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03

ATC	Substance	2004	2005	2006	2007	2008	2009	2010	2011	2012
J01D B01	Cefalexin	0.29	0.24	0.26	0.25	0.23	0.21	0.20	0.19	0.18
J01D B03	Cefalotin	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08
J01D B04*	Cefazolin		0.002	0.002	0.001	0.001				
J01D C02	Cefuroxim	0.14	0.13	0.12	0.12	0.11	0.10	0.09	0.09	0.08
J01D D01	Cefotaxim	0.07	0.08	0.09	0.09	0.10	0.11	0.11	0.12	0.12
J01D D02	Ceftazidim	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01D D04	Ceftriaxone	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03
J01D F01	Aztreonam	0.001	0.0005	0.0008	0.0008	0.0007	0.0006	0.0006	0.0005	0.0007
J01D H02	Meropenem	0.02	0.026	0.031	0.035	0.037	0.042	0.041	0.043	0.046
J01D H03	Ertapenem			0.000	0.001	0.001	0.002	0.002	0.002	0.002
J01D H51	Imipenem and enzyme inhibitor	0.005	0.005	0.004	0.004	0.003	0.002	0.002	0.002	0.002
J01E A01	Trimethoprim	0.76	0.73	0.70	0.68	0.64	0.60	0.56	0.55	0.51
J01E E01	Sulfamethoxazol and trimethoprim	0.34	0.33	0.34	0.34	0.34	0.33	0.31	0.32	0.36
J01F A01	Erythromycin	1.03	1.16	1.24	1.21	1.08	0.92	0.94	1.18	1.06
J01F A02	Spiramycin	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01F A09	Clarithromycin	0.37	0.39	0.40	0.43	0.37	0.31	0.34	0.37	0.39
J01F A10	Azithromycin	0.28	0.32	0.34	0.39	0.38	0.37	0.41	0.44	0.48
J01F F01	Clindamycin	0.20	0.23	0.25	0.26	0.28	0.28	0.31	0.32	0.33
J01GA01*	Streptomycin	0.0004	0.0002	0.0003	0.0002	0.0003	0.0002	0.0002	0.0002	0.0001
J01G B01	Tobramycin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01G B03	Gentamicin	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.05	0.05
J01G B06*	Amikacin	0.0003	0.0004	0.0009	0.0003	0.0007	0.0008	0.0009	0.001	0.001
J01M A01	Ofloxacin	0.05	0.05	0.04	0.04	0.04	0.03	0.03	0.03	0.02
J01M A02	Ciprofloxacin	0.47	0.52	0.57	0.62	0.66	0.67	0.70	0.71	0.72
J01MA12*	Levofloxacin		0.0003	0.0003	0.0008	0.0008	0.004	0.003	0.002	0.002
J01MA14*	Moxifloxacin				0.0007	0.001	0.001	0.004	0.006	0.004
J01X A01	Vancomycin	0.007	0.007	0.008	0.01	0.01	0.01	0.01	0.01	0.01
J01X A02	Teicoplanin	0.0007	0.0008	0.0008	0.0007	0.001	0.0007	0.0008	0.0008	0.0007
J01X B01	Colistin	0.003	0.004	0.005	0.004	0.004	0.005	0.004	0.004	0.004
J01X C01	Fusidic acid	0.008	0.006	0.006	0.006	0.006	0.005	0.004	0.005	0.005
J01X D01	Metronidazole	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.07
J01X E01	Nitrofurantoin	0.36	0.36	0.37	0.36	0.36	0.36	0.37	0.39	0.37
J01X X05	Methenamin	2.37	2.59	2.71	2.84	3.02	3.19	3.37	3.44	3.57
J01XX08	Linezolid	0.006	0.007	0.006	0.006	0.007	0.008	0.009	0.01	0.01
J01XX09	Daptomycin				0.000	0.000	0.000	0.0001	0.0004	0.0009
D06AX09/ R01AX06*	Mupirocin in kg ointment/cream (2%)	3.0	3.4	4.3	4.0	3.9	5.1	4.5	4.6	7.3
J04AB02	Rifampicin	0.003	0.003	0.003	0.004	0.003	0.004	0.004	0.004	0.005
J04A**	Rifampicin	0.038	0.047	0.052	0.052	0.067	0.087	0.086	0.082	0.086
A07AA09	Vancomycin	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002
A07AA11*	Rifaximin						0.0005	0.0013	0.0022	0.0043
A07AA12	Fidaxomicin									0.0001
P01AB01	Metronidazole	0.20	0.20	0.20	0.21	0.21	0.22	0.23	0.24	0.24

* Drugs not licensed at the Norwegian marked in 2012.
** Given as the amount of rifampicin in plain and combination products.

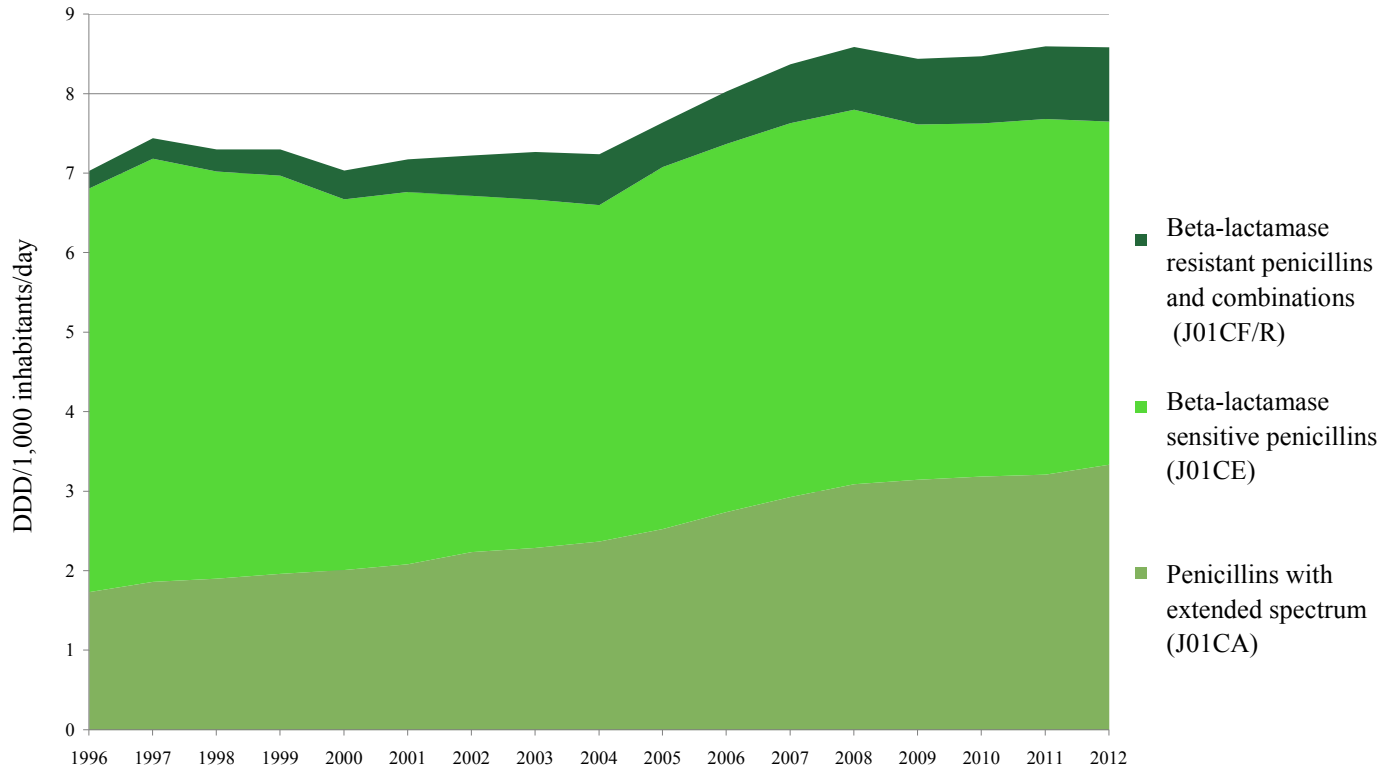

FIGURE 13. Sales of penicillins (J01C) in Norway 1996-2012 and changes within groups of penicillins.

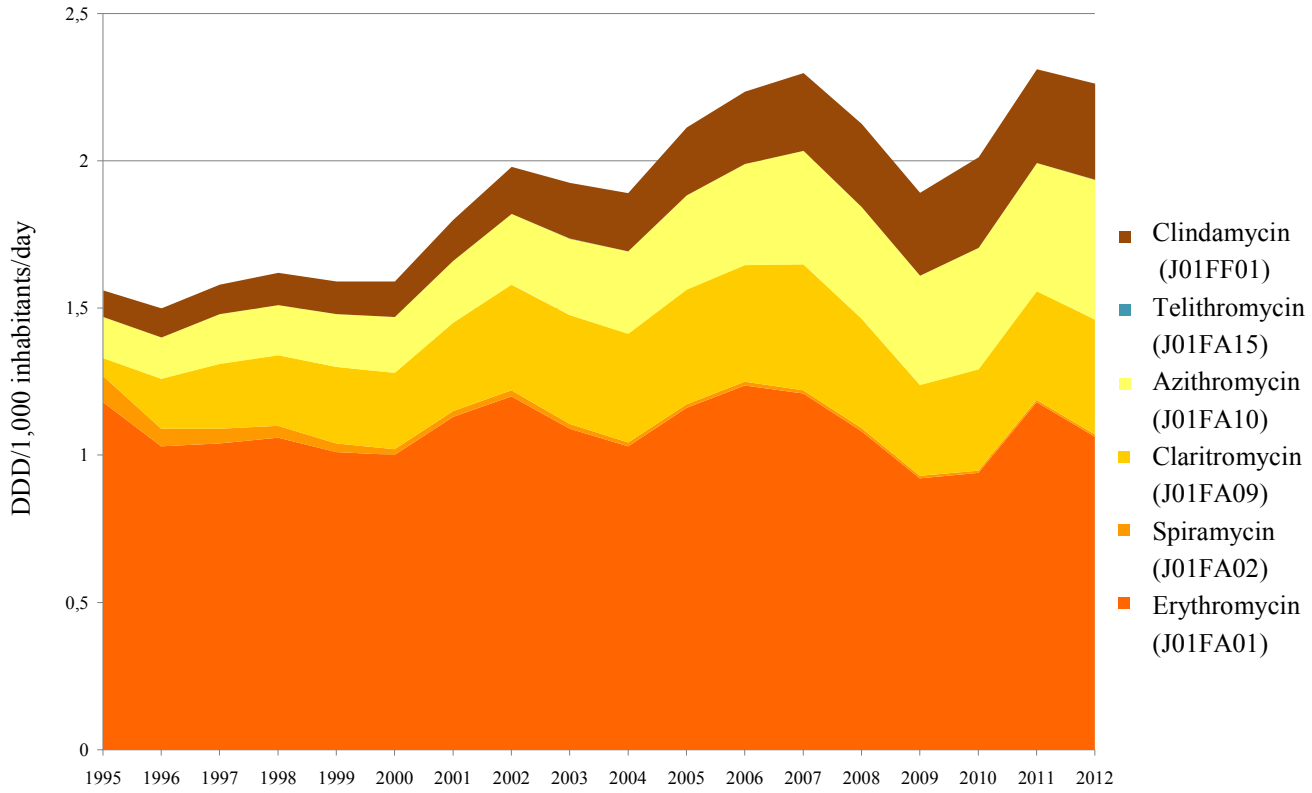

FIGURE 14. Sales of macrolides, lincosamides and streptogramins (J01F) in Norway 1995-2012.

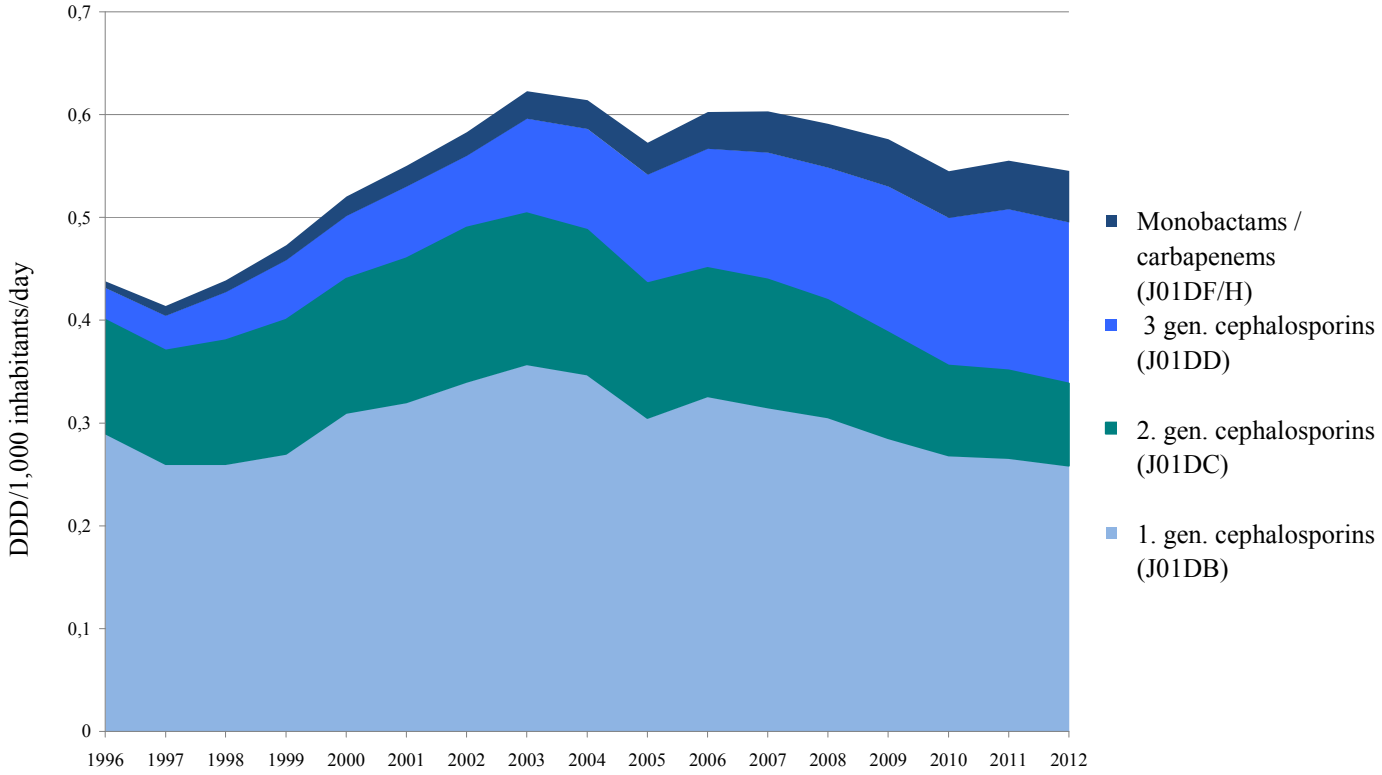

FIGURE 15. Sales of cephalosporins, monobactams and carbapenems (J01D) in Norway 1996-2012 and changes between generations of cephalosporins and monobactams/carbapenems.

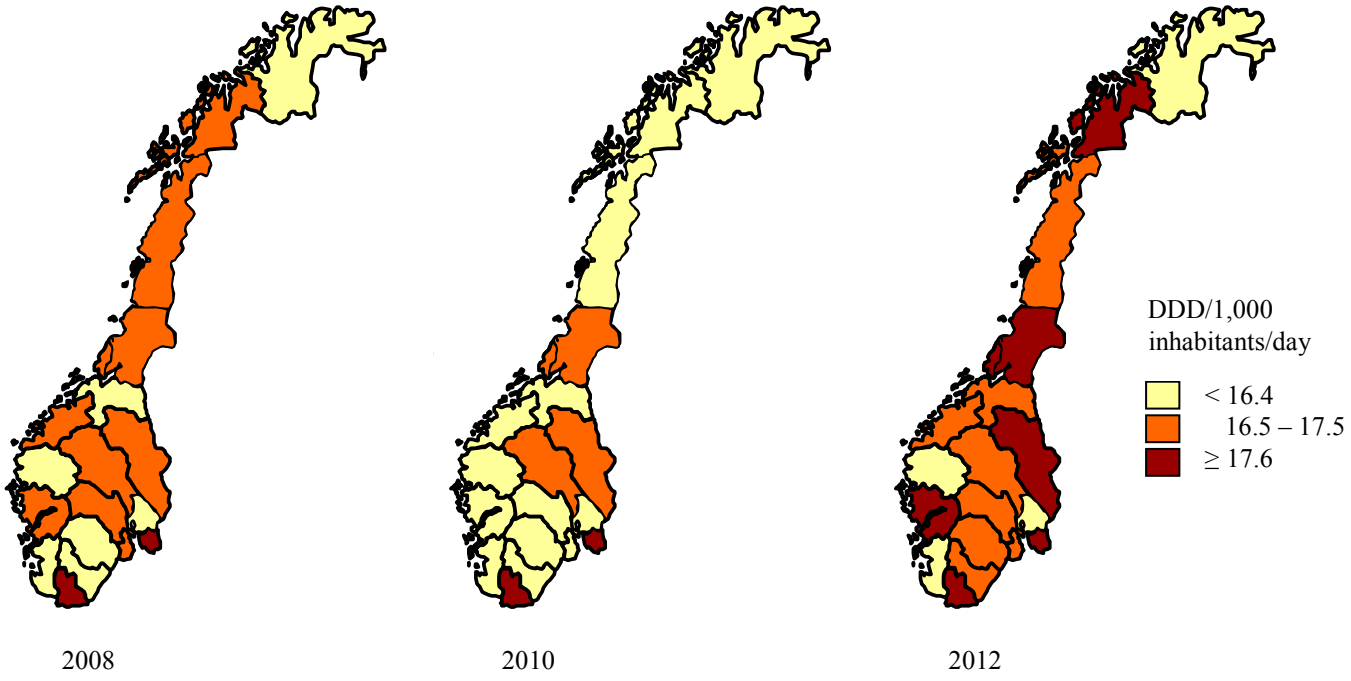

FIGURE 16. Sales of antibacterial agents for systemic use (ATC group J01, excl. methenamine) in the different counties of Norway in 2008, 2010 and 2012.

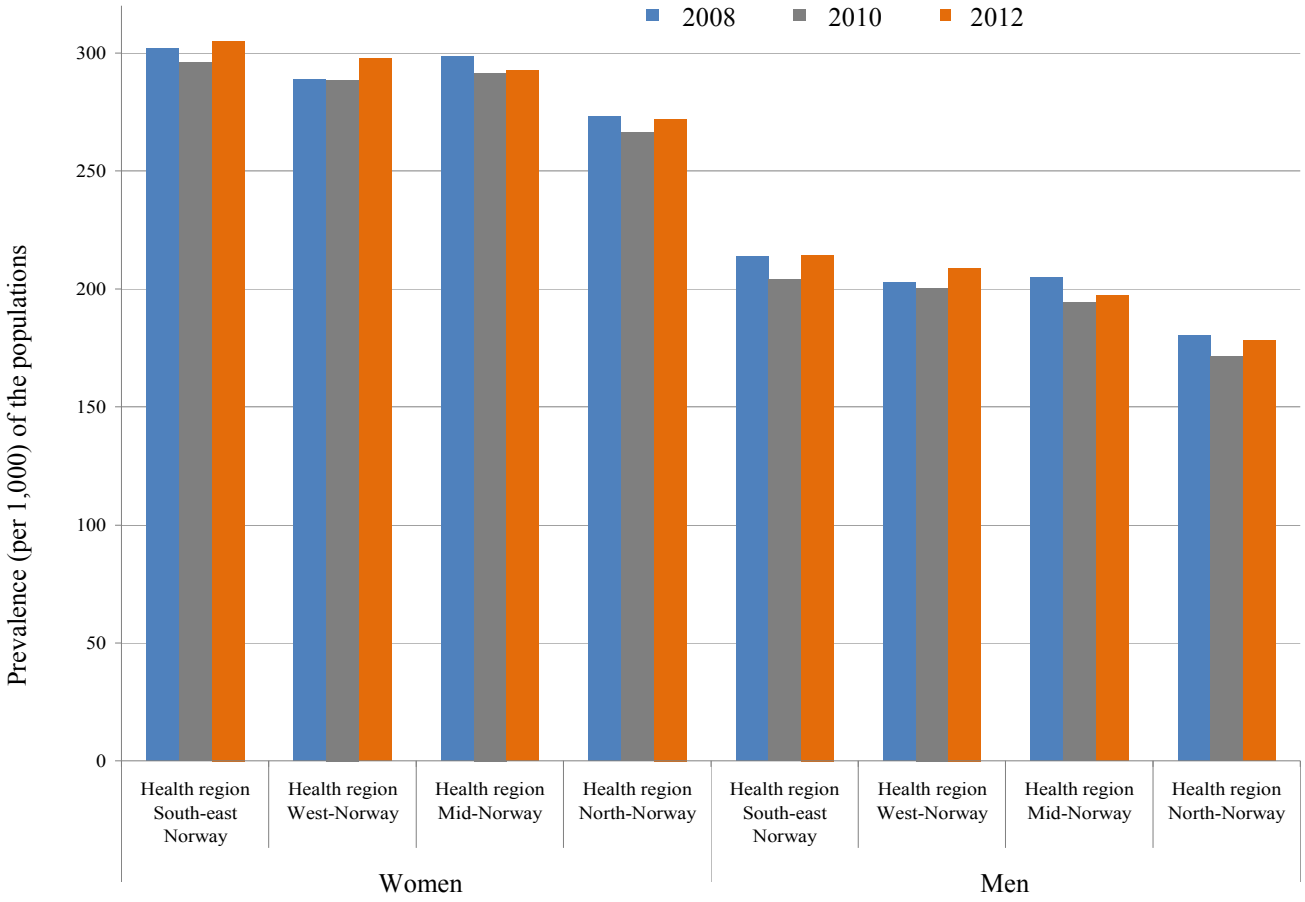

FIGURE 17. One year prevalence of systemic antibacterial use in ambulatory care by gender and health region in Norway for the years 2008, 2010 and 2012. Antibacterials for systemic use include ATC group J01, vancomycin (A07AA09), rifaximin (A07AA11), fidaxomicin (A07AA12) and metronidazole (P01AB01).

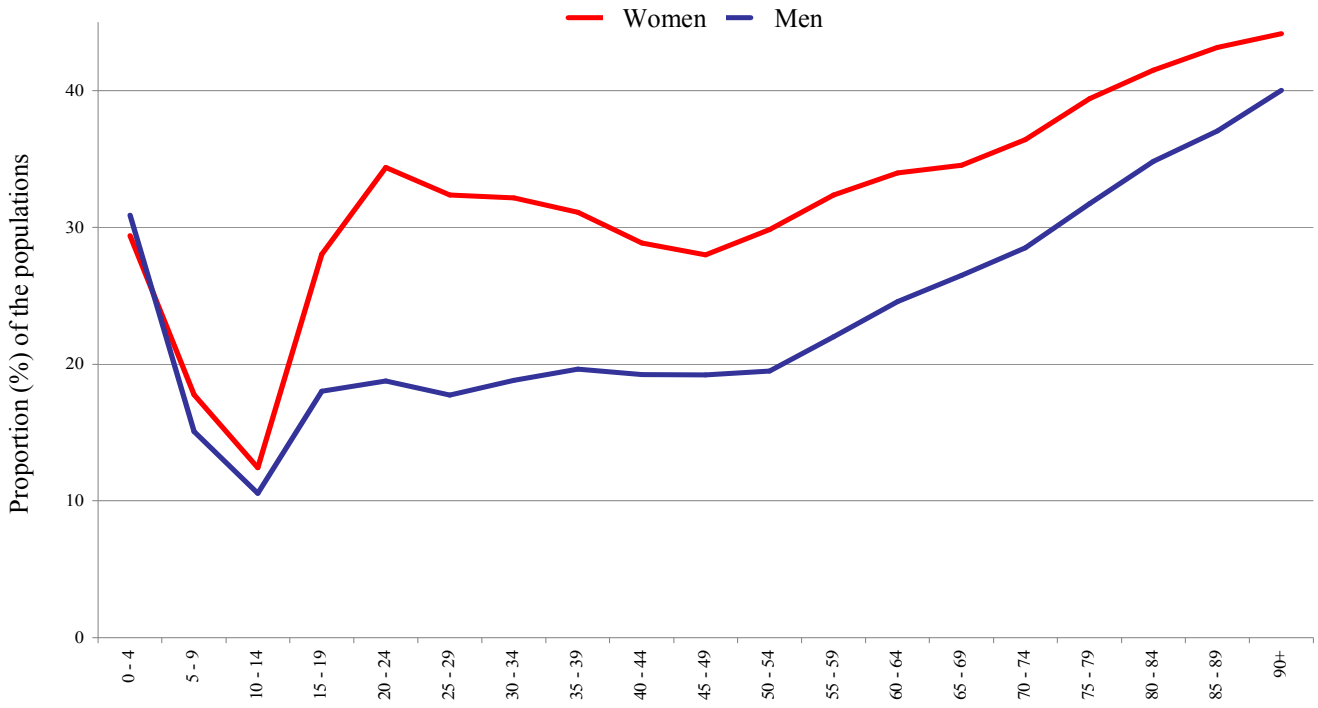

FIGURE 18. Proportion (%) of the population having dispensed at least one prescription of antibacterials (one year prevalence) in ambulatory care by gender and age (from 1 year to 90+ years) in Norway, 2012. Antibacterials included are antibacterials for systemic use (ATC group J01), oral vancomycin (A07AA09), rifaximin (A07AA11), fidaxomicin (A07AA12) and oral metronidazole (P01AB01). Prevalence in age groups above 65+ is adjusted according to persons from these age groups living outside institutions.

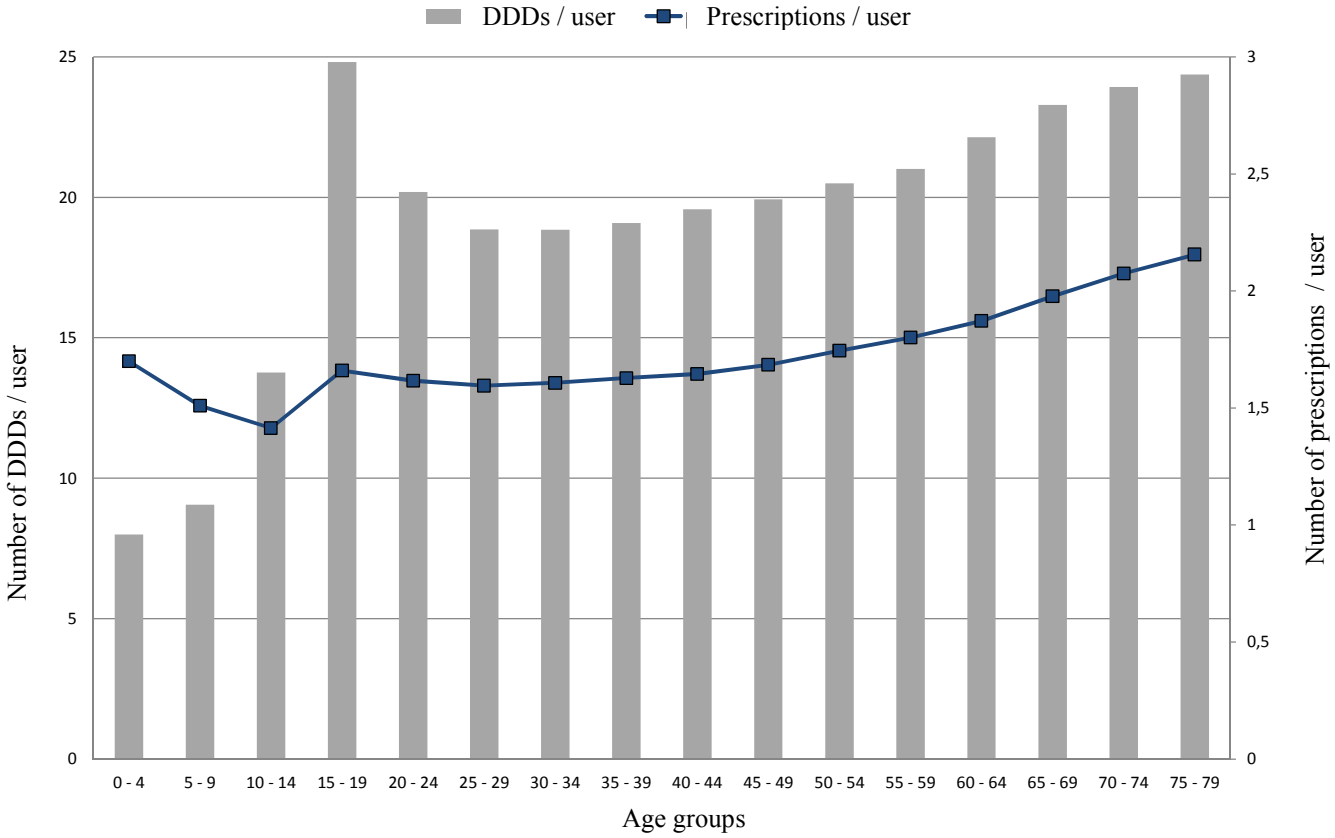

FIGURE 19. Mean number of prescriptions per person and mean number of DDDs per person among users of antibacterials in ambulatory care by age (from 1 year to 80 years) in Norway, 2012. Antibacterials included are antibacterials for systemic use (ATC group J01, excl. methenamine).

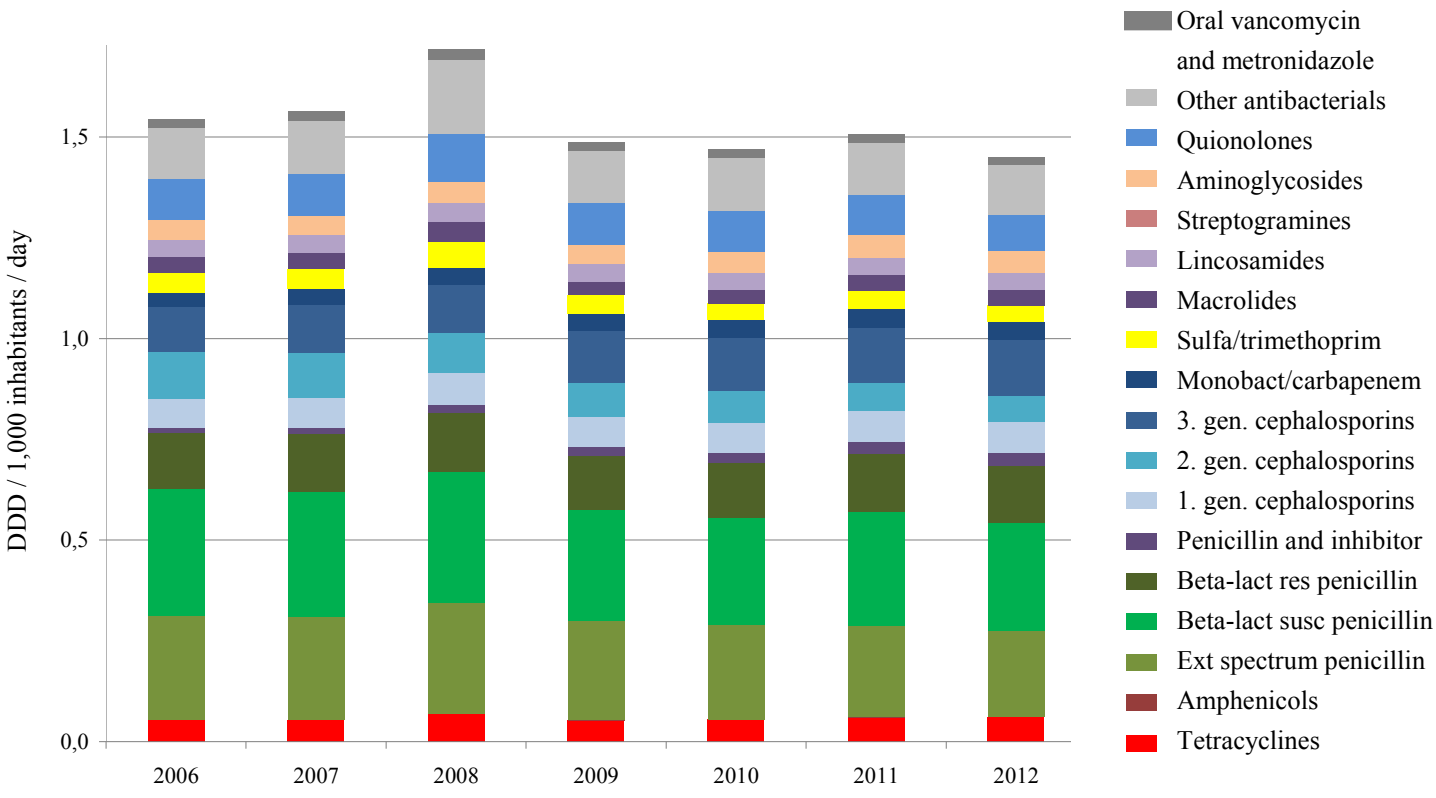

FIGURE 20. Distribution of antibacterial agents for systemic use in Norwegian hospitals 2006-2012, measured in DDD/1,000 inhabitants/day.

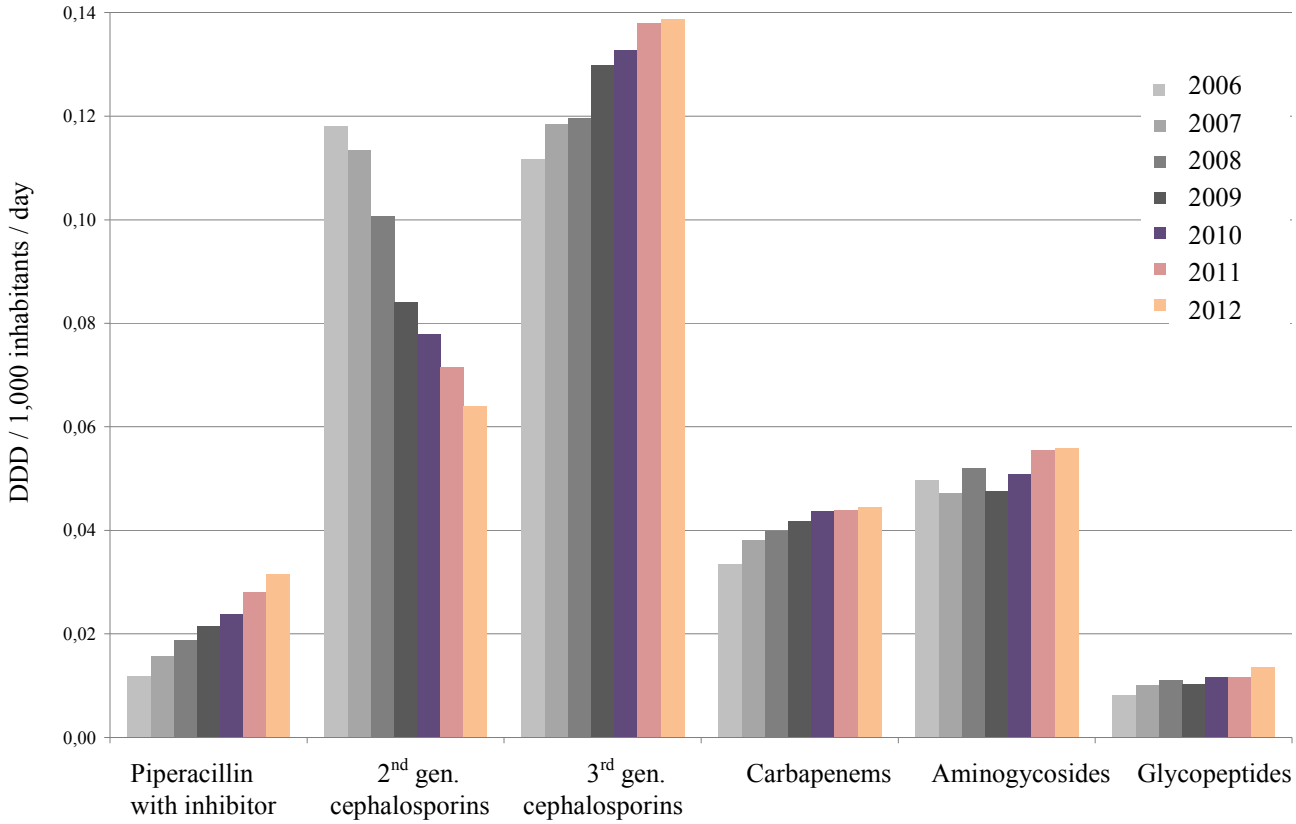

FIGURE 21. Distribution of selected antibacterial agents for systemic use in Norwegian hospitals 2006-2012, measured in DDD/1,000 inhabitants/day.

Antibiotic treatment in febrile neutropenia (FN)

Acute febrile neutropenia (FN) is important because it can rapidly develop into a fatal condition. FN also often causes reduced anti-cancer dose intensity and frequency leading to a worse condition for the cancer disease. FN usually occurs after chemotherapy with bone marrow suppressive side effects. The neutropenia often becomes present 7-14 days after the chemotherapy, but it may arrive both sooner or later. Acute FN is potentially lethal. All patients who have received chemotherapy during the last three weeks and who have measured a body temperature > 38°C, must immediately contact the nearest hospital or a medical doctor for clinical examination and measurement of polymorphonuclear neutrophilic granulocytes (PMN). If the patient is neutropenic (PMN < 1.0) or expected to become neutropenic within the next 24 hours, she should immediately be admitted to the hospital. Two blood culture sets should be drawn, and adequate intravenous antibiotic therapy should be started within one (maximum two hours). The few cases when a febrile neutropenic patient arrives too late to see a doctor represent the very few cases that may have a fatal outcome. It is important to make sure that the patient is adequately informed at discharge after chemotherapy, especially in cases where there are problems with language understanding.

Adequate antibiotic therapy includes use of at least one Gram-negative bactericidal drug (beta-lactam, aminoglycoside, or fluoroquinolone). Neutropenic bacterial sepsis is usually due to the patient's own flora (especially the gastrointestinal and the skin flora). The most commonly chosen antibiotic regimen in Norway consists of the combination of penicillin G 5 mill. IE x 4 plus an aminoglycoside (gentamicin or tobramycin) ≥ 6 mg/kg once daily. The aminoglycoside should be administrered first, and the first dose should rather be too high than too low. One dose of aminoglycoside may be given almost independently of renal function, but the aminoglycoside trough level should be measured before the next dose is given. The trough level must be < 0.5 mg/l to avoid nephrotoxicity. If the trough concentration is ≥ 0.5 mg/l, the time interval before the next dose should be increased whereas the total dose should not be reduced. Alternatively, one may switch to another drug.

Patients who are planned for future cis-platinum therapy for their cancer disease should not be given aminoglycosides to be certain not to compromise their anti-cancer therapy. The alternative then usually is a broad-spectrum beta-lactam antibiotic. Piperacillin/tazobactam (2 grams x 4) may be the best option. Cefotaxime (1 gram x 4) may be an alternative. One should avoid choosing a carbapenem (meropenem) as long as possible to reduce the total carbapenem consumption and thus the risk of carbapenem resistance.

If the patient is clinically stable the next day, most patients have a low risk for initial FN complications (< 5% during the first five days). A clinically stable patient should have stable cancer, no comorbidity, negative blood culture, expected neutropenia < 7 days, and age < 65. These patients may be considered for further oral antibiotic therapy (ciprofloxacin plus penicillin V) provided they have 24 hours supervision and daily contact with their medical doctor.

All other patients should remain isolated in the hospital and continue the treatment with intravenous antibiotics until the bone marrow shows signs of regeneration. In general, non-febrile neutropenic patients with a risk of FN ≥ 20% (or ≥ 10% if they also have comorbidity) should be given primary prophylaxis with G-CSF (1). After one episode with FN everybody should be offered secondary G-CSF prophylaxis after further cytotoxic chemotherapy. It is better to prevent FN with G-CSF than having to treat FN with antibiotics.

Seven trials, all Norwegian, have been conducted evaluating initial empiric therapy with penicillin and an aminoglycoside in FN. They all make the same conclusion. Penicillin G plus an aminoglycoside is an efficacious and safe initial empiric therapy provided the antibiotic regimen is modified if the clinical response is unsatisfactory. One of these trials (2) compared tobramycin 6 mg/kg given as one single daily dose versus given as three doses per day. All the 174 evaluable patients received penicillin 5 mill. IE x 4 besides the tobramycin. Forty percent of the patients in both groups had no modification of the antibiotic regimen during the course of FN. Patients who needed modifications of the antibiotic regimen had a mean time to modification of five days after they started antibiotic therapy. With these modifications all the patients had a successful outcome of their episodes with FN.

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2. Torfoss D *et al.* Tobramycin once versus three times daily, given with penicillin G, to febrile neutropenic cancer patients in Norway: a prospective, randomized, multicentre trial. J Antimicrob Chemother 2007; 59: 711-7

Dag Torfoss, Oslo University Hospital, Radiumhospitalet, Oslo.

Increasing use of azithromycin – change in therapy traditions

Azithromycin and clarithromycin have approximately the same antibiotic spectrum as erythromycin, but better oral absorption and fewer adverse effects than erythromycin. The mechanism of resistance for the macrolides is similar and cross-resistance is present. Since azithromycin has a high intracellular concentration and relatively low extracellular concentration, a wide distribution in tissues and a very long half-life (68 hours), there is also a possibility that the normal flora may be exposed to low levels of azithromycin for quite a long time, and thereby contributing to development of resistance.

In Norway, azithromycin is approved for chlamydia infections and gonorrhoeae, streptococcal pharyngitis/tonsillitis, sinusitis and otitis media (1). For several years, azithromycin has been recommended to be given as one dose (1 g) treatment for *Chlamydia trachomatis* infection, but in order to limit the risk of resistance development in general and in *Mycoplasma genitalium* in particular (2), the recommendations was changed in 2012 to doxycycline for 7 days (3). Although azithromycin has been available in the Norwegian market since 1995, it is the least used macrolide. However, a significant increase has been observed the latest years (Figure 22), with some shifts in the pattern of use. There are seasonal variations with higher use in the winter and lower in the summer, and in later years these fluctuations appear to have become larger. Secondly, Figure 23 shows that it is young adults who are the main users of azithromycin, but over time, use has increased in all age groups. However, since 2005 the increase is mainly observed among young children (age < 10 years) and in adults aged ≥ 35 years (Figure 23). Interestingly, in 2012 the prevalence went somewhat down for the 15-35 year olds (data not shown). A third observation is that both the number of individuals being prescribed azithromycin and the number of DDDs prescribed per user is increasing, giving an indication that azithromycin is prescribed for other indications than chlamydia and also that the treatment length or the doses have been increasing (Figure 24).

These observations (increased fluctuations, increased use in children and older adults, and increased number of DDDs per user) might suggest that use for respiratory tract infections is increasing while the use for sexually transmitted infections is stable. This is further corroborated by a stable, or possibly slightly declining, number of positive samples for genital chlamydia infections in recent years (4) (www.msis.no).

In conclusion, the use of azithromycin has increased over the years despite a stable occurrence of genital chlamydia, probably due to increased use for respiratory tract infections. Hopefully, new recommendations for treatment of sexually transmitted disease will lead to a decreased use in of azithromycin.

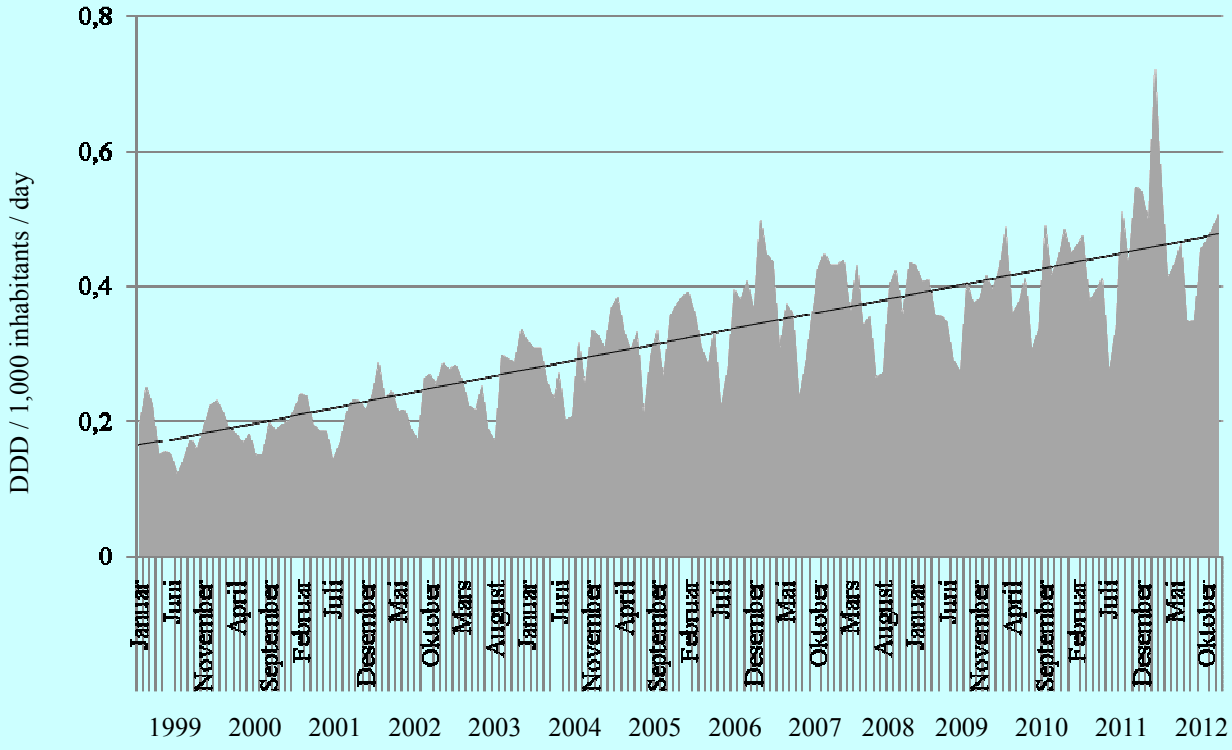

FIGURE 22. Changes in total sales of azithromycin in Norway, measured in DDD/1,000 inhabitants/day, 1999-2012. The oral DDD for azithromycin is 300 mg.

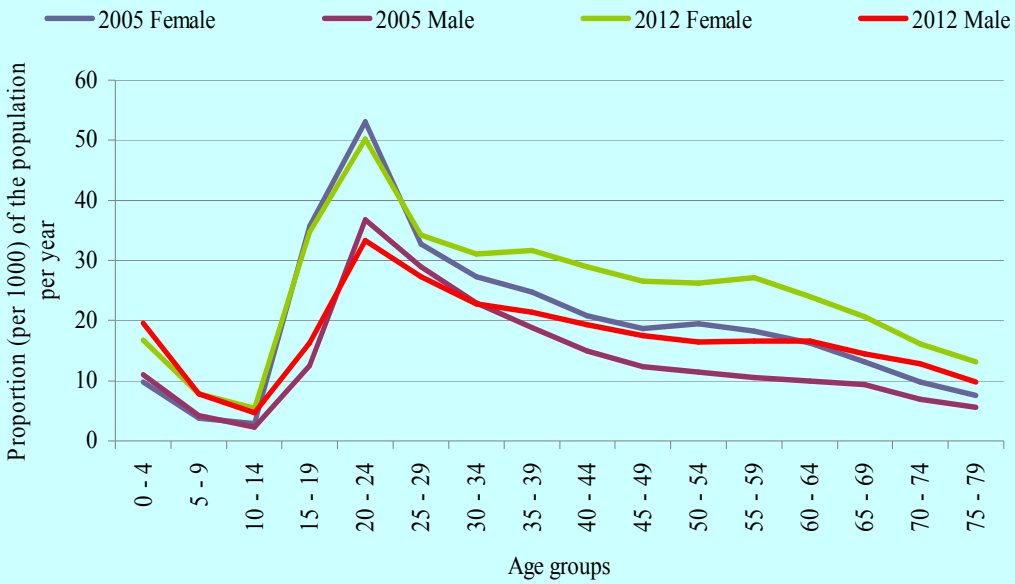

FIGURE 23. Proportion (per 1,000) of the population having dispensed at least one prescription of azithromycin in ambulatory care by gender and age in Norway shown for 2005 and 2012.

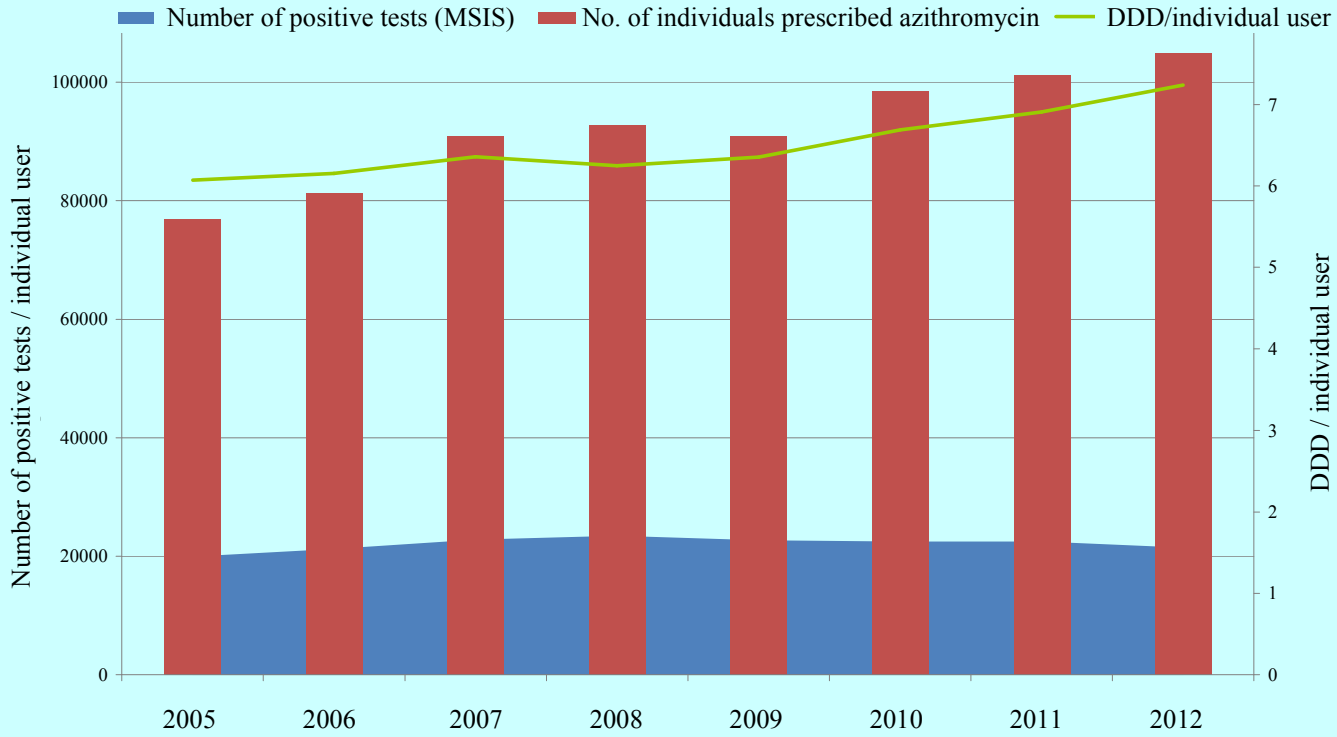

FIGURE 24. Annual changes in number of positive samples of *Chlamydia trachomatis*, number of individual users of azithromycin and number of DDDs prescribed per user in the period 2005-2012.

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1. Norsk legemiddelhåndbok for helsepersonell. http://legemiddelhandboka.no/ (10.05.2013).
2. Moi H, Vestrheim DF, Olsen AO. Reduce the use of one-dose azithromycin. Tidsskr Nor Laegeforen. 2011;131(7):673-4.
3. Lindbæk M, red. Nasjonale faglige retningslinjer for antibiotikabruk i primærhelsetjenesten. Oslo: Helsedirektoratet, 2013. www.helsebiblioteket.no/microsite/Antibiotikaretningslinjer (10.05.2013).
4. Smittevern: Smittevernboka. Oslo: Folkehelseinstituttet, 2013. http://www.fhi.no/artikler.

Hege Salvesen Blix, Department of Pharmacoepidemiology, Norwegian Institute of Public Health, Didrik Frimann Vestrheim and Martin Steinbakk, Department of Bacteriology and Immunology, Norwegian Institute of Public Health .

VI. OCCURRENCE OF ANTIMICROBIAL RESISTANCE

A. ANIMAL CLINICAL ISOLATES

Marianne Sunde, Madelaine Norström, Jannice Schau Slettemeås, Anne Margrete Urdahl

According to the NORM-VET plan, the clinical isolates included in 2012 were *Staphylococcus schleiferi* from infections in dogs. Sampling, laboratory methods and data processing are described in Appendix 3.

Staphylococcus schleiferi from dog

A total of 53 isolates of *Staphylococcus schleiferi* (of both ssp. *coagulans*/*schleiferi*) from clinical submissions were subjected to NORM-VET. The results are presented in Table 9 and in the text.

TABLE 9. *Staphylococcus schleiferi* from dog (n=53) in 2012.

	Resistance (%)		Distribution (%) of MIC values (mg/L)*													
Substance	%	[95% CI]	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256
Penicillin G**			60.4	30.2	1.9		1.9		1.9		3.8					
Oxacillin	3.8	[0.5-13.0]			15.1	49.1	18.9	13.2			1.9		1.9			
Cefalothin	3.8	[0.5-13.0]		9.4	86.8				1.9			1.9				
Gentamicin	0.0	[0.0-6.7]					96.2	3.8								
Kanamycin	1.9	[0.5-10.1]				30.2	35.8	28.3	1.9		1.9			1.9		
Ciprofloxacin	1.9	[0.5-10.1]		3.8	49.1	37.7	7.5		1.9							
Trimethoprim	1.9	[0.5-10.1]						30.2	58.5	7.5	1.9			1.9		
Clindamycin	0.0	[0.0-6.7]				100										
Erythromycin	1.9	[0.5-10.1]				98.1								1.9		
Chloramphenicol	0.0	[0.0-6.7]						1.9	49.1	49.1						
Tetracycline	1.9	[0.5-10.1]					98.1						1.9			
Fusidic acid	26.4	[15.2-40.3]		50.9	7.5	3.8	11.3	11.3	3.8	11.3						

*Bold vertical lines denote epidemiological cut-off values for resistance. 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.

**Resistance to penicillin G was based on beta-lactamase production. All isolates with a positive beta-lactamase test had a MIC value > 1 mg/L, and all beta-lactamase negative isolates had MIC values ≤ 1 mg/L.

RESULTS AND COMMENTS

The occurrence of resistance among *Staphylococcus schleiferi* was moderate. In total, 67.9% of the isolates were susceptible to all antimicrobial agents included. Altogether, 24.5% of the isolates were resistant to one antimicrobial agent (predominantly fusidic acid), 3.8% to two, 1.9% to three and 1.9% to four antimicrobial agents. One isolate was confirmed to be methicillin resistant (*mecA* detected by PCR). This isolate expressed resistance to oxacillin and cephalotin.

Comparison to previous years is difficult as species determination of *Staphylococcus* sp. (including *Staphylococcus schleiferi*) from dogs has only been performed once before, in 2008. In NORM-VET 2008, eight isolates of *Staphylococcus schleiferi* were tested and resistance to fusidic acid was the only detected resistance.

ESBL and AmpC producing *E. coli* in Norwegian poultry breeder holdings and broiler meat

Acquired resistance to cephalosporins among Gram-negative bacteria has called special attention in recent years. Production of extended-spectrum beta-lactamases (ESBLs) or transferable AmpC are major mechanisms behind such resistance. Among production animals, poultry seems to be associated with the highest prevalence of *Escherichia coli* and *Salmonella* producing ESBLs/AmpC. Like many other countries, broiler production in Norway has a high prevalence of *E. coli* resistant to third generation cephalosporins with 43% [95% CI: 36.7-49.2] positive broiler flocks (NORM-VET 2011). All isolates had a beta-lactam resistance profile corresponding to an AmpC phenotype. PCR and sequencing showed that all isolates contained the bla_{CMY-2} gene. This situation was surprising as there is no selection pressure from cephalosporin usage, and no commercial preparations for livestock containing cephalosporins are available on the market. The poultry production in Norway is, however, dependent on import of breeding animals and these animals are a likely source of resistant bacteria. A similar situation is also reported from other Scandinavian countries.

In NORM-VET 2012, the selective method for ESBL/AmpC positive *E. coli* was used for screening faecal boot swabs from 165 poultry breeder flocks. In addition, screening for ESBL/AmpC positive *E. coli* was performed on 205 broiler meat samples. See Appendix 3 for further description on sampling, microbiological methods and data processing.

TABLE 10. Antimicrobial resistance in isolates of *Escherichia coli* with ESBL bla_{CMY-2} from boot swab samples from poultry breeder holdings (n=12) and from broiler meat samples (n=66) in 2012.

Substance	Sample	Resistance (n)	Distribution (n) of MIC values (mg/L)*															
			0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	Breeder	1								11				1				
	Meat	0							31	35								
Chloramphenicol	Breeder	0								1	10	1						
	Meat	0								4	62							
Florfenicol	Breeder	0									8	4						
	Meat	0									27	39						
Ampicillin	Breeder	12													1	8	3	
	Meat	66												3	11	29	23	
Cefotaxime	Breeder	12									12							
	Meat	66									66							
Ceftazidime	Breeder	12									1	6	5					
	Meat	66								1	4	48	13					
Sulfamethoxazole	Breeder	3										2	5	2				3
	Meat	4										8	50	3	1			4
Trimethoprim	Breeder	2					2	8						2				
	Meat	2					25	39						2				
Gentamicin	Breeder	1						4	5	2	1							
	Meat	0						20	42	4								
Streptomycin	Breeder	1									5	4	2		1			
	Meat	2									16	47	1	1		1		
Kanamycin	Breeder	0										12						
	Meat	0										66						
Ciprofloxacin	Breeder	0		1	11													
	Meat	0		7	58				1									
Nalidixic acid	Breeder	0								11	1							
	Meat	1							2	55	7	1					1	
Colistin	Breeder	0						12										
	Meat	0						63	3									

*Bold vertical lines denote epidemiological cut-off values for resistance. 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

E. coli resistant to third generation cephalosporins were found in 7.3% (12 of 165) [95% CI: 3.8-12.4%] of the poultry breeder flocks, and in 32.2% (66 of 205) [95% CI: 25.9-39.1%] of the broiler meat samples. The detected prevalence in breeder samples was lower than the 43% prevalence found in broiler samples in NORM-VET 2011. The relatively high prevalence found in broiler meat is not surprising and corresponds to the prevalence found in broilers in NORM-VET 2011.

The presence of bacteria resistant to cephalosporins in food production animals and in the food chain is of 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. 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.

Marianne Sunde, Jannice Schau Slettemeås, Madelaine Norström and Anne Margrete Urdahl, Norwegian Veterinary Institute, Oslo.

B. INDICATOR BACTERIA FROM ANIMALS AND FOOD

Marianne Sunde, Madelaine Norström, Jannice Schau Slettemeås, Arve Lund, 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 resistance situation, detect trends and evaluate the effects of interventions.

In NORM-VET, *Escherichia coli* and *Enterococcus* spp. are used as indicator bacteria. In 2012, facal samples from reindeer and boot swabs from poultry breeder flocks, as well as *E. coli* from chicken fillet, 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 2012. Sampling, laboratory methods and data processing are described in Appendix 3.

Escherichia coli from wild reindeer, poultry and chicken fillet

E. coli isolates were obtained from 107 faecal samples from 134 wild reindeer in three different populations (79.9%). Samples from a total of 165 poultry breeder flocks were examined and *E. coli* isolates were obtained from 113 samples (68.5%). From broiler meat, *E. coli* isolates were obtained from 196 out of 205 samples (95.6%). One isolate per positive sample was susceptibility tested. The results are presented in Tables 11-12, in Figure 25, and in the text.

TABLE 11. Antimicrobial resistance in isolates of *Escherichia coli* (n=107) from faecal samples from wild reindeer in 2012.

Substance	Resistance (%) [95% CI*]		Distribution (%) of MIC values (mg/L)*															
			0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	2.8	[0.6-8.0]							47.7	47.7	1.9			2.8				
Chloramphenicol	0.0	[0.0-3.4]								10.3	68.2	21.5						
Florfenicol	0.0	[0.0-3.4]									56.1	43.0	0.9					
Ampicillin	1.9	[0.2-6.6]							16.8	61.7	19.6				0.9		0.9	
Cefotaxime	0.9	[0.0-5.1]		3.7	60.7	30.8	3.7	0.9										
Ceftazidime	3.7	[1.0-9.3]					73.8	22.4	2.8		0.9							
Sulfamethoxazole	2.8	[0.6-8.0]										13.1	39.3	39.3	5.6			2.8
Trimethoprim	0.0	[0.0-3.4]				11.2	48.6	36.4	2.8	0.9								
Gentamicin	2.8	[0.6-8.0]					3.7	76.6	15.0	1.9	2.8							
Streptomycin	6.5	[2.7-13.0]								2.8	64.5	23.4	2.8	2.8		2.8	0.9	
Kanamycin	0.9	[0.0-5.1]										99.1	0.9					
Ciprofloxacin	0.0	[0.0-3.4]	4.7	54.2	41.1													
Nalidixic acid	0.0	[0.0-3.4]							6.5	64.5	29.0							
Colistin	0.0	[0.0-3.4]						93.5	6.5									

*Bold vertical lines denote epidemiological cut-off values for resistance. 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 12. Antimicrobial resistance in isolates of *Escherichia coli* from faecal boot swab samples from poultry breeder flocks (n=113) and from broiler meat (n=196) in 2012.

Substance	Sample	Resistance (%) [95% CI*]		Distribution (%) of MIC values (mg/L)*															
				0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	Breeder	5.3	[2.0-11.2]							42.5	52.2			0.9		3.5		0.9	
	Meat	2.0	[0.6-6.5]							45.9	50.0	1.5			0.5	1.5	0.5		
Chloramphenicol	Breeder	0.0	[0.0-3.2]								4.4	69.9	25.7						
	Meat	0.0	[0.0-1.9]								6.1	77.0	16.8						
Florfenicol	Breeder	0.0	[0.0-3.2]									54.0	43.4	2.7					
	Meat	0.0	[0.0-1.9									58.7	41.3						
Ampicillin	Breeder	15.0	[9.0-23.0]							17.7	48.7	17.7	0.9			0.9	1.8	12.4	
	Meat	5.6	[2.8-9.1]							13.8	65.8	13.8	1.0				0.5	5.1	
Cefotaxime	Breeder	0.9	[0.0-4.8]		14.2	58.4	24.8	1.8				0.9							
	Meat	0.5	[0.0-2.8]		8.2	56.6	32.7	2.0				0.5							
Ceftazidime	Breeder	2.7	[0.6-7.6]					65.5	31.9	1.8				0.9					
	Meat	1.0	[0.1-3.6]					73.0	26.0	0.5			0.5						
Sulfamethoxazole	Breeder	8.8	[4.3-15.7]										23.9	41.6	23.0	2.7			8.8
	Meat	7.7	[4.3-12.3]										12.8	42.3	33.2	4.1			7.7
Trimethoprim	Breeder	4.4	[1.4-10.0]				5.3	38.9	48.7	2.7			0.9		3.5				
	Meat	2.6	[0.8-5.9]				5.1	39.3	48.0	5.1					2.6				
Gentamicin	Breeder	0.0	[0.0-4.8]					2.7	58.4	36.3	2.7								
	Meat	0.0	[0.0-1.9]					2.0	62.2	33.7	2.0								
Streptomycin	Breeder	1.8	[0.2-6.2]								1.8	54.9	36.3	5.3		0.9		0.9	
	Meat	3.1	[1.1-6.5]									56.1	37.8	3.1	2.6		0.5		
Kanamycin	Breeder	0.9	[0.0-4.8]										99.1	0.9					
	Meat	0.0	[0.0-1.9										100.0						
Ciprofloxacin	Breeder	0.9	[0.0-4.8]	4.4	53.1	41.6			0.9										
	Meat	2.6	[0.8-5.9]	2.0	52.0	43.4	0.5		2.0										
Nalidixic acid	Breeder	0.9	[0.0-4.8]							6.2	49.6	40.7	2.7					0.9	
	Meat	2.0	[0.6-6.5]							8.2	59.7	28.1	1.5	0.5	0.5		0.5	1.0	
Colistin	Breeder	0.9	[0.0-4.8]						92.9	6.2			0.9						
	Meat	0.0	[0.0-1.9						90.8	9.2									

*Bold vertical lines denote epidemiological cut-off values for resistance. 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.

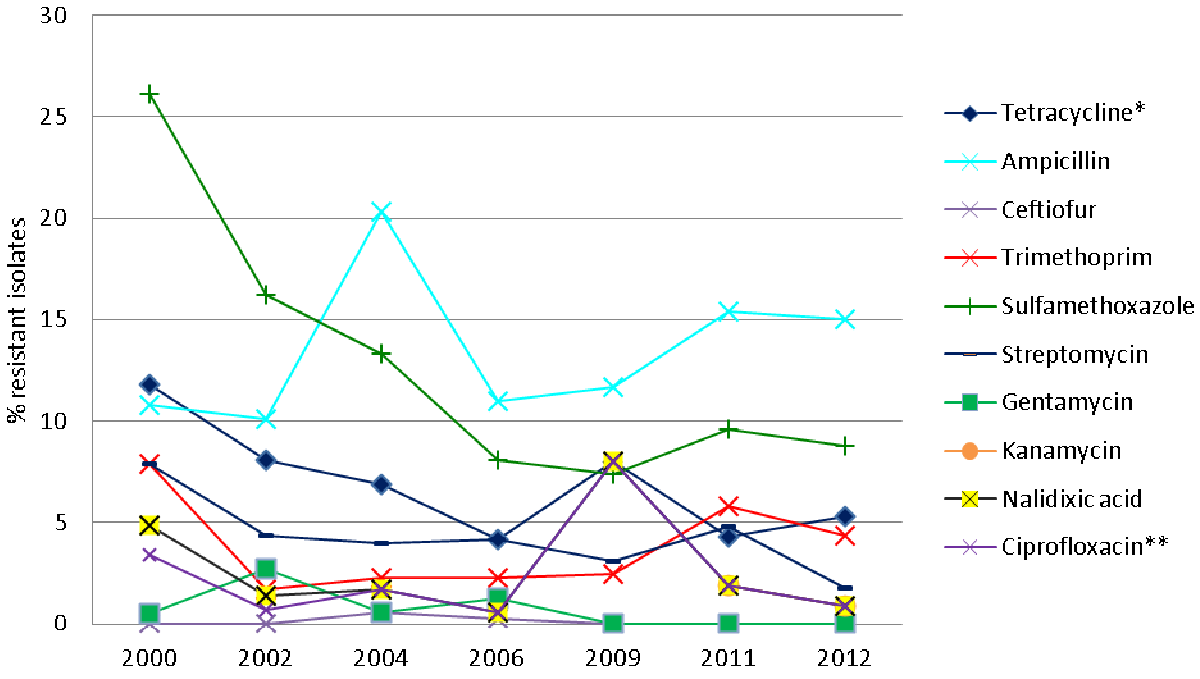


FIGURE 25. Prevalence of resistance to various antimicrobials in *E. coli* from poultry, mainly broilers, isolated in 2000-2012. The cut-off values used in NORM-VET 2012 were applied. *Oxytetracycline in 2002 and 2004. **Enrofloxacin before 2006.

RESULTS AND COMMENTS

WILD REINDEER

The data indicate a low occurrence of resistance among *E. coli* from reindeer faecal samples. In total, 89.7% of the isolates were susceptible to all antimicrobial agents included. Altogether, 4.7% of the isolates were resistant to one antimicrobial agent (predominantly streptomycin or ceftazidime), 0.9% to two (ceftazidime and gentamicin), 2.8% to three and 1.9% to four antimicrobial agents. However, for a few isolates, MIC values for kanamycin, gentamicin and ceftazidime were just above the cut-off value. These were all considered to be part of the normal distribution and should not be regarded as true resistance.

One isolate expressed high MIC values to cephalosporines. Upregulated AmpC production due to chromosomal mutation in the promotor area was found to be the mechanism behind this cephalosporin resistance.

Wild reindeer have been included in NORM-VET only once before, in 2003. Compared to the 2003 results, there has been an increase in susceptibility as 89.7% of the isolates in 2012 were susceptible to all antimicrobial agents tested, compared to 76.2% in 2003 (p=0.03). However, the results from 2003 were based on only 42 isolates and the confidence intervals are slightly overlapping. The highest prevalence of resistance was seen for streptomycin with 6.5% in 2012 compared to 24% in 2003.

Resistance was more common in *E. coli* from wild reindeer with 10.3% of the isolates being resistant to at least one of the antimicrobials included, compared to *E. coli* from other cervids where 2.2% of the isolates were resistant to at least one of the antimicrobials included. However, these other cevids were tested in NORM-VET 2002 and the situation may not be the same today.

POULTRY BREEDER

The data indicate a moderate occurrence of resistance among *E. coli* from poultry breeder flocks. In total, 74.3% of the isolates were susceptible to all antimicrobial agents included. Altogether, 16.8% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin), 5.3% to two (mainly ampicillin and sulfamethoxazole), 0.9% to three and 2.7% to four antimicrobial agents. Resistance to ampicillin was the most frequently identified resistance determinant, followed by resistance to sulfamethoxazole, tetracycline and trimethoprim. The resistance to sulfamethoxazole seems to stabilise just below 10% after the decrease observed in the years 2000-2006 (Figure 25).

Resistance to the fluoroquinolone ciprofloxacin and to the quinolone nalidixic acid was identified in 0.9% of the isolates compared to 1.9% of the isolates from broiler in 2011 (Figure 25). However, caution should be made comparing with data from previous years as these have mainly been from broilers, while this year´s samples were from poultry breeders. Also, the observed decrease was nonsignificant.

By using a non-selective method, one isolate was resistant to third generation cephalosporines and the $bla_{\text{CMY-2}}$ gene was identified. A selective method was used to detect ESBL/AmpC positive *E. coli* in the same material and with that method, 12 samples were positive (see separate presentation on page 36).

BROILER MEAT

The data indicate a moderate occurrence of resistance among *E. coli* from broiler meat samples. In total, 82.6% of the isolates were susceptible to all antimicrobial agents included. Altogether, 9.7% of the isolates were resistant to one antimicrobial agent (predominantly sulfamethoxazole), 6.1% to two (mainly sulfamethoxazole and ampicillin), 0.5% to three and 1.0% to four antimicrobial agents.

In contrast to the results from poultry breeders this year and previously in broilers, resistance to sulfamethoxazole was the most frequently identified resistance determinant in *E. coli* from broiler meat samples, followed by resistance to ampicillin and streptomycin. It is also in contrast to the results from NORM-VET 2006, the last time samples from broiler meat were included. At that time resistance to ampicillin and streptomycin were the most frequently identified resistance determinants, both with 7.6% resistant isolates, although resistance to sulfamethoxazole was close by with 6.7% resistant isolates.

By using a non-selective method, one isolate was resistant to third generation cephalosporines and the $bla_{\text{CMY-2}}$ gene was identified. A selective method was used to detect ESBL/AmpC positive *E. coli* in the same material and with that method, 66 samples were positive (see separate presentation on page 36).

Methicillin resistant *Staphylococcus aureus* (MRSA)

Resistance to critically important antimicrobials represents a significant public health problem. In 2012, screening for methicillin resistant *Staphylococcus aureus* (MRSA) was performed on samples from swine. Sampling, laboratory methods and data processing are described in Appendix 3. The data are presented in the text.

MRSA from bovine mastitis

A total of 117 isolates of *Staphylococcus aureus* from clinical mastitis in cattle were susceptibility tested for methicillin using a disk diffusion method (cefoxitin) method. No methicillin resistant *S. aureus* (MRSA) was detected. This is in accordance with previous results in NORM-VET 2010 where no MRSA was detected among 195 *S. aureus* isolates from bovine mastitis.

MRSA from swine

Samples from a total of 175 swine herds were screened for the presence of MRSA. One positive sample was identified, giving a prevalence of MRSA in Norwegian swine holdings at 0.6% [95% CI: 0.0-3.1]. The isolate belonged to clonal complex 398 (CC398), *spa*-type t034. This is the first detection of MRSA CC398 in a Norwegian swine holding. In two previous surveys of MRSA in Norwegian swine herds conducted in 2008, no isolates of livestock-associated MRSA were detected. However, in 2011, a total of 1,033 slaughter pigs from 207 different farms (five animals per farm, but analysed as one pooled sample) were sampled by nasal swabs at eleven different slaughterhouses. MRSA CC398, *spa*-type t034 was detected in six pooled samples (3%), all originating from the same slaughterhouse. An attempt to identify positive swine holdings was unsuccessfull. Follow-up sampling at the slaughterhouse shortly after showed that MRSA CC398, *spa*-type t034 was present in the environment and could have contaminated the slaughtered pigs that were included in the screening, giving an overestimation of the MRSA prevalence in swine herds in Norway.

Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli*

Acquired resistance to cephalosporins among Gram-negative bacteria has become almost epidemic in recent years. Production of extended-spectrum beta-lactamases or transferable AmpC are major mechanisms behind such resistance. In 2012, screening for ESBL producing *E. coli* was performed on samples from swine, wild reindeer, poultry breeders and broiler meat. Sampling, laboratory methods and data processing are described in Appendix 3. The data are presented in the text and in a separate presentation on page 36.

ESBL producing *Escherichia coli* from swine

A total of 169 faecal boot swabs from swine holdings were screened for the presence of ESBL producing *E. coli* using a selective method. ESBL producing *E. coli* was not detected from any of the 169 holdings indicating a prevalence below 2.1%. In a previous survey in 2011 *E. coli* resistant to third generation cephalosporins was found in one of 194 (0.5%) swine holdings. Further investigations showed that the isolate contained a $bla_{\text{TEM-52}}$ gene. This was the first detection of an ESBL positive *E. coli* from swine in Norway.

ESBL producing *Escherichia coli* from wild reindeer

A total of 134 faecal samples from three wild reindeer populations were screened for the presence of ESBL producing *E. coli* using a selective method. ESBL producing *E. coli* was not detected in any of the 134 samples.

C. ZOONOTIC AND NON-ZOONOTIC ENTEROPATHOGENIC BACTERIA

Astrid Louise Wester, Madelaine Norström, Marianne Sunde, Anne Margrete Urdahl, Ulf R. Dahle

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 good 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. Additionally, 35 isolates of *Salmonella* spp. from reptiles collected during the period 2010-2012 were included. The data are presented in Tables 13-14 and in the text.

TABLE 13. Antimicrobial resistance in *Salmonella* spp. (n=25) from animals (cattle=1, dog=16, cat=4, horse=4); *S.* Typhimurium (n=7) and other *Salmonella* spp. (n=18) in 2012.

	Resistance (n)	Distribution (n) of MIC values (mg/L)*															
Substance		0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	4							2	19						4		
Chloramphenicol	0								1	18	6						
Florfenicol	0									16	9						
Ampicillin	3						2	19	1						3		
Cefotaxime	0			10		14	1										
Sulfamethoxazole	4												1	11	9		4
Trimethoprim	1					11	13					1					
Gentamicin	0					1	22	2									
Streptomycin	4								1		8	12	1			3	
Kanamycin	0							1	22	2							
Ciprofloxacin	2		3		20			1									
Nalidixic acid	2									22	1					1	1

*Bold vertical lines denote epidemiological cut-off values for resistance. 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.

TABLE 14. Antimicrobial resistance in *Salmonella* spp. (n=35) of diagnostic submissions from reptiles during the years 2010-2012.

	Resistance (n)	Distribution (n) of MIC values (mg/L)*															
Substance		0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	0						1	24	9	1							
Chloramphenicol	1								4	18	12		1				
Florfenicol	1								2	22	10		1				
Ampicillin	0						4	29	2								
Cefotaxime	0			16	15	3	1										
Sulfamethoxazole	1											5	6	17	6		1
Trimethoprim	0					24	9	2									
Gentamicin	0					1	21	12	1								
Streptomycin	8								1	9	8	9	6	2			
Kanamycin	0							3	26	6							
Ciprofloxacin	10		19	6		5	3	2									
Nalidixic acid	10								2	23			4			3	3

*Bold vertical lines denote epidemiological cut-off values for resistance. 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

ANIMALS

In 2012, a total of 25 isolates of *Salmonella* spp. from animals were susceptibility tested. The seven isolates of *S.* Typhimurium included three each from cats and horses and one from a dog. The remaining *Salmonella* isolates belonged to several different serovars and originated from dogs (n=15), and one each from a cattle, horse and a cat. Two isolates showed resistance to fluoroquinolones.

The emerging multi-resistant *S. enterica* subsp. *enterica* serovar 4,[5],12:i- was isolated from four different animal species including cattle, horse, and dog. Three presented a multi-resistant profile against tetracyclines, ampicillin, streptomycin and sulfamethoxazole, whereas the last isolate was resistant to streptomycin, trimethoprim, sulfamethoxazole, nalidixic acid and ciprofloxacin.

REPTILES

In total, ten isolates of *Salmonella* spp. from reptiles were resistant to fluoroquinolones, eight to streptomycin and one to chloramphenicol and florfenicol and sulfamethoxazole. Only one of the isolates was multi-resistant; to fluoroquinolones, chloramphenicol and florfenicol.

Salmonella from human clinical specimens

In 2012 the National Reference Laboratory (NRL) for Enteropathogenic Bacteria at the Norwegian Institute of Public Health (NIPH) performed antimicrobial resistance (AMR) testing on a total of 1,393 unique *Salmonella* isolates from human infections. As indicated in Table 15, 18.3% was reported as acquired in Norway, 75.7% was acquired abroad, whereas the place of origin was unknown for 6.0%.

BLOOD CULTURE ISOLATES

A total of 60 strains were isolated from blood culture; 3 strains of *S.* Typhimurium and its monophasic variant, 17 *S.* Enteritidis, 26 *S.* species, 9 *S.* Typhi and 5 *S.* Paratyphi (Figure 26). The 26 *S.* species isolates represented 20 different serovars, *S.* Heidelberg was the most frequent (n=4). Among these isolates, 21 were susceptible to all antimicrobials tested. Five isolates (all acquired abroad) were resistant to two, three, or more, drugs (Figure 27).

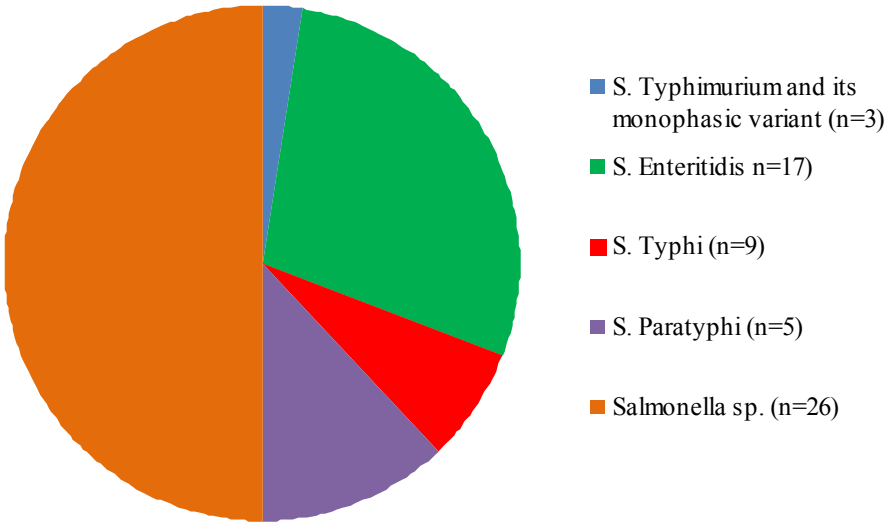


FIGURE 26. Distribution of *Salmonella* serovars isolated from blood cultures (n=60) in 2012.

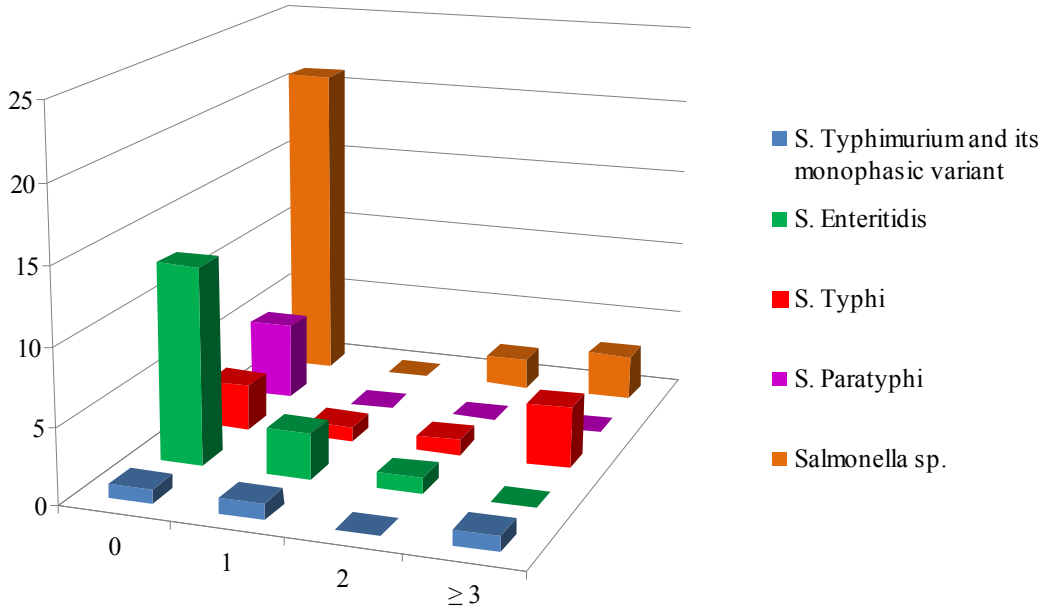


FIGURE 27. *Salmonella* isolated in blood culture 2012 (n=60) with the number of isolates resistant to none, one, two, or three or more antimicrobials.

ALL ISOLATES OF SALMONELLA

The numbers of *S.* Typhi and *S.* Paratyphi isolates remain low. For 2012 the total numbers of isolates were 15 and 8, respectively. As many as 40% of the *S.* Typhi isolates were MDR (resistant to ≥ 3 antimicrobial groups) (i.e resistance to ampicillin 40%, ciprofloxacin 13.3%, trimethoprim-sulfamethoxazole 46.7% and chloramphenicol 46.7%), whereas none of the *S.* Paratyphi strains were MDR. A total of 18 strains carried extended spectrum beta-lactamases (ESBL). Eleven of these were *S.* Typhimurium or its monophasic variant (7 $ESBL_A$ and 4 $ESBL_M$), 3 were *S.* Enteritidis (2 $ESBL_A$ and 1 $ESBL_M$), 1 was *S.* Grumpensis with $ESBL_A$, 1 was *S.* Krefelt, and 1 was *S.* Albany. Both of these latter isolates carried $ESBL_M$.

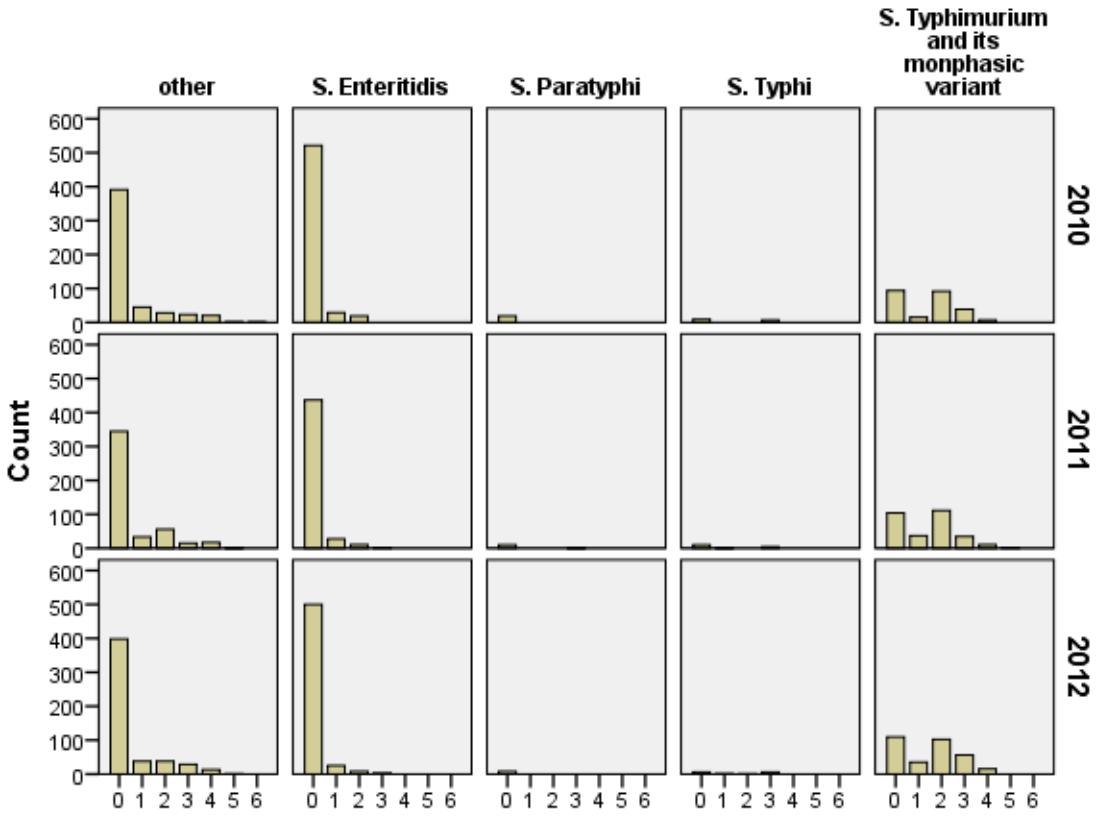


FIGURE 28. Distribution of number of antimicrobials that *Salmonella* isolates were resistant to; by species and by year.

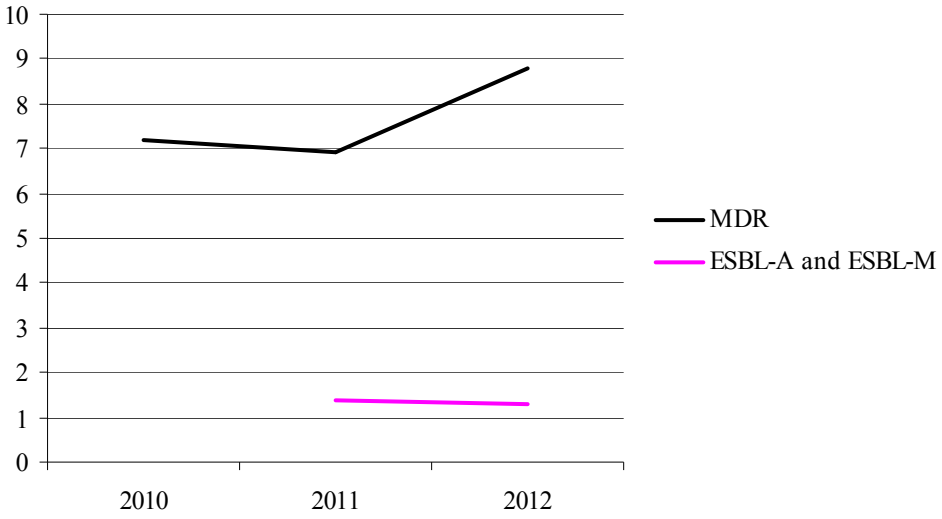


FIGURE 29. Percentage of all *Salmonella* isolates in 2010-2012 that were resistant to ≥ 3 groups of antimicrobials (MDR) and carrying ESBL of either $ESBL_A$ or $ESBL_M$ type. None of the differences shown are statistically significant.

TABLE 15. Distribution of human isolates of *Salmonella* serovars (n=1,394) in 2012 according to place of acquisition.

	Place of acquistion		
	Norway	Abroad	Unknown
S. Typhimurium including *S. enterica* serovar 4,[5],12:i- (n=317)	107	192	18
S. Enteritidis (n=536)	45	465	26
S. Typhi (n=15)	0	13	2
S. Paratyphi (n=5)	1	6	1
Other *Salmonella* (n=517)	102	378	37
Total (n=1,393)	**255 (18.3%)**	**1,054 (75.7%)**	**84 (6.0%)**

The dominating serovars were *S.* Typhimurium (n=172) and its monphasic variant (n=145), with 317 (22.8%) of the isolates, and *S.* Enteritidis with 536 (38.5%) of the isolates. Of these, 107 (33.7%), and 44 (8.3%) were reported as infected in Norway, respectively. From 2010 on, phage typing has not been performed, and thus results on *S.* Typhimurium definite phage type (DT) 104 are not available. DT 104 is of special concern, however, because of carriage of a specific pattern of MDR, namely resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, thus the term ACSSuT resistance profile. To mirror, to some extent, the development regarding *S.* Typhimurium DT 104, numbers are given for isolates showing the ACSSuT resistance profile.

The results of the AMR for 2012 strains are presented in Tables 16-19, in Figures 30-33, and in the text. Sampling, laboratory methods, and data handling are described in Appendix 4.

TABLE 16. Human isolates of domestically acquired isolates of *Salmonella* Typhimurium-group (n=107) during 2012, including domestically acquired *S. enterica* serovar 4,[5],12:i:- (n=39), and isolates of either of the two serovars with the ACSSuT resistance profile (n=10). 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 [1]	≤ 8	> 8	49.5	-	50.5
Chloramphenicol [1]	≤ 8	> 8	86.9	-	13.1
Tetracycline [2]	≥ 13	< 13	52.3	-	47.7
Nalidixic acid [2]	≥ 19	< 19	89.7	-	10.3
Ciprofloxacin [1]	≤ 0.5	> 1	100	-	0
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	97.2	0.0	2.8

[1] EUCAST clinical breakpoint for *Enterobacteriaceae* 2013, version 3.1. [2] Epidemiological cut-off values based on zone distribution evaluations. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

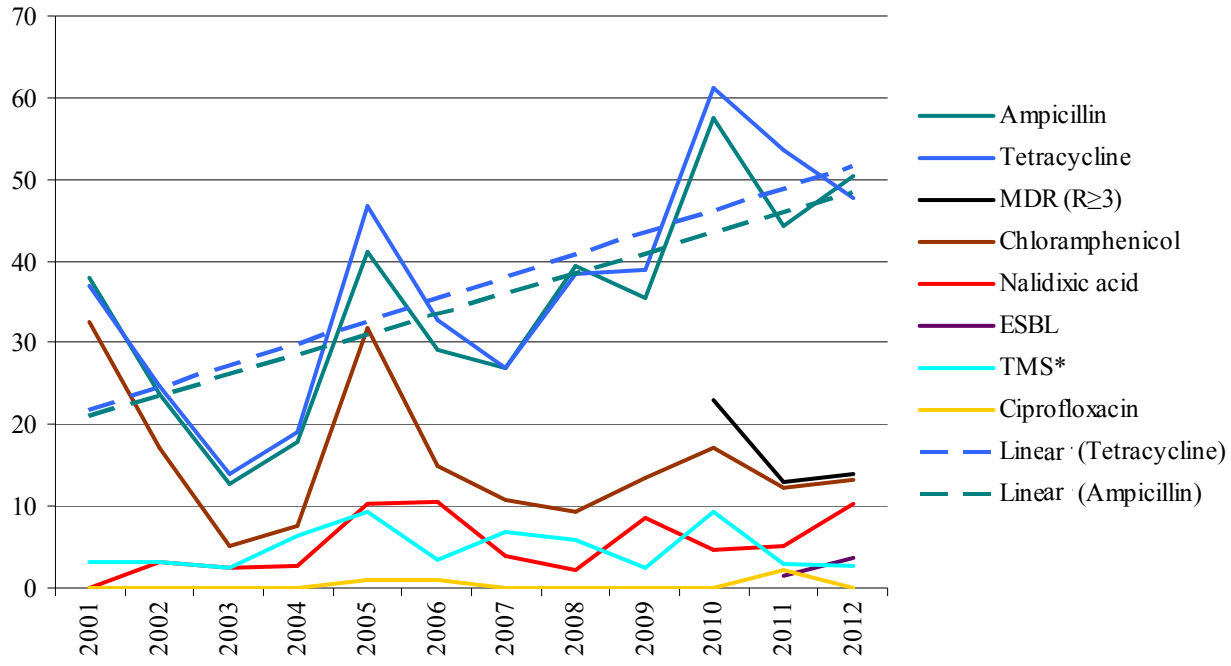


FIGURE 30. Percentage of resistance to various antimicrobial agents in *Salmonella* Typhimurium-group (including *S. enterica* serovar 4,[5],12:i:- and isolates with the ACSSuT resistance profile) from humans infected in Norway 2001-2012; with linear trend lines generated in Excel when relevant. *TMS=Trimethoprim-sulfamethoxazole.

TABLE 17. Human isolates of *Salmonella* Typhimurium-group acquired abroad during 2012 (n= 192), including *S. enterica* serovar 4,[5],12:i:- (n= 97) and isolates of either of the two serovars with the ACSSuT resistance profile (n= 48). 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 [1]	≤ 8	> 8	33.3	-	66.7
Chloramphenicol [1]	≤ 8	> 8	72.9	-	27.1
Tetracycline [2]	≥ 13	< 13	34.9	-	65.1
Nalidixic acid [2]	≥ 19	< 19	74.0	-	26.0
Ciprofloxacin [1]	≤ 0.5	> 1	100	-	0
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	95.8	0.0	4.2

[1] EUCAST clinical breakpoint for *Enterobacteriaceae* 2013. [2] Epidemiological cut-off values based on zone-distribution evaluations. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

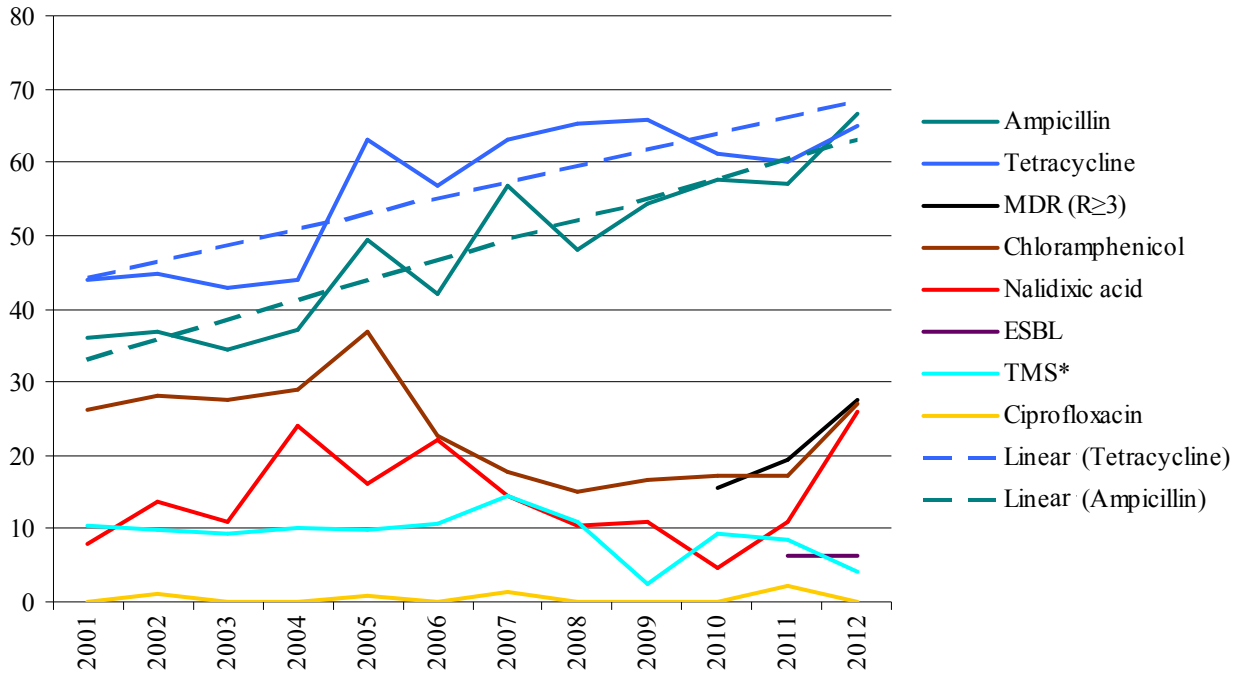


FIGURE 31. Percentage of resistance to various antimicrobial agents in human *Salmonella* Typhimurium-group (including *S. enterica* serovar 4,[5],12:i:- and isolates of either of the two serovars with the ACSSuT resistance profile) from humans infected outside Norway 2001-2012, with linear trend lines generated in Excel when relevant. *TMS=Trimethoprim-sulfamethoxazole.

TABLE 18. Human isolates of *Salmonella* Enteritidis (n=536 [#]), acquired during 2012, 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 [1]	≤ 8	> 8	94.4	-	5.6
Chloramphenicol [1]	≤ 8	> 8	99.5	-	0.5
Tetracycline [2]	≥ 13	< 13	97.8	-	2.2
Nalidixic acid [2]	≥ 19	< 19	77.4	-	22.6
Ciprofloxacin [1]	≤ 0.5	> 1	100.0	-	0.0
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	99.4	0.0	0.6

Place of infection; Norway (n=45), abroad (n=465), unknown (n=26). [1] EUCAST clinical breakpoint for *Enterobacteriaceae* 2013, version 3.1. [2] Epidemiological cut-off values based on zone-distribution evaluations. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

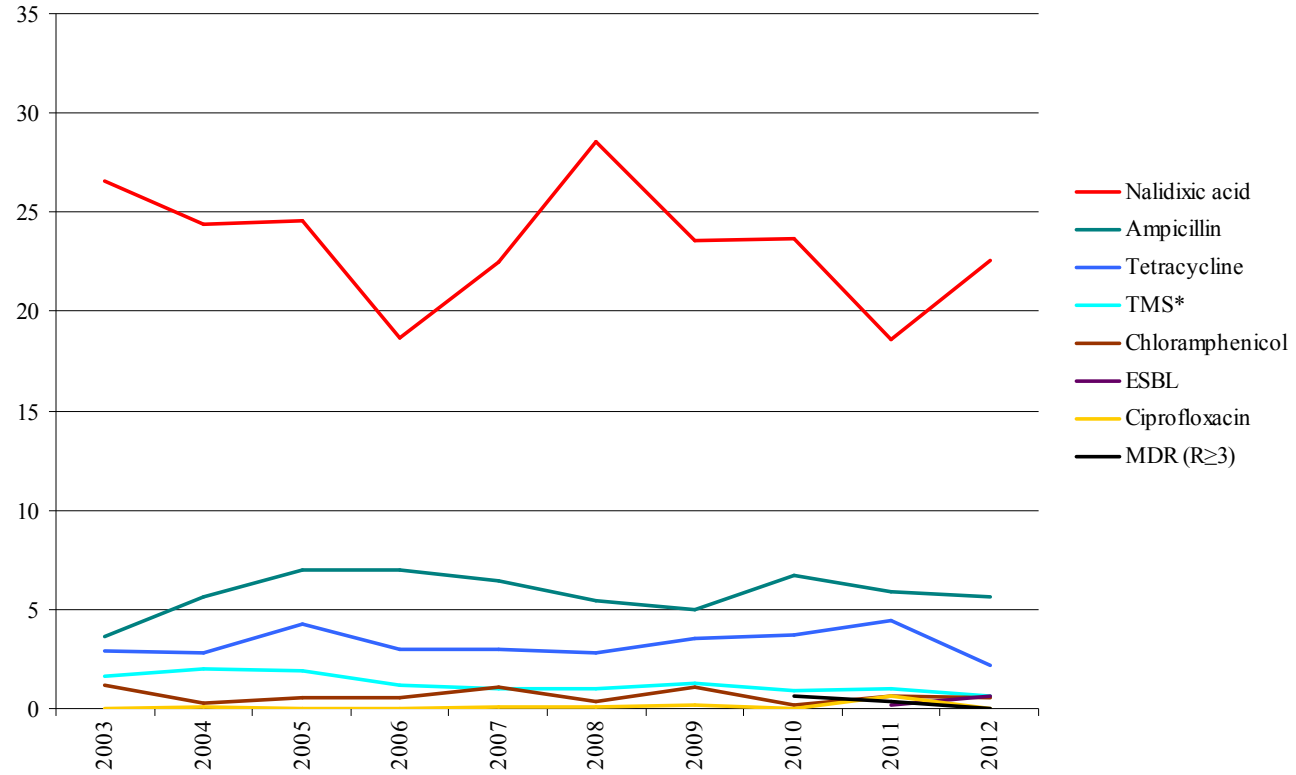


FIGURE 32. Percentage of resistance to various antimicrobial agents in *Salmonella* Enteritidis from humans in 2003-2012. *TMS=Trimethoprim-sulfamethoxazole.

TABLE 19. Human isolates of *Salmonella* spp. (including *S.* Paratyphi B variant Java, but excluding *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi) (n=517 [#]), acquired during 2012, 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 [1]	≤ 8	> 8	85.5	-	14.5
Chloramphenicol [1]	≤ 8	> 8	96.1	-	3.9
Tetracycline [2]	≥ 13	< 13	80.9	-	19.1
Nalidixic acid [2]	≥ 19	< 19	82.0	-	18.0
Ciprofloxacin [2]	≤ 0.5	>1	98.6	-	1.4
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	90.3	0.0	9.7

Place of infection; Norway (n=102), abroad (n=378), unknown (n=37). [1] EUCAST clinical breakpoint for *Enterobacteriaceae* 2013, version 3.1 . [2] Epidemiological cut-off values based on zone-distribution evaluations. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

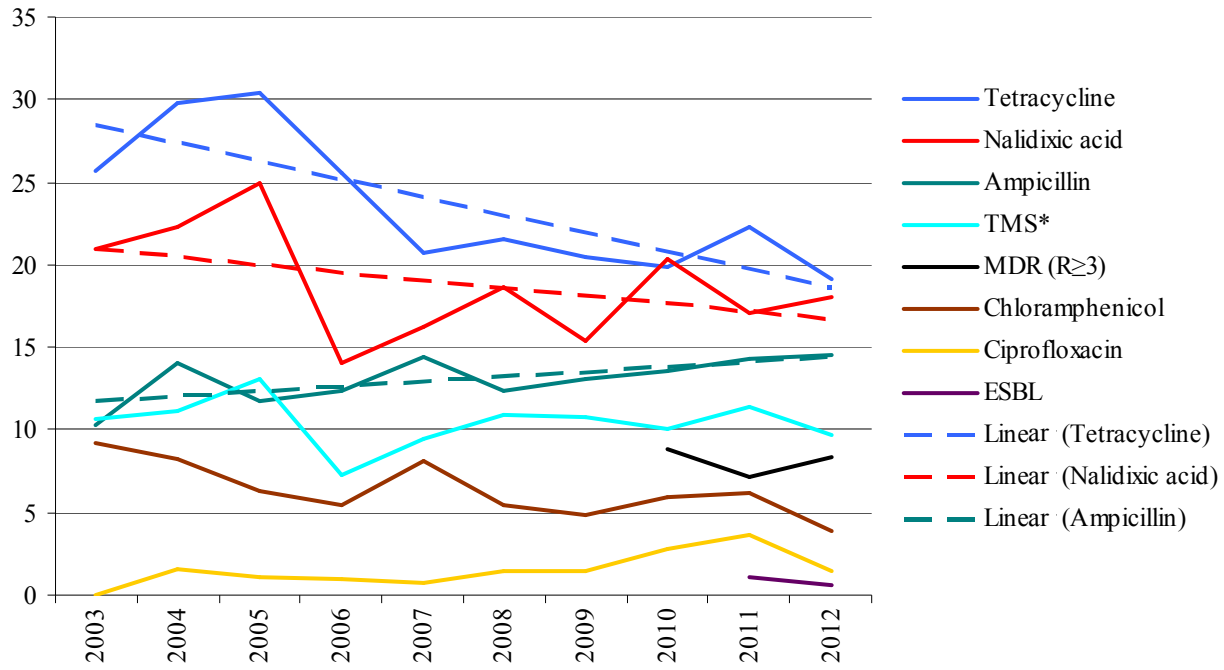


FIGURE 33. Percentage of resistance to various antimicrobial agents in *Salmonella* spp. (including *S.* Paratyphi B variant Java; but excluding *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi) from humans in 2003-2012, with linear trend lines generated in Excel when relevant. *TMS=Trimethoprim-sulfamethoxazole.

RESULTS AND COMMENTS

The overall tendencies were that most resistance was found in the *Salmonella* Typhimurium-group, and that the rate of drug resistant strains assigned to 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 approaching 50% for domestically acquired isolates and 70% in those acquired abroad. Several countries report an increase in multiresistant *S. enterica* serovar 4,[5],12:i-. In Norway, the number of isolates assigned to this serovar has increased steadily over the last years; with 59, 43, 87, 127 and 145 isolates in 2008, 2009, 2010, 2011 and 2012, respectively. The corresponding proportions of this serovar within the *Salmonella* Typhimurium-group were around 20% in 2008 and 2009, 35% in 2010, 42% in 2011, and 46% in 2012. However, MDR was less frequent among *S. enterica* serovar 4,[5],12:i- (8.3%) than in *S.* Typhimurium (34.3%). The majority of *S.* Enteritidis isolates were acquired abroad (465 compared to 45 acquired in Norway). The proportions of *S.* Enteritidis isolates resistant to the given antimicrobial agents were 23% for nalidixic acid, ~5% for ampicillin, and less than 5% for the other drugs (Figure 32). There is still a very low level of resistance to ciprofloxacin. The prevalence of antimicrobial resistance in *S.* Enteritidis was stable.

With regard to *Salmonella* spp. (including 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 stable (Table 19 and Figure 33). Resistance to nalidixic acid was most common, followed by resistance to tetracycline and ampicillin. Resistance to ciprofloxacin was observed in 1.5% of the isolates, whereas 14.5% were resistant to ampicillin.

CAMPYLOBACTER SPP.

Campylobacter spp. from human cases

Of the 2,933 cases of human campylobacteriosis registered in Norway in 2012, 48% were reported as acquired abroad. Based on epidemiological data on 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. Thus outbreaks with less clear epidemiological links may very well have been over-looked, and the AMR results presented may therefore be underestimated or overestimated.

Susceptibility testing was performed on a total of 250 isolates of *C. jejuni* (86 from patients infected in Norway, 142 from patients infected abroad and 22 from patients where the origin of infection was unknown), 17 *C. coli* isolates, one *C. lari* isolate and one isolate without species annotation. EUCAST clinical breakpoints and epidemiological cut-off values have been used. The results for *C. jejuni* are presented in Table 20-21, Figures 34-35, and in the text.

TABLE 20. *Campylobacter jejuni* isolates from patients infected in Norway (n=86). Distribution (%) of antimicrobial susceptibility categories.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline [1]	≤ 2	> 2	86.0	-	14.0
Erythromycin [1]	≤ 4	> 4	100.0	-	0.0
Gentamicin [2]	≤ 2	> 2	96.5	-	3.5
Nalidixic acid [2]	≤ 16	> 16	80.2	-	19.8
Ciprofloxacin [1]	≤ 0.5	> 0.5	86.0	-	14.0

[1] Clinical breakpoint according to EUCAST 2012 version 3.1. [2] Epidemiological cut-off values according to EUCAST web-pages by June 2013.

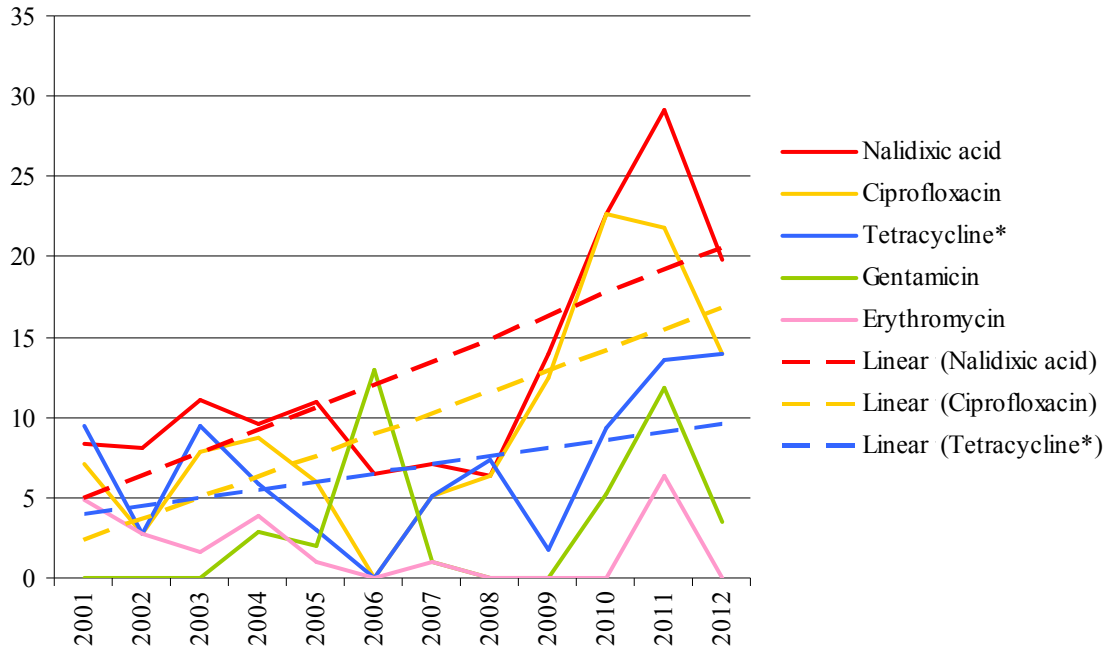


FIGURE 34. Prevalence of resistance in *Campylobacter jejuni* isolated from humans infected in Norway 2001-2012 to various antimicrobials. *Doxycycline before 2006.

TABLE 21. *Campylobacter jejuni* isolates from patients infected outside Norway (n=142). Distribution (%) of antimicrobial susceptibility categories.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline[1]	≤ 2	> 2	44.4	-	55.6
Erythromycin[1]	≤ 4	> 4	98.6	-	1.4
Gentamicin[2]	≤ 2	> 2	100.0	-	0.0
Nalidixic acid[2]	≤ 16	> 16	19.7		80.3
Ciprofloxacin[1]	≤ 0.5	> 0.5	24.6	-	75.4

[1] Clinical breakpoint according to EUCAST 2012 version 3.1. [2] Epidemiological cut-off values according to EUCAST web-pages by June 2013.

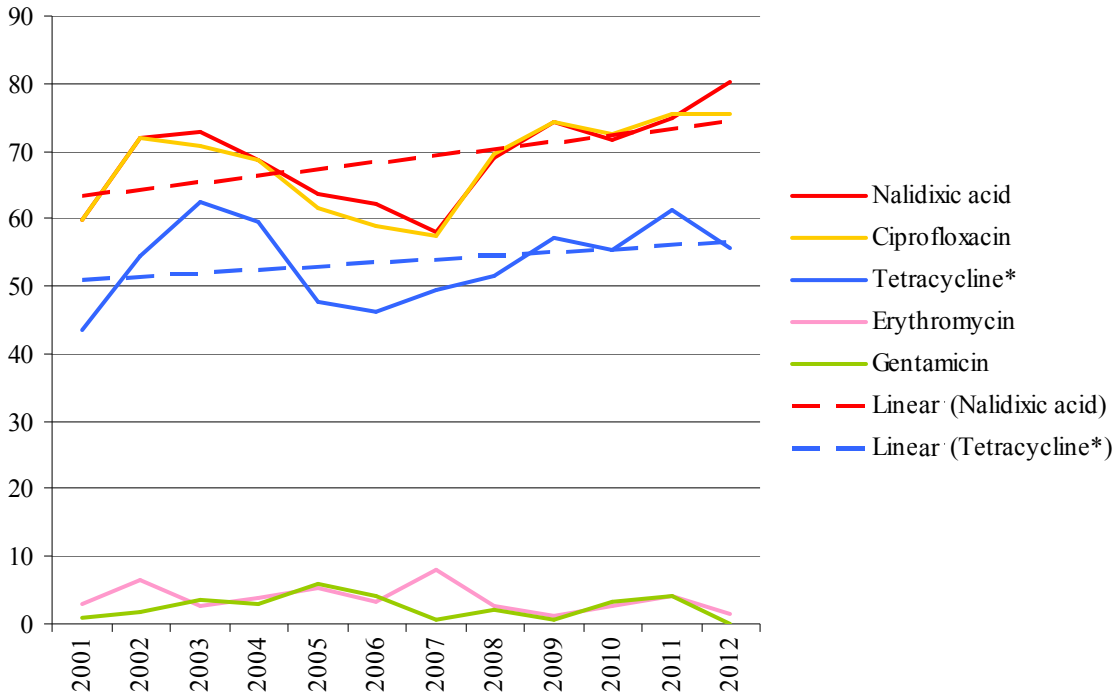


FIGURE 35. Prevalence of resistance to various antimicrobial agents in *Campylobacter jejuni* from humans infected outside Norway 2001-2012. *Doxycycline before 2006.

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 19% of isolates from infections acquired abroad were susceptible to all antimicrobial agents tested compared to 74.2% of the isolates from patients infected in Norway. The main differences between the two groups were seen for quinolones (ciprofloxacin and nalidixic acid) with 75.5% resistance in isolates acquired abroad versus 30.0% resistance in isolates acquired in Norway ($p<0.001$), and tetracycline with 61.2% resistance in isolates acquired abroad versus 13.6% resistance for those acquired in Norway ($p<0.001$).
For strains acquired abroad, there is no significant change in resistance rates to any antimicrobials tested. For domestically acquired strains, however, changes did reach statistical significance for erythromycin ($p=0.01$) and gentamicin ($p<0.001$), and in the proportion of MDR (from 0% in 2010 to 5.5% in 2011, $p=0.005$). On the other hand, even when comparing 2011 numbers to the numbers in 2008, the apparent increase in tetracycline resistance shown in Figure 34 did not reach statistical significance. Yet there are highly significant changes for both ciprofloxacin ($p=0.003$) and nalidixin ($p<0.001$) when comparing to 2008 data.

Thirteen *C. coli* isolates were acquired abroad, and two were acquired in Norway. Nine of the thirteen isolates acquired abroad were resistant to at least one of the antimicrobial agents tested. One *C. lari* and one *C. upsaliensis* isolates were acquired in Norway and were sensitive to the antibiotics tested.

Yersinia enterocolitica from human cases

A total of 42 strains of pathogenic *Yersinia enterocolitica* were analysed in 2012. Thirty-seven belonged to serogroup 3 (19 of these were acquired in Norway, 11 abroad and 7 with unknown place of acquisition). Three strains belonged to serogroup 9, of which one was acquired in Norway and two strains were acquired abroad. One single strain belonged to serogroup O:5.27 biotype 2 and was domestically acquired. All *Y. enterocolitica* isolates were tested for drug susceptibility. The results are presented in Table 22 and Figures 36-37.

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 reported on.

TABLE 22. *Yersinia enterocolitica* serogroups O:3, O:9 and O:5,27 isolates from human cases (n=42). 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 (mm) *		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≥ 14	< 14	4.8	-	95.2
Chloramphenicol	≥ 17	< 17	92.9	-	7.1
Tetracycline	≥ 14	< 14	97.6	-	2.4
Nalidixic acid	≥ 19	< 19	92.9	-	7.1
Ciprofloxacin	≥ 22	< 22	100.0	-	0.0
Trimethoprim-sulfamethoxazole **	≥ 16	< 16	92.9	-	7.1

* As of June 2013 EUCAST recommendations for clinical or epidemiological cut-off values for *Yersinia enterocolitica* are unavailable. The cut-off values used are therefore based on evaluations of the distribution of zone diameters for each antimicrobial. ** Cut-off values for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

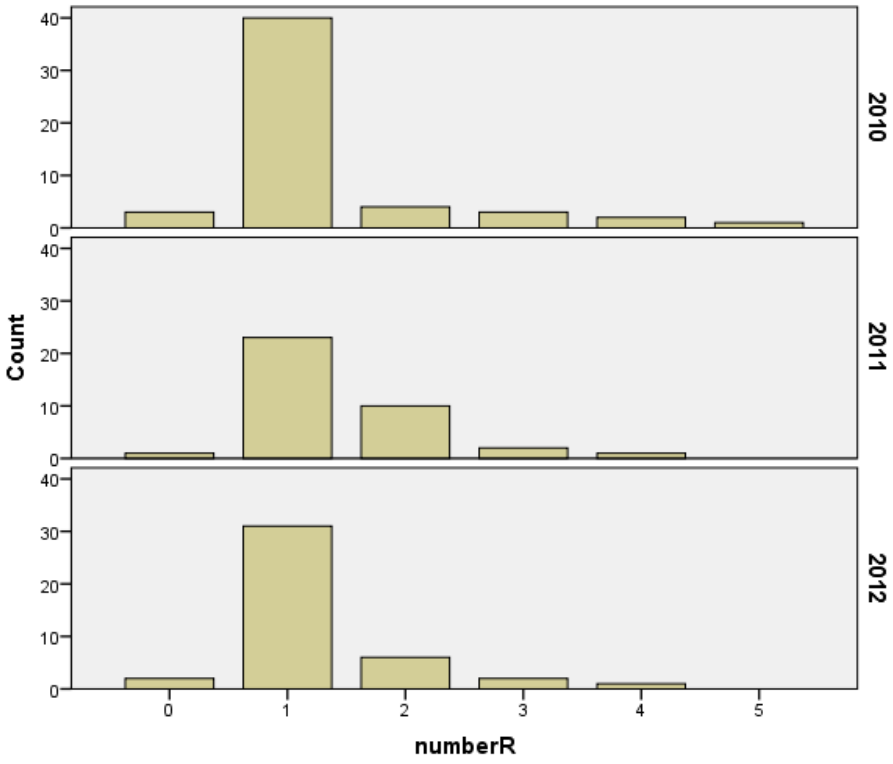


FIGURE 36. Distribution of number of antimicrobials that *Y. enterocolitica* isolates were resistant to; by year.

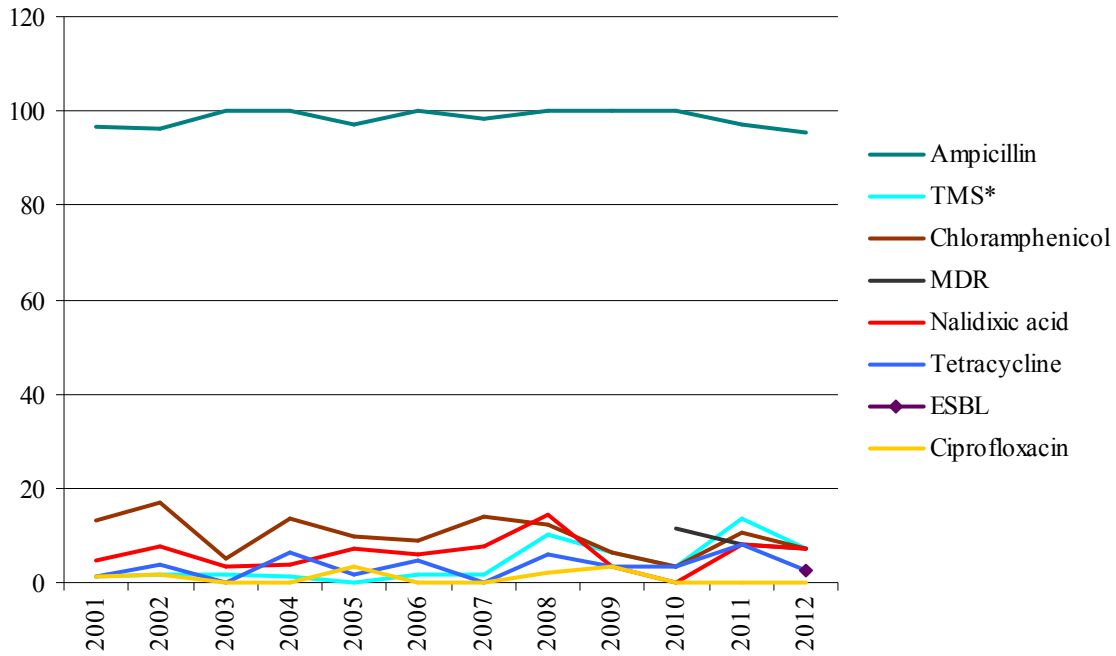


FIGURE 37. Prevalence of resistance to various antimicrobials in *Yersinia enterocolitica* from humans in Norway 2001-2012. *TMS=Trimethoprim-sulfamethoxazole.

RESULTS AND COMMENTS

Almost all isolates of pathogenic *Yersinia enterocolitica* expressed intrinsic resistance to ampicillin. In accordance with the data on zone diameters published on 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 2001-2012. However, when EUCAST establishes clinical breakpoints or epidemiological cut-off values for *Y. enterocolitica*, it may be possible to judge with more statistical weight on this matter.

One *Y. enterocolitica* serogroup O:9 carried AmpC-type of ESBL.

Shigella spp. from human cases

In 2012, ten (12.8%) of the 78 unique isolates *of Shigella* were domestically acquired. 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 78 *Shigella* isolates that were tested for drug susceptibility was as follows: *S. sonnei* 44 (56.4%); *S. flexneri* 30 (38.5%); *S. boydii* 3 (3.8%); and *S. dysenteriae* 2 (1.3%) (Figure 38). Multi-resistance was defined as resistance to three or more antimicrobial categories.

The results for *S. sonnei* and *S. flexneri* are presented in Table 23 and Figure 39, and in Table 24 and Figure 40, respectively.

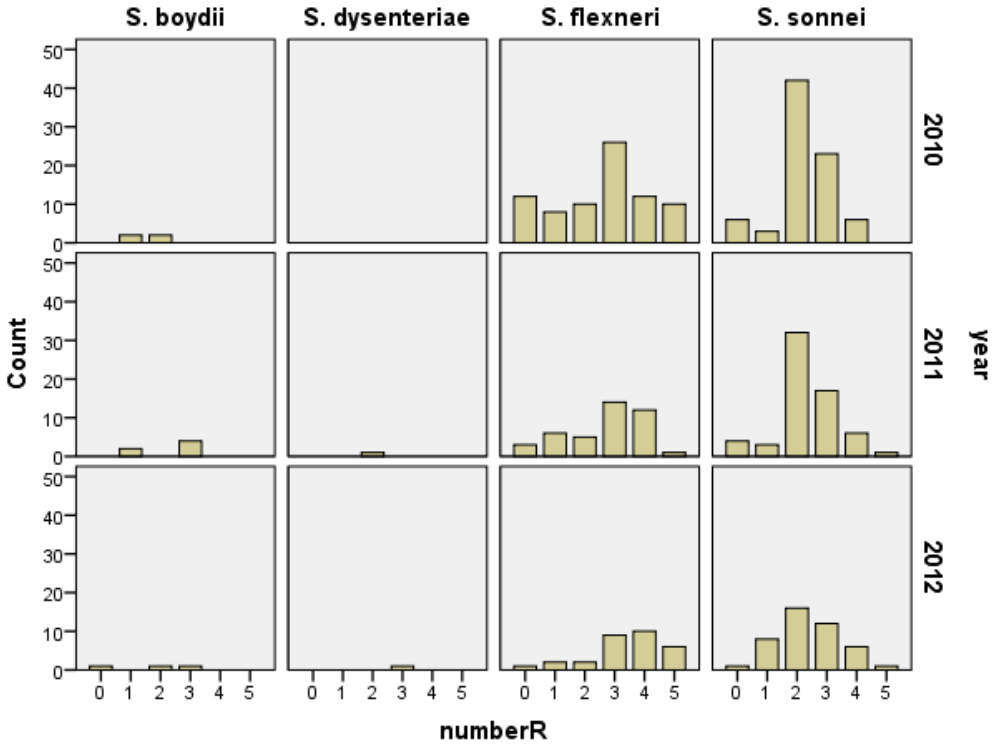


FIGURE 38. Distribution of number of antimirobials that *Shigella* isolates were resistant to; by species and by year.

TABLE 23. *Shigella sonnei* isolates from human cases (n=44). 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 [1]	≤ 8	> 8	79.5	-	20.5
Chloramphenicol [1]	≤ 8	> 8	93.2	-	6.8
Tetracycline [2]	≥ 14	< 14	31.8	-	68.2
Nalidixic acid [2]	≥ 19	< 19	52.3	-	47.7
Ciprofloxacin [1]	≤ 0.5	> 1	86.4	0.0	13.6
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	9.1	4.5	86.4

[1] EUCAST breakpoint for *Enterobacteriaceae* 2013, version 3.1. [2] Epidemiological cut-off values based on zone-distribution evaluations. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

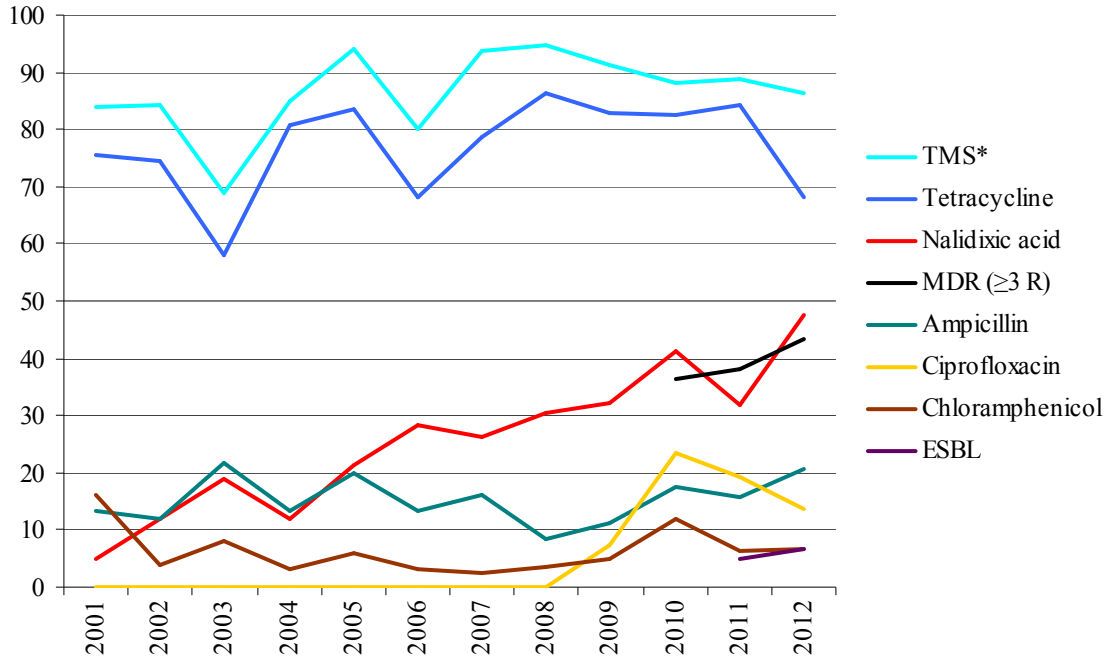


FIGURE 39. Prevalence of resistance to various antimicrobial agents in *Shigella sonnei* from humans in Norway 2001-2012. *TMS=Trimethoprim-sulfamethoxazole.

TABLE 24. *Shigella flexneri* isolates from human cases (n=30). 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 [1]	≤ 8	> 8	26.7	-	73.3
Chloramphenicol [1]	≤ 8	> 8	20.0	-	80.0
Tetracycline [2]	≥ 14	< 14	13.3	-	86.7
Nalidixic acid [2]	≥ 19	< 19	63.3	-	36.7
Ciprofloxacin [1]	≤ 0.5	> 1	76.7	0.0	23.3
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	30.0	3.3	66.7

[1] EUCAST clinical breakpoint for *Enterobacteriaceae* 2013 version 3.1. [2] Epidemiological cut-off values based on zone-distribution evaluations. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

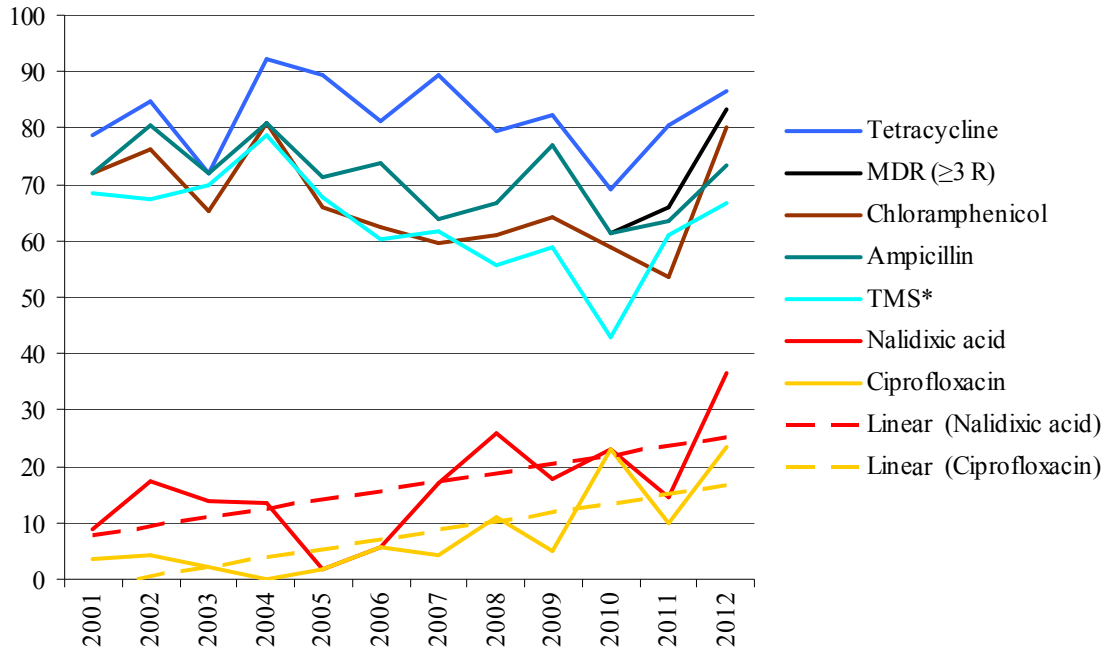


FIGURE 40. Prevalence of resistance to various antimicrobial agents in *Shigella flexneri* from humans in Norway 2001-2012. *TMS=Trimethoprim-sulfamethoxazole.

RESULTS AND COMMENTS

The resistance patterns for *S. sonnei* have been fairly stable during the period 2001-2012. The increase in resistance to nalidixic acid however, has been going on since 2001, and resistance to ciprofloxacin appeared to start increasing in 2008/2009. A similar development appears for resistance to nalidixic acid and ciprofloxacin in *S. flexneri* isolates, but here an increase in resistance to the other drugs included, were observed over the last 2-3 years. As shown in Figure 38 multi-resistance was frequent for all *Shigella* tested; also for the few isolates of *S. dysenteriae* (n=1) and *S. boydii* (n= 3). The percentages of resistance to three or more categories of antimicrobials in *S. sonnei* and *S. flexneri* were 43.2% and 83.3% respectively. Two of the three *S. boydii* isolates were MDR, and the single strain of *S. dysenteriae* was MDR as well.

Three strains had reduced susceptibility to cefpodoxime. All these were *S. sonnei*, and all were phenotypically characterised as $ESBL_A$ producers with inhibitory effect of clavulanic acid.

D. HUMAN CLINICAL ISOLATES

Gunnar Skov Simonsen, Martin Steinbakk, Petter Elstrøm, Kjersti Wik Larssen, Frode Width-Gran, Didrik Vestrheim, Andreas Radtke, Karin Rønning, Cecilie Torp Andersen, Susanne Dudman, Olav Hungnes

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 25, 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 25. Number of blood culture isolates in 2012, proportion of all isolates, and proportion of isolates excluding possible skin contaminants (coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp.) 2008-2012. The table is based on data from the information systems of all laboratories in Norway except one in 2012.

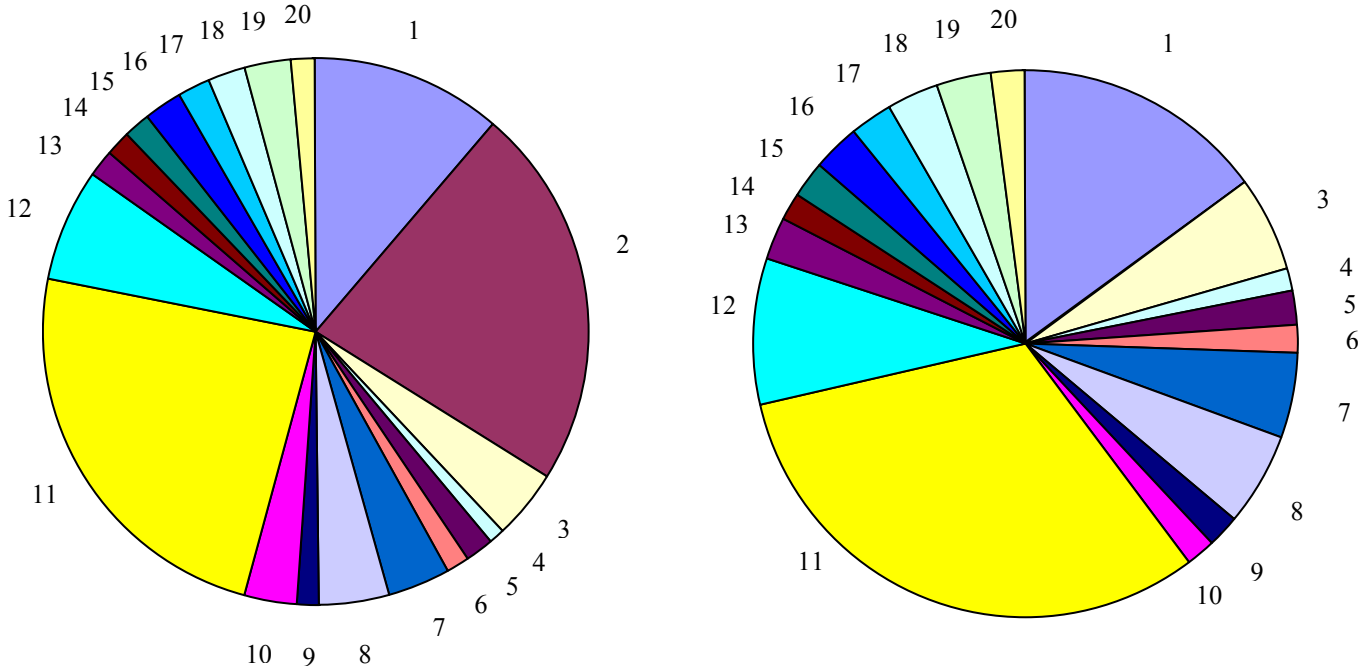
Species	No. of isolates 2012	% of all isolates					% of isolates excluding skin flora				
		2008	2009	2010	2011	2012	2008	2009	2010	2011	2012
Staphylococcus aureus	1,428	10.6	10.6	11.4	11.0	11.3	13.9	13.9	14.5	14.2	15.0
Coagulase negative staphylococci	2,831	21.3	22.3	19.3	20.6	22.5	-	-	-	-	-
Streptococcus pneumoniae	531	6.6	6.2	5.7	5.3	4.2	8.7	8.2	7.3	6.8	5.6
Streptococcus pyogenes	122	1.3	1.3	1.3	1.4	1.0	1.7	1.7	1.6	1.8	1.3
Streptococcus agalactiae	205	1.6	1.4	1.5	1.6	1.6	2.0	1.8	1.9	2.1	2.1
Beta-haemolytic streptococci group C and G	153	1.4	1.1	1.3	1.2	1.2	1.9	1.4	1.7	1.6	1.6
Viridans- and non-haemolytic streptococci	483	3.9	3.5	4.7	4.1	3.8	5.1	4.7	5.9	5.2	5.1
Enterococcus faecalis	509	4.0	4.6	4.6	4.1	4.0	5.2	6.0	5.9	5.2	5.3
Enterococcus faecium	186	1.4	1.3	1.7	1.8	1.5	1.9	1.7	2.1	2.3	2.0
Other Gram positive aerobic bacteria	386	3.4	2.7	2.8	2.9	3.1	1.5	1.5	1.4	1.6	1.9
Escherichia coli	3,006	22.8	23.0	23.4	24.0	23.9	29.9	30.2	29.6	30.9	31.4
Klebsiella spp.	824	5.8	6.5	6.8	6.1	6.5	7.6	8.6	8.7	7.9	8.6
Enterobacter spp.	239	1.9	1.9	1.6	1.8	1.9	2.5	2.5	2.1	2.3	2.5
Proteus spp.	169	1.5	1.5	1.7	1.7	1.3	2.0	2.0	2.2	2.2	1.8
Other *Enterobacteriaceae*	257	2.1	1.9	2.3	2.2	2.0	2.8	2.6	2.9	2.9	2.7
Pseudomonas spp.	210	1.8	1.9	1.8	1.5	1.7	2.4	2.5	2.2	2.0	2.2
Other Gram negative aerobic bacteria	249	2.1	1.9	2.3	2.2	2.0	2.8	2.5	2.9	2.8	2.6
Bacteroides spp.	286	2.3	2.2	2.0	2.2	2.3	3.0	2.9	2.6	2.9	3.0
Other anaerobic bacteria	348	2.5	2.3	2.3	2.8	2.8	2.8	2.8	2.6	3.4	3.3
Yeasts	182	1.8	1.9	1.5	1.5	1.4	2.3	2.5	1.9	1.9	2.0
Total	**12,604**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

As seen in Table 25 and Figure 41, aerobic Gram-positive and Gram-negative bacteria represented 54.2% and 39.3% 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 22.5% of all isolates. 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 39.9% aerobic Gram-positives and 51.8% aerobic Gram-negatives.

Among the aerobic Gram-positives, the prevalence of *S. pneumoniae* has steadily declined even when the bacteria considered skin contaminants are excluded, from 12.1% in 2005 to 5.6% in 2012. The proportions accounted for by other streptococci and enterococci have been fairly stable over the five-year period. The prevalence of coagulase negative staphylococci increased to 22.5%, but this is still within the range seen in recent years.

E. coli (31.4%) and other *Enterobacteriaceae* (15.6%) accounted for the vast majority of aerobic Gram-negative isolates, but the proportions have remained relatively unchanged since 2005. *Pseudomonas* spp. (1.8%) 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 5.1% (6.3% excluding skin flora) which is at the same level as in 2011 (5.0%; 6.3% excluding skin flora). Yeasts accounted for 1.4% (2.0% excluding skin flora) which is unchanged from earlier years. The major pathogens among anaerobes were members of *Bacteroides* spp. (2.3%/3.0%) and among yeasts *Candida albicans* (1.0%/1.3%). 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 41. Distribution of all blood culture isolates (left, n=12,604) and blood culture isolates excluding common skin contaminants (right, n=9,535) such as coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp. Data were retrieved from the information systems of all Norwegian laboratories except for one in 2012.

Escherichia coli in blood cultures

TABLE 26. *Escherichia coli* blood culture isolates (n=1,646). 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	58.1	-	41.9
Piperacillin-tazobactam	≤ 8	> 16	96.0	2.7	1.3
Cefuroxime*	≤ 0.5	> 8	2.6	90.8	6.6
Cefotaxime	≤ 1	> 2	94.3	0.4	5.3
Ceftazidime	≤ 1	> 4	94.7	1.0	4.3
Cefepime	≤ 1	> 4	94.9	1.1	4.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	94.1	0.2	5.7
Ciprofloxacin	≤ 0.5	> 1	88.3	0.4	11.3
Tigecycline	≤ 1	> 2	99.8	0.1	0.1
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	72.6	0.5	26.9
ESBL	Negative	Positive	94.5	-	5.5

*The wild type is defined as intermediately susceptible indicating that the drug is active at low dosage in urinary tract infections and at high dosage (1.5 g x 3) in systemic infections. **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* correspond to EUCAST breakpoints except for cefuroxime where the wild type is defined as intermediately susceptible by NWGA. The wild type was recategorised as susceptible to ampicillin from 2012 which is in line with EUCAST.

The vast majority of isolates remained fully susceptible to broad-spectrum antimicrobial agents such as cefotaxime (94.3%), ceftazidime (94.7%), cefepime (94.9%), gentamicin (94.1%), piperacillin-tazobactam (96.0%), tigecycline (99.8%) and meropenem (100.0%) (Table 26). However, for several of these agents there was a reduction in the prevalence of susceptibility by approximately one percentage point from 2011 to 2012.

The increasing prevalence of non-susceptibility to gentamicin since 2004 was again noticable with 0.2% I and 5.7% R in 2012 compared to 0.3% I and 4.4% R in 2011 (Figure 42). The prevalence of gentamicin resistance is now approximately five times higher than a decade ago. A high proportion of gentamicin non-susceptible isolates (37/97, 38.1%) also produced ESBL enzymes. They were retrieved from 18 different laboratories across the country. The prevalences at individual laboratories (0-11.2%) varied widely due to relatively small numbers. When aggregated by region there were no major geographical differences (South/East 6.7%, West 6.6%, Mid/North 5.0%).

The prevalence of non-suscpetibility to ciprofloxacin was 11.7% (0.4% I and 11.3% R) in 2012 compared to 9.1% in 2011 (0.2% I and 8.9% 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 43. A similar association between quinolone use and resistance in systemic *E. coli* isolates is also reported internationally. The resistance rates for ampicillin (41.9% in 2012, 39.6% in 2011) and trimethoprim-sulfamethoxazole (26.9% in 2012, 24.1% in 2011) are also increasing.

In 2012, 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 Etests. A total of 90 isolates (5.5%) were reported as ESBL positive which is a significant increase from 3.1% in 2010 and 3.3% in 2011 (Figure 45). The isolates originated from 17 different laboratories in all parts of the country with up to 12 isolates from each institution. Estimates at laboratory level (0-9.6%) are uncertain due to small numbers. When aggregated at regional level the prevalence of ESBL was somewhat lower in the middle and northern parts of the country (3.1%) compared to the western (6.1%) and south-eastern parts (6.0%). All ESBL isolates were non-susceptible to ampicillin and cefuroxime, and most of them were also non-susceptible to cefotaxime (89/90), ceftazidime (77/90) and cefepime (72/90). Many isolates were intermediately (22/90) or even fully susceptible (65/90) to piperacillin-tazobactam as well as susceptible to amoxicillin-clavulanic acid (37/90). Most displayed co-resistance to ciprofloxacin (73/90), gentamicin (37/90) and/or trimethoprim-sulfamethoxazole (74/90). All were fully susceptible to tigecycline and meropenem. Twelve additional isolates were reported as non-susceptible to cefotaxime (n=5) and/or ceftazidime (n=10) without being confirmed as ESBL producers.

All *E. coli* ESBL isolates were molecularly characterised which revealed a predominance of CTX-M groups 1 (n=66) and 9 (n=17). The remaining seven isolates harboured SHV (n=2), derepressed chromosomally encoded AmpC (n=3), or plasmid encoded CMY (n=2) enzymes.

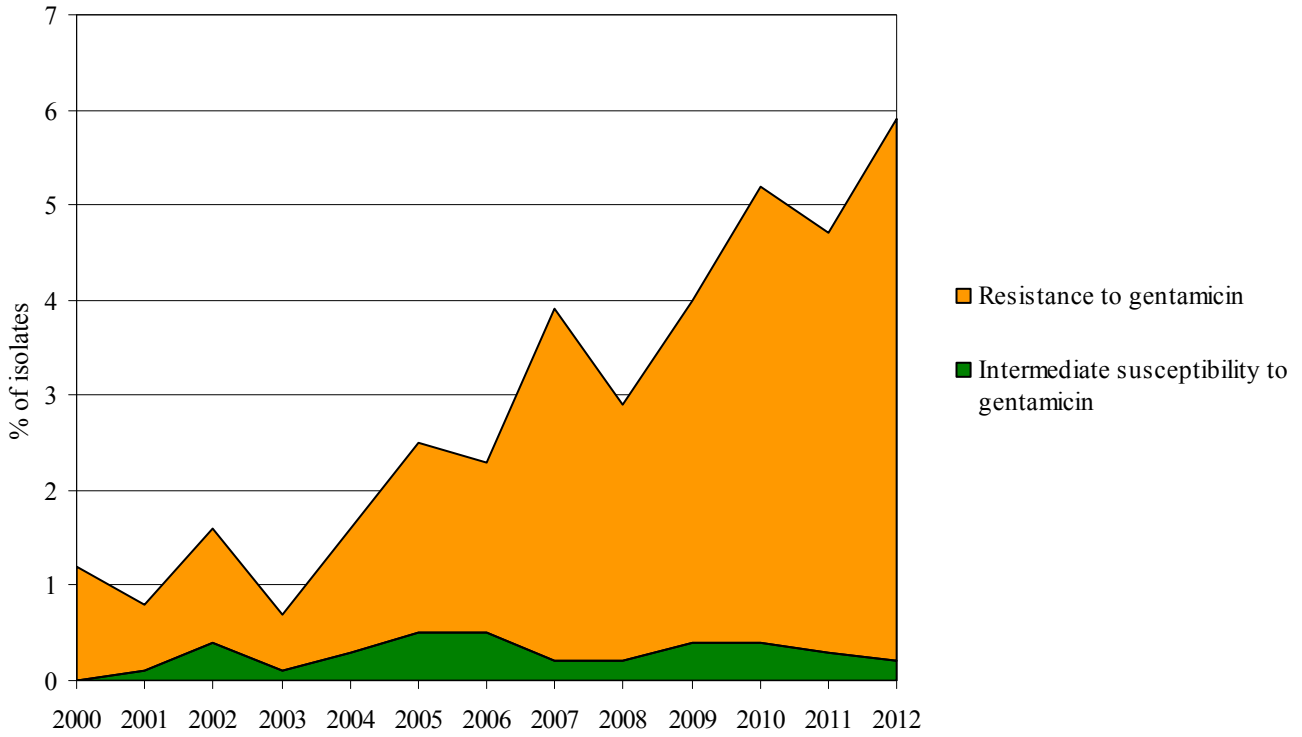


FIGURE 42. Prevalence of intermediate susceptibility and resistance to gentamicin in *Escherichia coli* blood culture isolates 2000-2012.

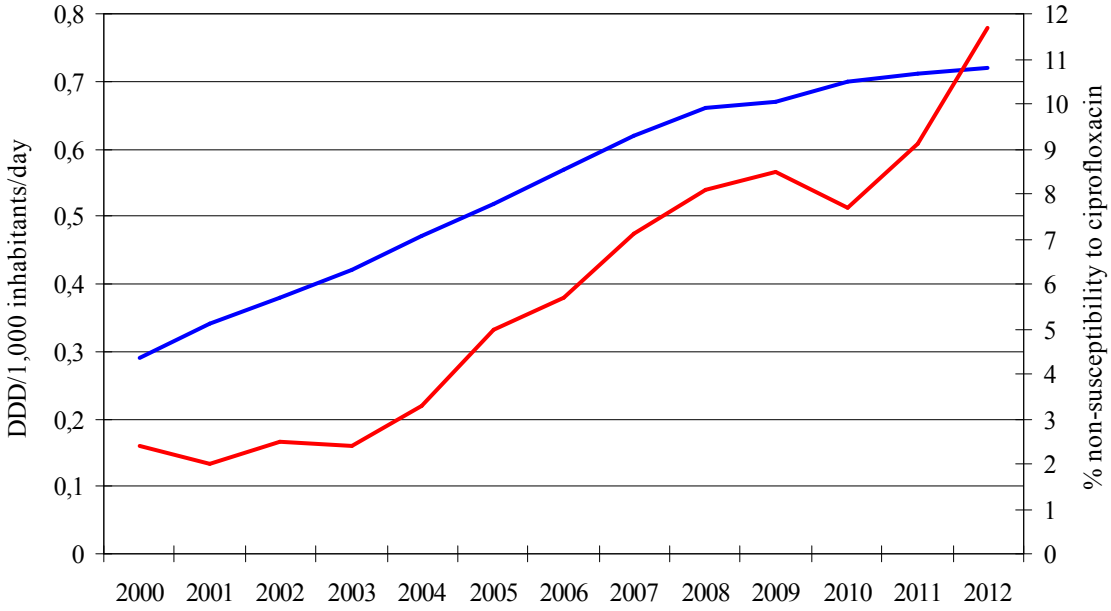


FIGURE 43. Usage of ciprofloxacin (blue) and prevalence of ciprofloxacin non-susceptibility in *Escherichia coli* blood culture isolates as defined by the 2013 breakpoints (red) 2000-2012.

Escherichia coli in urine

TABLE 27. *Escherichia coli* urinary tract isolates (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
Ampicillin	≤ 8	> 8	66.2	-	33.8
Mecillinam	≤ 8	> 8	95.8	-	4.2
Amoxicillin-clavulanic acid	≤ 8	> 8	93.6	-	6.4
Cefuroxime*	≤ 0.5	> 8	2.8	94.2	3.0
Cefotaxime	≤ 1	> 2	97.6	0.4	2.0
Ceftazidime	≤ 1	> 4	97.7	0.3	2.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	97.0	0.0	3.0
Ciprofloxacin	≤ 0.5	> 1	93.0	0.1	6.9
Nitrofurantoin	≤ 64	> 64	99.0	-	1.0
Trimethoprim	≤ 2	> 4	74.5	0.2	25.3
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	76.2	1.0	22.8
ESBL	Negative	Positive	97.8	-	2.2

*The wild type is defined as intermediately susceptible indicating that the drug is active at low dosage in urinary tract infections and at high dosage (1.5 g x 3) in systemic 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 2012 is shown in Table 27 and the results 2000-2012 are shown in Figure 44. As for *E. coli* blood culture isolates, wild type isolates were reclassified as susceptible to ampicillin from 2012 and the results since 2000 have been recalculated accordingly.

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 35%. Around 25% of *E. coli* isolates are resistant to trimethoprim and trimethoprim-sulfamethoxazole. The prevalence of resistance to mecillinam increased from 4.4% in 2010 to 8.7% in 2011, but reverted to 4.2% in 2012. 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 has been relatively stable around 3.5-5.5% over the last years. The prevalence in 2012 was 7.0% with 0.1% intermediate susceptibility and 6.9% resistance. This is a significant increase from 5.4% non-susceptibility in 2011 and 5.8% in 2010. The corresponding rates for blood culture isolates were 0.4% intermediate susceptibility and 11.3% resistance in 2012. 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 cause for great concern.

Of note, 6.4% of the isolates were resistant to amoxicillin-clavulanic acid. The trail of isolates below the breakpoint may be due to technical difficulties in the interpretation of the sone sizes or indicate low level resistance caused by induction of chromosomally encoded AmpC beta-lactamases. Other mechanisms of resistance may also be involved.

In total, 21 isolates (2.2%) were reported as ESBL producers. This prevalence is a slight increase from 2011 (1.6%) and confirms the increasing trend from 0.5% in 2009 and 1.4% in 2010. As seen in Figure 45, the prevalence of *E. coli* ESBL is still significantly lower in urine than in blood culture isolates (5.5%), but there is an increasing trend in both specimen types. The ESBL-positive isolates were retrieved from eleven different laboratories in all parts of the country. Six isolates were found in hospitalised patients while the others were detected in samples submitted from outpatient clinics (n=6), nursing homes (n=1), general practitioners (n=6) or other sources (n=2). The ESBL strains were generally resistant to ampicillin (21/21) and non-susceptible to cefuroxime (21/21), cefotaxime (21/21) and ceftazidime (19/21), but a majority was registered as *in vitro* susceptible to mecillinam (20/21). The clinical relevance of this finding is doubtful, since mecillinam is not stable for most beta-lactamases. Most of the ESBL isolates were non-susceptible to quinolones (n=15) and trimethoprim-sulfamethoxazole (n=18), but remained susceptible to nitrofurantoin (n=19) and gentamicin (n=14). All isolates were susceptible to carbapenems. Urinary tract ESBL-isolates were not subjected to molecular characterisation in 2012, but resistance determinants in such isolates have previously been closely aligned with findings among blood culture isolates.

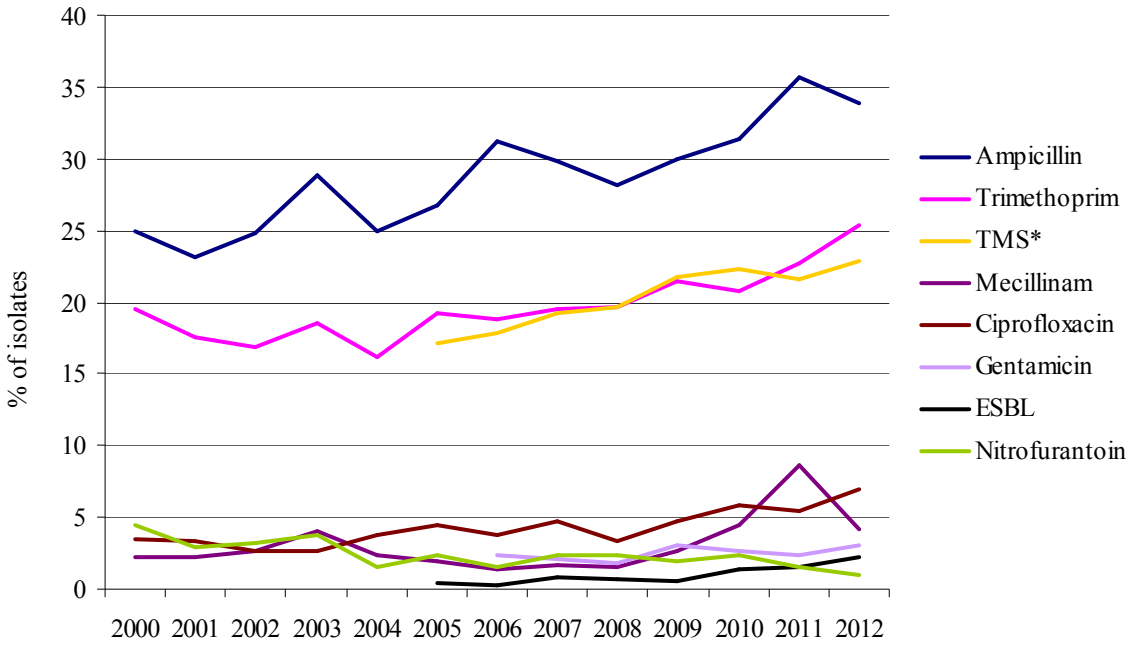


FIGURE 44. Prevalence of resistance to various antimicrobial agents in urinary tract *E. coli* isolates 2000-2012. The breakpoint for resistance to ampicillin was changed from R > 16 mg/L to R > 8 mg/L in 2005. For all years, isolates previously classified as intermediately susceptible have been categorised as susceptible according to 2013 EUCAST guidelines. *TMS=Trimethoprim-sulfamethoxazole.

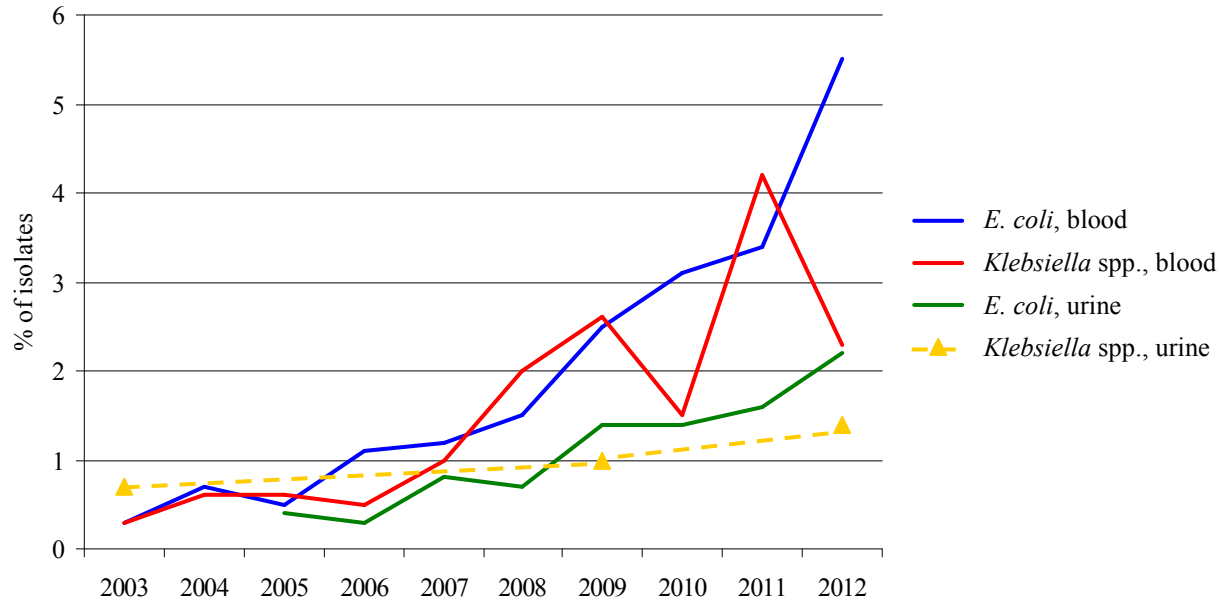


FIGURE 45. Prevalence of ESBL production among *E. coli* and *Klebsiella* spp. isolates from blood and urine 2003-2012.

Only minor differences were seen when the isolates were separated according to the location of the patients sampled (Table 28). The results should be interpreted with caution as the number of isolates from each patient location is limited. Isolates retrieved at hospital outpatient clinics were overall more resistant to ampicillin, mecillinam, gentamicin, ciprofloxacin, trimethoprim and trimetoprim-sulfamethoxazole compared to isolates from other locations. Surprisingly, isolates from nursing homes were relatively less resistant than isolates from other locations. It should be noted that there is no national consensus on the indication for urine culture in these settings, and the rates from different locations are therefore not directly comparable.

TABLE 28. Non-susceptibility of *Escherichia coli* urinary tract isolates (n=912) according to location of patients sampled. Only antibiotics relevant for treatment of urinary tract infections are included. Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Hospital ward (n=166)	Outpatient clinic (n=110)	Nursing home (n=71)	General practice (n=565)
Ampicillin	38.6	44.5	21.1	32.2
Mecillinam	5.4	9.1	1.4	3.2
Amoxicillin-clavulanic acid	7.8	8.2	4.2	6.0
Gentamicin	3.6	5.5	1.4	2.8
Ciprofloxacin	6.6	9.1	5.6	6.9
Nitrofurantoin	0.6	1.8	1.4	1.1
Trimethoprim	27.7	31.8	14.1	25.2
Trimethoprim-sulfamethoxazole	25.3	32.7	11.3	23.4
ESBL	3.6	5.5	1.4	1.1

Amoxicillin-clavulanic acid can be prescribed in Norway but is not widely marketed. The consumption is consequently very low comprared to other Western countries. The distribution of zone diameters for amoxicillin-clavulanic acid is shown in Figure 46. In total, 6.4% of the isolates were categorised as resistant by use of the NWGA breakpoints for lower urinary tract infections. An even higher proportion (12.4%) was resistant among blood culture isolates, but breakpoints for systemic infections are not given. The trailing left side of the distribution may be caused by poorly optimised agar medium or induction of the chromosomal AmpC enzyme of *E. coli* and/or reduced permeability of the cell wall.

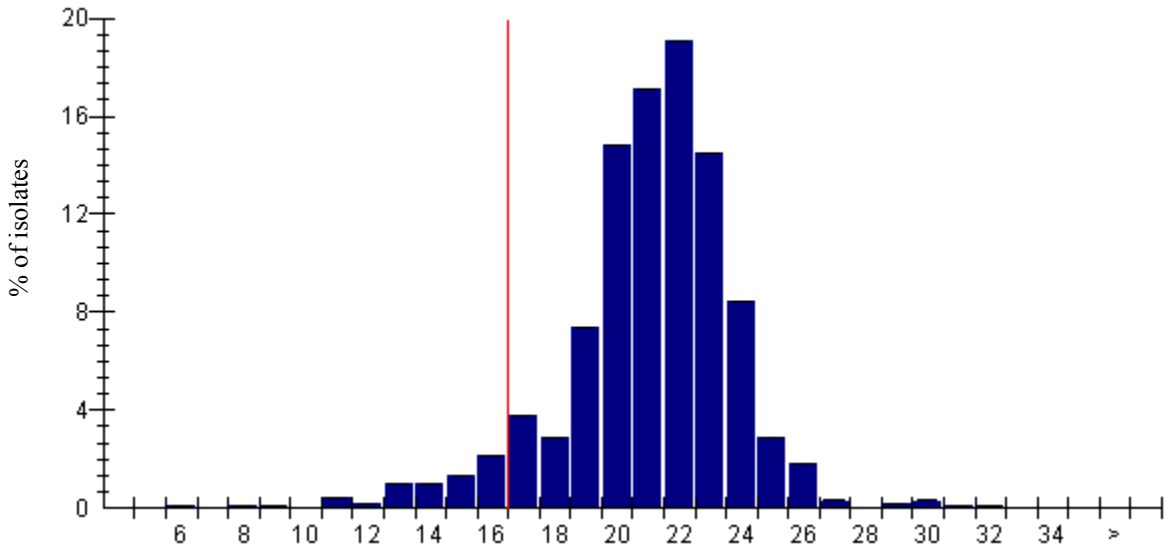


FIGURE 46. Distribution of zone diameters for amoxicillin-clavulanic acid (20 + 10 µg) among 955 urinary tract *E. coli* isolates. The vertical red line indicates the breakpoint between susceptible (≥ 17 mm) and resistant (< 17 mm) isolates.

Klebsiella spp. in blood cultures

TABLE 29. *Klebsiella* spp. blood culture isolates (n=681). 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	93.2	5.3	1.5
Cefuroxime*	≤ 0.5	> 8	3.1	89.3	7.6
Cefotaxime	≤ 1	> 2	97.5	0.4	2.1
Ceftazidime	≤ 1	> 4	96.6	1.5	1.9
Cefepime	≤ 1	> 4	97.3	1.2	1.5
Meropenem	≤ 2	> 8	99.9	0.1	0.0
Gentamicin	≤ 2	> 4	99.0	0.3	0.7
Ciprofloxacin	≤ 0.5	> 1	95.0	1.9	3.1
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	86.5	0.6	12.9
ESBL	Negative	Positive	97.7	-	2.3

*The wild type is defined as intermediately susceptible indicating that the drug is active at low dosage in urinary tract infections and at high dosage (1.5 g x 3) in systemic infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 30. *Klebsiella pneumoniae* blood culture isolates (n=545). 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	93.1	6.2	0.7
Cefuroxime*	≤ 0.5	> 8	3.1	88.6	8.3
Cefotaxime	≤ 1	> 2	97.2	0.2	2.6
Ceftazidime	≤ 1	> 4	95.8	1.8	2.4
Cefepime	≤ 1	> 4	97.1	1.1	1.8
Meropenem	≤ 2	> 8	99.8	0.2	0.0
Gentamicin	≤ 2	> 4	98.9	0.2	0.9
Ciprofloxacin	≤ 0.5	> 1	94.1	2.0	3.9
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	83.9	0.7	15.4
ESBL	Negative	Positive	97.1	-	2.9

*The wild type is defined as intermediately susceptible indicating that the drug is active at low dosage in urinary tract infections and at high dosage (1.5 g x 3) in systemic infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 31. *Klebsiella oxytoca* blood culture isolates (n=126). 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	93.6	1.6	4.8
Cefuroxime*	≤ 0.5	> 8	3.2	91.2	5.6
Cefotaxime	≤ 1	> 2	98.4	1.6	0.0
Ceftazidime	≤ 1	> 4	100.0	0.0	0.0
Cefepime	≤ 1	> 4	98.4	1.6	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	99.2	0.8	0.0
Ciprofloxacin	≤ 0.5	> 1	98.4	1.6	0.0
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	97.6	0.0	2.4
ESBL	Negative	Positive	100.0	-	0.0

*The wild type is defined as intermediately susceptible indicating that the drug is active at low dosage in urinary tract infections and at high dosage (1.5 g x 3) in systemic infections. **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 545 *K. pneumoniae* (80.0%), 126 *K. oxytoca* (18.5%), and 10 (1.5%) isolates not identified to the species level, giving a total of 681 *Klebsiella* spp. isolates (Tables 29-31). The species distribution was not significantly changed from 2011. As for *E. coli*, the Norwegian Working Group for Antibiotics (NWGA) has defined the *Klebsiella* spp. wild type as intermediately susceptible to cefuroxime. The breakpoints for antimicrobial agents included in the *Klebsiella* surveillance protocol were not changed in 2012. Tigecycline and amoxicillin-clavulanic acid are not included in the table as EUCAST has not defined breakpoints for these agents. The *E. coli* breakpoints for tigecycline intersected the wild type population and were clearly not suitable for *Klebsiella* spp. (data not shown).

The majority of *Klebsiella* spp. isolates remained susceptible to aminoglycosides. The peak of 4.6% non-susceptibility observed in 2011 reverted to 1.0% in 2012 which is more in line with the results from previous years. The prevalence of non-susceptibility to aminoglycosides was similar in *K. oxytoca* isolates (0.8%) and *K. pneumoniae* (1.1%) in 2012. Aminoglycoside resistance in common *Enterobacteriaceae* species is a cause for great concern as aminoglycosides have traditionally been used in the empirical regimen for 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 5.0% non-susceptibility (1.9% intermediate susceptibility and 3.1% resistance) observed in 2012 was at the same level as in previous years. Non-susceptibility to ciprofloxacin is still more common in *K. pneumoniae* (5.9%) than in *K. oxytoca* (1.6%). Non-susceptibility to trimethoprim-sulfamethoxazole declined from 17.9% in 2010 and 17.1% in 2011 to 12.9% in 2012. As for ciprofloxacin, the prevalence of resistance to trimethoprim-sulfamethoxazole was significantly lower in *K. oxytoca* (2.4%) than in *K. pneumoniae* (15.4%).

A comparison of ESBL rates and non-susceptibility to beta-lactam antibiotics between species is complicated by the diagnostic challenges of the chromosomal K1 beta-lactamase in *K. oxytoca*. Most *Klebsiella* spp. isolates were susceptible to cefotaxime (97.5%), ceftazidime (96.6%) and the beta-lactam/beta-lactamase inhibitor combination piperacillin-tazobactam (93.2%), see Figure 47. The rates of non-susceptibility to third generation cephalosporins decreased by approximately two percentage points in 2012 compared to 2011 and were at the same level as the years 2008-2010.

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 Etests. The prevalence of phenotypically confirmed ESBL isolates declined from 4.2% in 2011 to 2.3% in 2012 (Figure 45). The 16 ESBL isolates originated from nine different laboratories and were all identified as *K. pneumoniae*, thus constituting 2.3% in this species compared to 5.5% in 2011. The ESBL isolates were generally non-susceptible to cefuroxime (16/16), ceftazidime (14/16) and cefotaxime (14/16), and co-resistance was frequently seen to ciprofloxacin (8/16), trimethoprim-sulfamethoxazole (15/16) and gentamicin (4/16). Many iolates were intermediately (2/16) or even fully (12/16) susceptible to piperacillin-tazobactam. Molecular characterisation of the ESBL isolates at the Reference Centre for Detection of Antimicrobial Resistance (K-Res) confirmed the presence of CTX-M groups 1 (n=9) and 9 (n=1). The remaining isolates contained broad-spectrum (n=4) or wild type variants of SHV. A single isolate (0.1%) displayed reduced susceptibility to meropenem and contained a KPC determinant compatible with carbapenemase production.

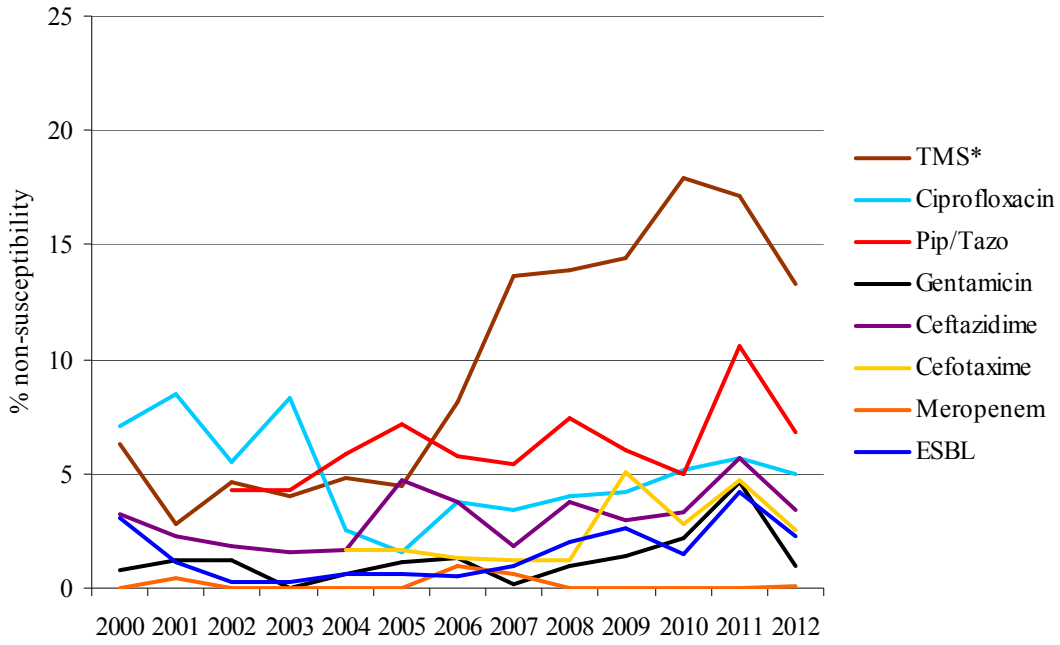


FIGURE 47. Prevalence of non-susceptibility to various antimicrobial agents in *Klebsiella* spp. blood culture isolates 2000-2012. *TMS=Trimethoprim-sulfamethoxazole.

Klebsiella spp. in urine

TABLE 32. *Klebsiella* spp. urinary tract isolates (n=857). 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	90.5	-	9.5
Piperacillin-tazobactam	≤ 8	> 16	93.1	4.0	2.9
Cefuroxime*	≤ 0.5	> 8	4.8	87.7	7.5
Cefotaxime	≤ 1	> 2	97.7	0.4	1.9
Ceftazidime	≤ 1	> 4	97.3	1.3	1.4
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	98.1	0.5	1.4
Ciprofloxacin	≤ 0.5	> 1	96.5	2.1	1.4
Trimethoprim	≤ 2	> 4	81.5	0.9	17.6
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	76.4	1.5	22.1
ESBL	Negative	Positive	98.6	-	1.4

*The wild type is defined as intermediately susceptible indicating that the drug is active at low dosage in urinary tract infections and at high dosage (1.5 g x 3) in systemic infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Klebsiella spp. isolates have previously been included in the NORM surveillance programme in 2001, 2003 and 2009. 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 or amoxicillin-clavulanic acid.

In general, the rates of resistance to urinary tract antibiotics were slightly lower in *Klebsiella* spp. than in *E. coli* isolates (Tables 32-34). The vast majority of isolates are still susceptible to gentamicin (98.1% compared to 99.1% in 2009), ciprofloxacin (96.5% compared to 97.1% in 2009) and meropenem (100%). The comparable rates for *E. coli* were 97.0% for gentamicin, 93.0% for

ciprofloxacin and 100% for meropenem. Susceptibility to trimethoprim (81.5% compared to 80.3% in 2009) and trimethoprim-sulfamethoxazole (76.4% compared to 83.3% in 2009) were similar to the findings in *E. coli* (74.5% and 76.2%, respectively), but the resistance rate for trimethoprim being lower than for trimethoprim-sulfamethoxazole may indicate technical problems with the susceptibility test results.

All *Klebsiella* isolates are by definition resistant to ampicillin due to the chromosomal SHV beta-lactamase. Wild type strains are in Norway categorised as intermediately susceptible to cefuroxime. 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 Etests. Only twelve isolates were reported as ESBL positive of which ten were *K. pneumoniae* and two were unspeciated. The twelve ESBL isolates were retrieved from eight different laboratories and originated from hospitals (n=2), general practices (n=6), long term health care facilities (n=3) and an unknown location (n=1). The 1.4% ESBL rate (2.2% in *K. pneumoniae*) represented a modest increase from 1.0% in 2009 and lower than the 2.3% rate (2.9% in *K. pneumoniae*) found in blood culture isolates. The twelve ESBL isolates were generally resistant to trimethoprim (n=11) and trimethoprim-sulfamethoxazole (n=11), but remained susceptible to gentamicin (n=6), ciprofloxacin (n=6), mecillinam (n=9) and piperacillin-tazobactam (n=9). All isolates were fully susceptible to meropenem.

TABLE 33. *Klebsiella pneumoniae* urinary tract isolates (n=454). 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	93.0	-	7.0
Piperacillin-tazobactam	≤ 8	> 16	95.0	3.5	1.5
Cefuroxime*	≤ 0.5	> 8	4.6	89.2	6.2
Cefotaxime	≤ 1	> 2	97.8	0.0	2.2
Ceftazidime	≤ 1	> 4	96.7	1.1	2.2
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	97.8	0.2	2.0
Ciprofloxacin	≤ 0.5	> 1	95.3	2.9	1.8
Trimethoprim	≤ 2	> 4	74.2	0.9	24.9
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	77.7	0.9	21.4
ESBL	Negative	Positive	97.8	-	2.2

*The wild type is defined as intermediately susceptible indicating that the drug is active at low dosage in urinary tract infections and at high dosage (1.5 g x 3) in systemic infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 34. *Klebsiella oxytoca* urinary tract isolates (n=86). 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	83.7	-	16.3
Piperacillin-tazobactam	≤ 8	> 16	87.2	2.3	10.5
Cefuroxime*	≤ 0.5	> 8	5.8	82.6	11.6
Cefotaxime	≤ 1	> 2	95.4	2.3	2.3
Ceftazidime	≤ 1	> 4	97.7	2.3	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	97.6	1.2	1.2
Ciprofloxacin	≤ 0.5	> 1	98.8	0.0	1.2
Trimethoprim	≤ 2	> 4	91.8	1.2	7.0
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	96.5	0.0	3.5
ESBL	Negative	Positive	100.0	-	0.0

*The wild type is defined as intermediately susceptible indicating that the drug is active at low dosage in urinary tract infections and at high dosage (1.5 g x 3) in systemic infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

Staphylococcus aureus in blood cultures

TABLE 35. *Staphylococcus aureus* blood culture isolates (n=1,141). 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	96.3	0.0	3.7
Clindamycin	≤ 0.25	> 0.5	98.8	0.4	0.8
Fusidic acid	≤ 1	> 1	94.1	-	5.9
Ciprofloxacin	≤ 1	> 1	97.5	-	2.5
Gentamicin	≤ 1	> 1	99.3	-	0.7
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	99.1	0.6	0.3
Tetracycline	≤ 1	> 2	95.7	0.4	3.9
Tigecycline	≤ 0.5	> 0.5	99.9	0.0	0.1
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.4	0.4	0.2
Beta-lactamase	Negative	Positive	28.2	-	71.8
Cefoxitin screen	Negative	Positive	99.0	-	1.0
MRSA (*mecA*)	Negative	Positive	99.0	-	1.0

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

RESULTS AND COMMENTS

Eleven methicillin resistant *S. aureus* (MRSA) isolates were detected in the NORM surveillance system in 2012 (Table 35) corresponding to a prevalence of 1.0%. This is at the same level as 0.7% in 2008, 0.4% in 2009, 1.0% in 2010 and 0.5% in 2011. The resistance phenotype was confirmed by *mecA* PCR in all cases. The isolates originated from ten different hospitals.

Laboratory screening for MRSA in NORM is performed using cefoxitin disks. All MRSA isolates had cefoxitin zone diameters below the screening breakpoint. Only a few MRSA isolates were concomitantly resistant to ciprofloxacin (2/11), clindamycin (1/11), erythromycin (3/11), tetracycline (2/11) or fucidic acid (1/11). All MRSA isolates were fully susceptible to gentamicin, linezolid, rifampicin and trimethoprim-sulfamethoxazole. The results from susceptibility testing of all Norwegian MRSA isolates are presented on page 72. A single methicillin susceptible *S. aureus* (MSSA) isolate was reported to have a cefoxitin zone diameter below the screening breakpoint. This was not further investigated.

The findings are in accordance with reports from the databases of the participating laboratories where 16 out of 1,556 (1.0%) *S. aureus* blood culture isolates were MRSA. None of the 20 *S. aureus* isolates recovered from cerebrospinal fluids were methicillin resistant, thus bringing the total number of systemic MRSA isolates to 16/1,576 (1.0%). The Norwegian Surveillance System for Communicable Diseases (MSIS) reported a total number of 1,210 MRSA cases in 2012. This is a 14% increase from 1,059 cases in 2011. The cases reported to MSIS were predominantly skin and soft tissue infections and colonisations. The 575 MRSA infections were at the same level as 563 in 2011. The number of MRSA colonisations has increased steadily from 402 in 2009, 481 in 2010 and 496 in 2011, to 635 in 2012 (+28%). Further information about MRSA cases in MSIS is presented on page 71.

A total of 42 *S. aureus* isolates (3.7%) were non-susceptible to erythromycin. This is a further decrease from 5.4% in 2010 and 4.3% in 2011. The macrolide resistance phenotypes were determined by the double disk diffusion (DDD) test. Only one isolate (2%) was constitutively MLS_B resistant, 34 (81%) were inducibly MLS_B resistant and seven (17%) displayed efflux mediated M type resistance. These figures represent 0.1%, 3.0% and 0.6% of all *S. aureus* isolates from blood cultures, respectively. The distribution of macrolide resistance phenotypes was similar to the results from previous years.

The prevalence of resistance to fusidic acid increased slightly to 5.9% in 2012 compared to 4.2% in 2011. The 2.5% prevalence of ciprofloxacin resistance was at approximately the same level as 3.9% in 2010 and 2.0% in 2011. There were no significant changes for gentamicin, rifampicin or trimethoprim-sulfamethoxazole. All isolates were fully susceptible to linezolid. A single isolate was phenotypically resistant to tigecycline. This has not previously been reported from Norway, and the isolate will therefore be further investigated. Vancomycin was not included in the susceptibility test panel in 2012.

Figure 48 shows the prevalence of non-susceptibility to various antimicrobials. A total of 71.8% of the isolates were beta-lactamase positive which is unchanged from previous years. A subgroup analysis revealed only minor differences in resistance rates to other antimicrobials between beta-lactamase positive and beta-lactamase negative isolates.

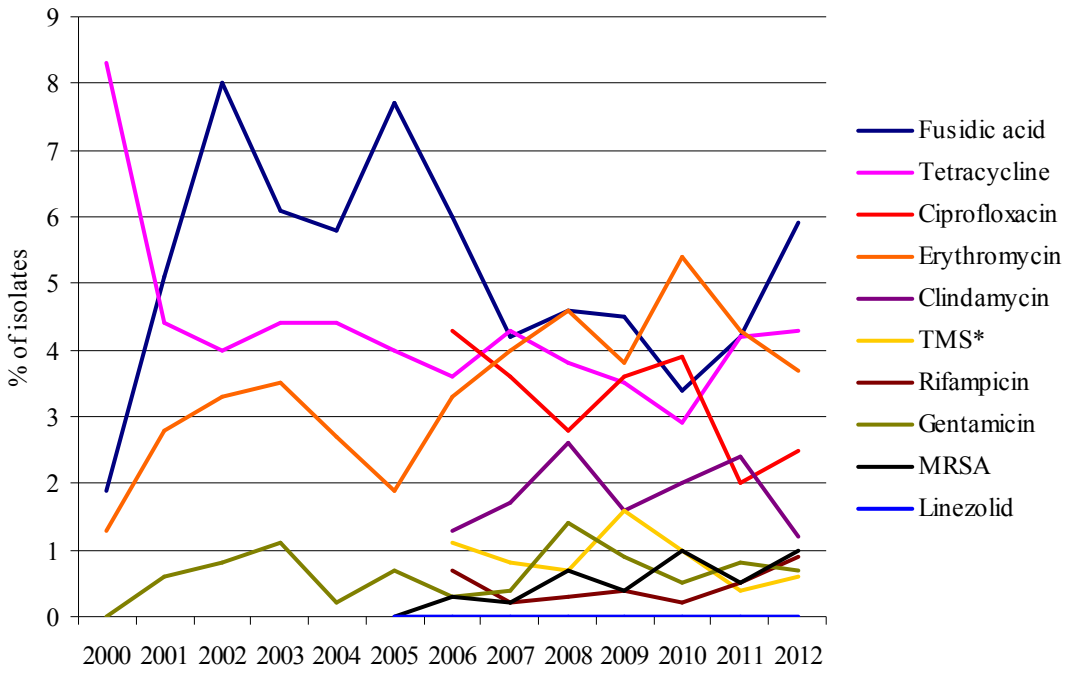


FIGURE 48. Prevalences of non-susceptibility to various antimicrobial agents among *Staphylococcus aureus* blood culture isolates 2000-2012. Doxycycline was replaced by tetracycline in 2006. *TMS=Trimethoprim-sulfamethoxazole.

Staphylococcus aureus in wound specimens

TABLE 36. *Staphylococcus aureus* isolates from wound specimens (n=956). 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	94.1	0.1	5.8
Clindamycin	≤ 0.25	> 0.5	97.3	0.3	2.4
Fusidic acid	≤ 1	> 1	90.5	-	9.5
Ciprofloxacin	≤ 1	> 1	97.5	-	2.5
Gentamicin	≤ 1	> 1	99.7	-	0.3
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	99.7	0.2	0.1
Tetracycline	≤ 1	> 2	94.9	0.7	4.4
Tigecycline	≤ 0.5	> 0.5	100.0	0.0	0.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.0	0.6	0.4
Beta-lactamase	Negative	Positive	23.3	-	76.7
Cefoxitin screen	Negative	Positive	99.1	-	0.9
MRSA (*mecA*)	Negative	Positive	99.3	-	0.7

*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. Seven out of 956 (0.7%) isolates were confirmed as MRSA by *mecA* PCR. The prevalence was at the same level as in 2010 (0.6%) and 2011 (1.3%), and also as in blood cultures (1.0%, see above). The MRSA isolates originated from patients admitted to hospital (n=2), outpatient clinics (n=2), nursing homes (n=2) and general practitioners (n=1) in different parts of the country. Several MRSA isolates displayed non-susceptibility to fusidic acid (4/7), ciprofloxacin (3/7), erythromycin (2/7), tetracycline (2/7), clindamycin (1/7) and/or gentamicin (1/7). All MRSA isolates were fully susceptible to linezolid, rifampicin, trimethoprim-sulfamethoxazole and tigecycline. Two of 949 isolates (0.2%) were reported with zone diameters below the cefoxitin screening breakpoint without being confirmed as MRSA.

The high prevalence of resistance to fusidic acid in *S. aureus* wound isolates remained essentially unchanged at 9.5% compared to 9.9% in 2011 (Table 36 and Figure 49). 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 (5.9%).

For other antimicrobial agents such as trimethoprim-sulfamethoxazole, gentamicin, rifampicin, and tetracycline there were only minor changes from 2011 to 2012, 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 and tigecycline.

A total of 56 (5.9%) isolates were non-susceptible to erythromycin which is a slight decrease from 6.5% in 2011. Fifty-three of these isolates were further examined for determination of resistance phenotype. The majority (31/53, 58% of macrolide resistant isolates) were inducibly resistant to clindamycin, thus representing the $iMLS_B$ phenotype. Minor proportions were either constitutively resistant to clindamycin (n=11) or low-level resistant to erythromycin (n=11), expressing efflux mediated M type resistance. The findings are in accordance with the results from blood culture isolates.

A total of 76.7% of the isolates were beta-lactamase positive compared to 74.8% in 2011. Resistance to fusidic acid was more common among the 733 beta-lactamase positive isolates (9.8%) than among the 223 beta-lactamase negative ones (8.5%). A similar trend was seen for erythromycin (6.4% vs 3.6%), tetracycline (5.0% vs 2.2%), ciprofloxacin (3.0% vs 0.9%) and clindamycin (2.7% vs 1.3%). There were no significant differences for other antimicrobial agents.

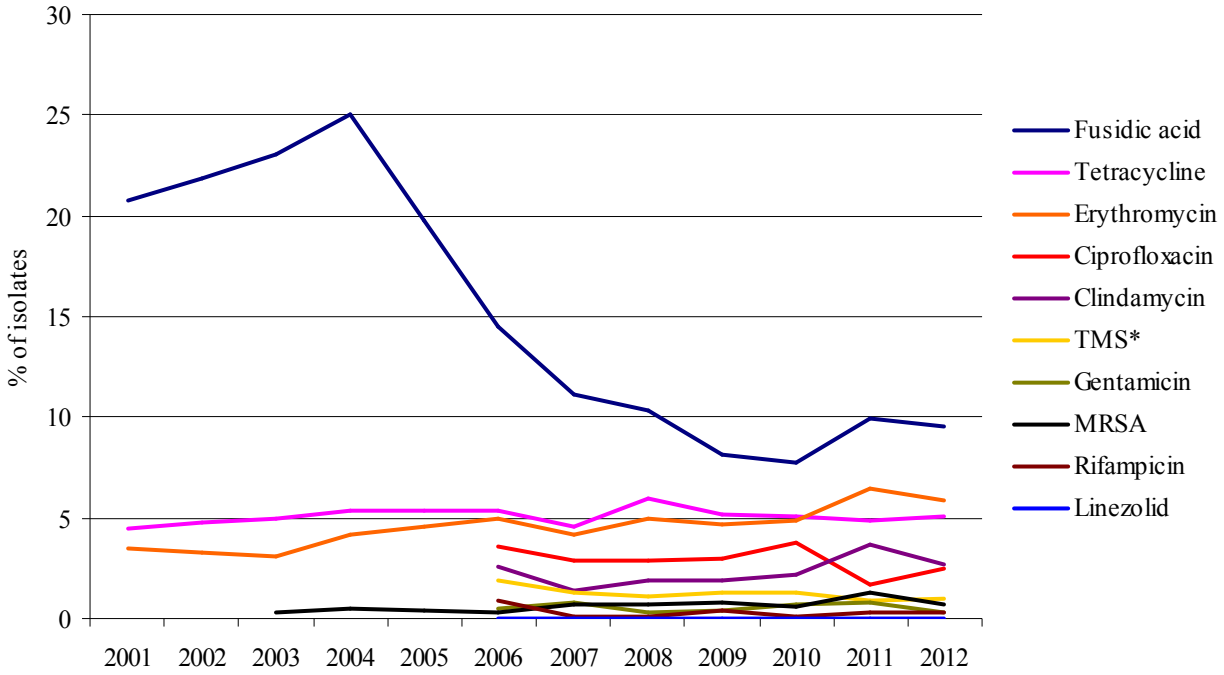


FIGURE 49. Prevalence of non-susceptibility to various antimicrobial agents among *Staphylococcus aureus* wound isolates 2000-2012. Doxycycline was replaced by tetracycline in 2006. *TMS=Trimethoprim-sulfamethoxazole.

Methicillin resistant *Staphylococcus aureus* (MRSA) infections in humans 2012

Infections with methicillin resistant *Staphylococcus aureus* (MRSA) have been notifiable in Norway since 1995 and colonisation was made notifiable in 2005. 1,210 cases of MRSA were reported in 2012 (24 per 100,000 person-years). 575 (47%) of the cases had an active clinical infection and 635 were colonised at the time of diagnosis (Figure 50).

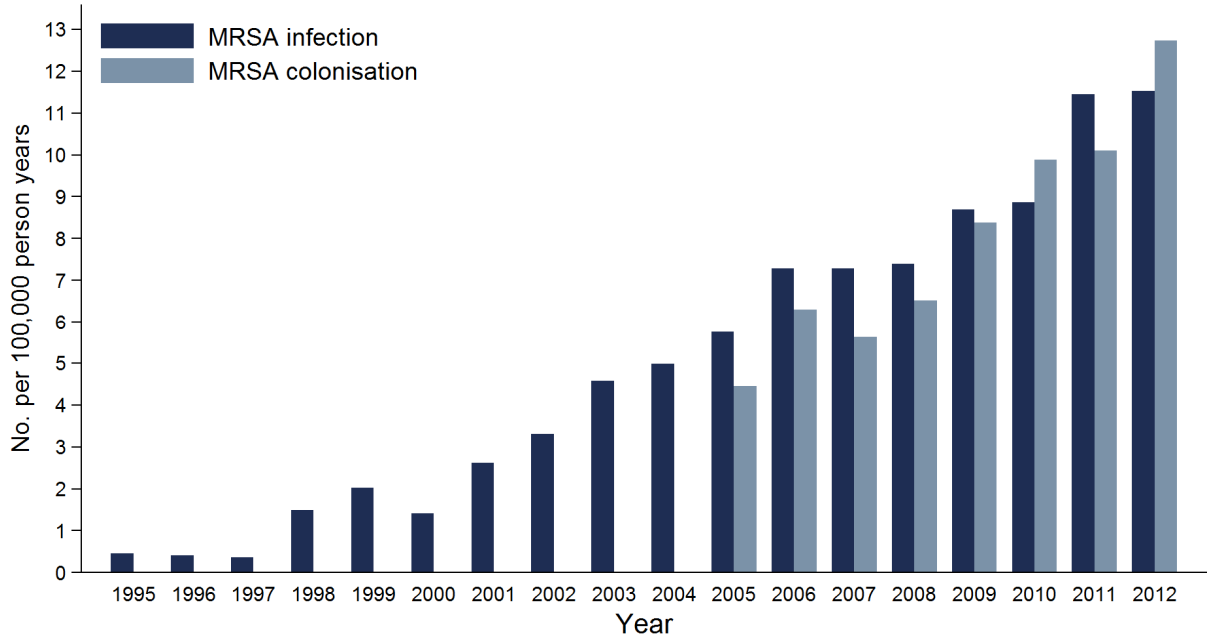


FIGURE 50. Number of MRSA cases per 100,000 person-years in Norway 1995-2012, by infection and colonisation.

The aim of the Norwegian MRSA guidelines is to prevent MRSA from becoming endemic in health-care institutions. 282 (23%) of persons notified with MRSA in 2012 were inpatients at the time of diagnosis, while 65 (5%) were residents in nursing homes and 805 (67%) were diagnosed in the community. 52 (4%) of the reported MRSA cases in 2012 were health-care workers. The increase in number reported with MRSA can in total be attributed to persons infected abroad or in the community, while there has been no increase in cases associated to the Norwegian health-care institutions in the last seven years (Figure 51).

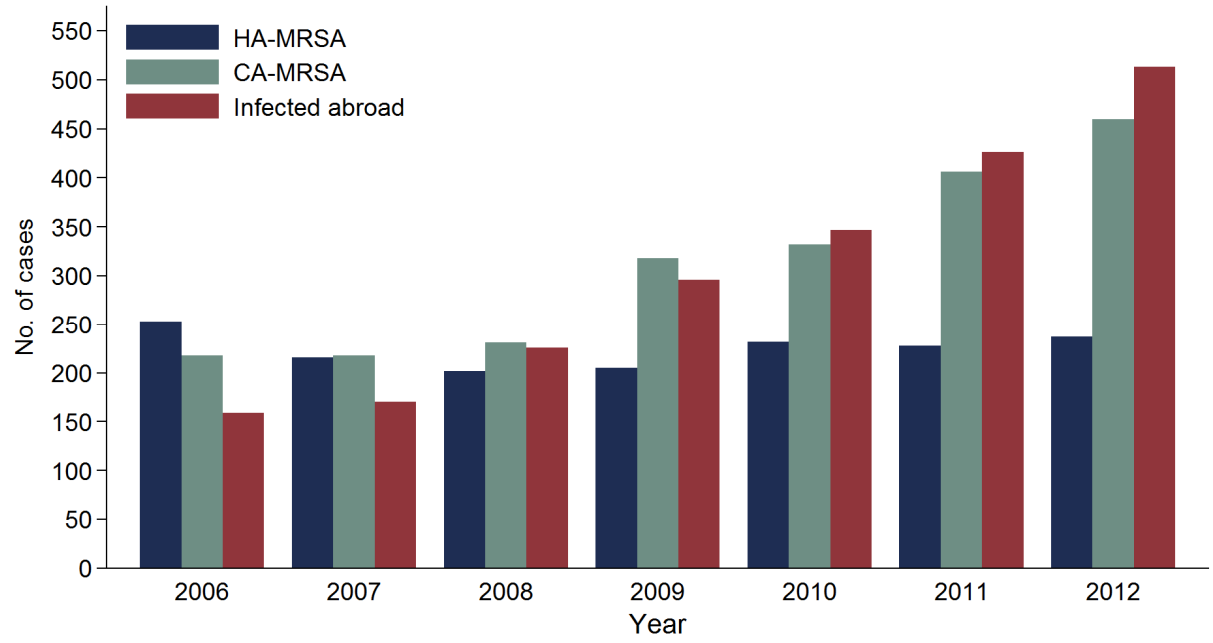


FIGURE 51. Reported cases of MRSA infections and colonisations in Norway 2006-2012, by health-care associated, community associated and imported cases.

The Reference Laboratory for MRSA, St. Olav University Hospital, Trondheim, received 1,156 MRSA isolates in 2012. 54 isolates were not sent to the reference laboratory but only reported to MSIS. A total of 224 different spa-types were identified and the six most frequent were (spa-type, n (%)): t002, n=130 (10.7%), t019, n=120 (9.9%), t008, n=76 (6.3%), t127, n=46 (3.8%), t223, n=46 (3.8%) and t044, n=39 (3.2%). A total of 104 spa-types were reported as single events.

Based on spa-type, all isolates were assigned to MLST clonal complex. A total of 864 isolates (71.4%) occurred in the six most prevalent clusters (CC, n (%)): CC5, n=252 (20.8%), CC30, n=194 (16.0%), CC8, n=152 (12.6%), CC22, n=119 (9.8%), CC45, n=77 (6.4%), and CC1, n=70 (5.8%).

The Reference Laboratory found five livestock-associated (LA) MRSA, three spa-type t034 and two t011, as well as two isolates positive for *mecC*.

Susceptibility testing was performed on 1,161 MRSA isolates collected in 2012 with the EUCAST 2012 agar diffusion method and analysed with breakpoints from NWGA 2012 (Table 37). In total, 34.5% of the strains (n=400) were sensitive to all antibiotics tested except beta-lactams. The highest proportions of resistance were found for erythromycin (36.9%) followed by clindamycin (25.3%). 294 strains were resistant to clindamycin, of which 174 strains (59.2%) were inducibly resistant. 275 isolates (23.7%) were resistant to tetracycline. The lowest rates of resistance were found towards linezolid (0.0%), quinupristin-dalfopristin (0.0%), mupirocin (0.9%) and rifampicin (1.8%).

TABLE 37. Susceptibility characterisation of methicillin resistant *Staphylococcus aureus* (MRSA) from 2012 (n=1,161). Standard agar diffusion method ad modum EUCAST 2012. Breakpoints from NWGA 2012.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	62.8	0.3	36.9
Clindamycin	≤ 0.25	> 0.5	73.8	0.9	25.3
Fusidic acid	≤ 1	> 1	88.3	-	11.7
Norfloxacin	≤ 4	> 4	77.3	-	22.7
Gentamicin	≤ 1	> 1	90.3	-	9.7
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	97.6	0.6	1.8
Tetracycline	≤ 1	> 2	76.1	0.3	23.7
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	95.5	2.1	2.4
Mupirocin	≤ 1	> 256	93.9	5.2	0.9
Quinupristin-dalfopristin	≤ 1	> 2	99.2	0.8	0.0

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

Enterococcus spp. in blood cultures

TABLE 38. *Enterococcus* spp. blood culture isolates (n=557). 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	78.0	0.5	21.5
Imipenem	≤ 4	> 8	74.7	2.9	22.4
Gentamicin*	≤ 128	> 128	-	68.9	31.1
Linezolid	≤ 4	> 4	99.5	-	0.5
Tigecycline	≤ 0.25	> 0.5	100.0	0.0	0.0
Vancomycin	≤ 4	> 4	98.2	-	1.8

*The wild type is defined as intermediately susceptible.

TABLE 39. *Enterococcus faecalis* blood culture isolates (n=386). 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	97.2	1.8	1.0
Gentamicin*	≤ 128	> 128	-	74.4	25.6
Linezolid	≤ 4	> 4	99.7	-	0.3
Tigecycline	≤ 0.25	> 0.5	99.5	0.0	0.0
Vancomycin	≤ 4	> 4	100.0	-	0.0

*The wild type is defined as intermediately susceptible.

TABLE 40. *Enterococcus faecium* blood culture isolates (n=144). 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	18.1	2.1	79.8
Imipenem	≤ 4	> 8	15.3	3.5	81.2
Gentamicin*	≤ 128	> 128	-	52.8	47.2
Linezolid	≤ 4	> 4	98.6	-	1.4
Tigecycline	≤ 0.25	> 0.5	100.0	0.0	0.0
Vancomycin	≤ 4	> 4	99.3	-	0.7

*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 38. The surveillance in NORM 2012 included 386 (69.2%) *E. faecalis* isolates (63.9% in 2011), 144 (25.9%) *E. faecium* isolates (30.2% in 2011) and 27 (4.9%) unspeciated enterococcal isolates (5.9% in 2011). The ratio of *E. faecalis* to *E. faecium* isolates was 3.5 in 2009, 2.5 in 2010, 2.1 in 2011 and 2.7 in 2012. Further surveillance will show whether the declining proportion of *E. faecalis* among enterococcal isolates has now stabilised. 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 was expanded to include imipenem and tigecycline, whereas the breakpoints for interpretation remained unchanged from 2011.

E. faecalis was universally susceptible to ampicillin (Table 39). The prevalence of resistance to ampicillin in *E. faecium* remained stable at 79.8% compared to 81.1% in 2011 and 81.3% in 2010 (Table 40). Imipenem has not previously been surveyed, but as expected the results closely mirrored those for ampicillin.

The prevalence of high-level gentamicin resistance (HLGR) in *E. faecalis* was 25.6% which is unchanged from 26.0% in 2010 (Figure 52), and the prevalence of HLGR in *E. faecium* has apparently stabilised around 45-50%. Almost all (65/68) HLGR *E. faecium* isolates were concomitantly non-susceptible to ampicillin. Conversely, 65 of 118 (55.1%) 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 harbors 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 the western and south-eastern parts of the country. Ten isolates were reported as vancomycin resistant in NORM 2012 (1.8%), but only one *E. faecium* isolate contained transferable glycopeptide resistance confirmed by positive *vanA* PCR. The remaining vancomycin resistant isolates were registered as either *E. gallinarum* (n=5) or *E. casseliflavus* (n=4) which are inherently low-level resistant to vancomycin due to expression of the VanC ligase.

Three enterococcal isolates were verified as linezolid resistant. An *E. faecalis* strain originated from one hospital whereas two *E. faecium* strains were isolated at another. A possible link between the two *E. faecium* isolates is presently being investigated. The two *E. faecium* isolates were both ampicillin resistant, and all three displayed HLGR. None of them were VRE. The emergence of linezolid resistant enterococci is of course ominous as linezolid is often used as an antimicrobial of last resort against multi-resistant strains.

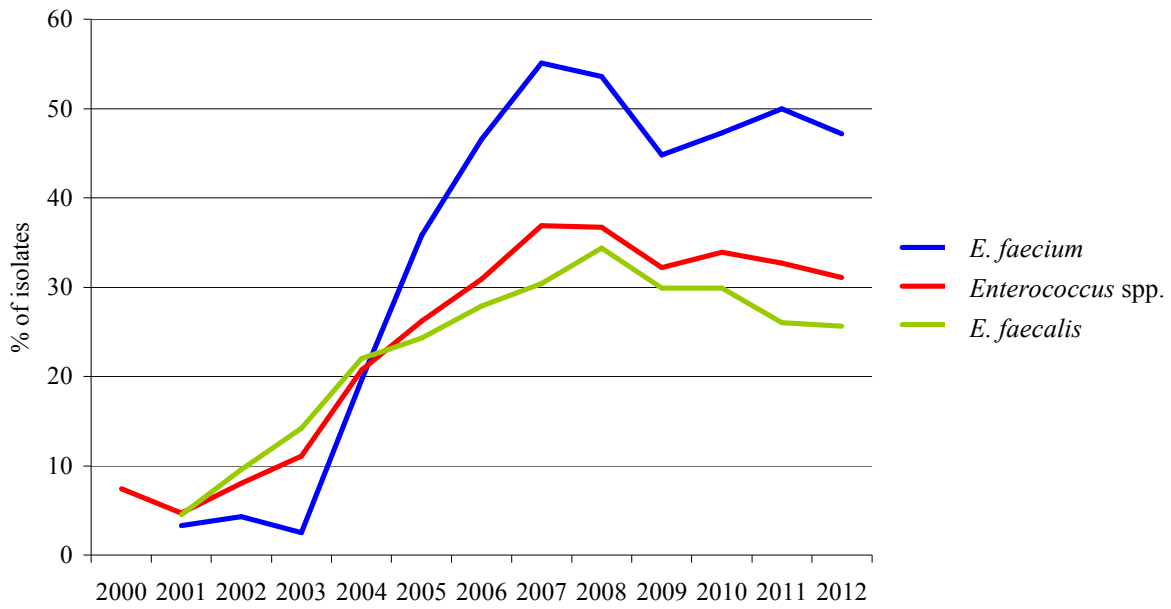


FIGURE 52. Prevalence of high-level resistance to gentamicin in blood culture isolates of *E. faecalis*, *E. faecium* and all enterococci combined during 2000-2012. The breakpoint for high-level resistance was decreased from R ≥ 1,024 mg/L to R > 128 mg/L in 2004.

The effect of antibiotic treatment on normal flora microbes in children with cancer, children with cystic fibrosis, and healthy controls

Antibiotics are essential in the treatment of children with cancer, who undergo anti-cancer chemotherapy that causes severe immunosuppression, and in children with cystic fibrosis (CF) who often develop chronic lung infections. Antibiotics are in fact life-saving for these children, but less is known about the effect of antibiotics on their commensal flora. Several studies have shown a correlation between antibiotic consumption and the development of antibiotic resistance (1). Most studies, however, have been population based and few have followed individual subjects longitudinally.

The main aim of our study was to investigate the consequences of exposure to extensive antimicrobial treatment on the normal flora microbes in children. Our main hypothesis was that children who receive large amounts of antimicrobials (e.g. pediatric cancer patients and children with CF) have a higher occurrence of resistant microbes and a higher *Candida* colonization rate than healthy children with little or no antibiotic exposure. During the years 1999-2001, 2003-2005, and 2006-2008 we included 45 children with cancer, 37 children with CF, and 70 healthy, age-matched controls who all were followed with repeated sampling. In total, 566 throat swabs and 545 fecal samples were collected.

In our *Candida* studies (2,3) we found that colonisation with *Candida* was common (> 50%) in both sick children (cancer and CF) undergoing repeated broad-spectrum antibiotic therapy and healthy children exposed to little or no antibiotic treatment. In fact, there were few significant differences between the two patient groups and the healthy controls. *Candida* colonisation was usually stable over time with only one or two species. *Candida albicans* was the most prevalent *Candida* species, and by using multilocus sequence typing we found that most children were colonised with the same *C. albicans* genotype over time. Certain antibiotics (e.g. amoxicillin, 3rd generation cephalosporins, and azithromycin) seemed to increase both the rate and load of *Candida* colonisation, whereas other antibiotic agents such as phenoxymethylpenicillin, metronidazole, and trimethoprim–sulphamethoxazole (TMS) showed little or no effect. There was no evidence of the development of antifungal resistance over time in any of the three study groups.

We also studied the occurrence of antibiotic resistance over time in fecal enterobacteria of children with cancer compared to healthy controls (4). We found less differences than between the two groups expected. This may, in part, be explained by the fact that the first line treatment for febrile neutropenia in Norwegian pediatric cancer patients was, and still is, the combination ampicillin+gentamicin rather than broader regimens (e.g. 3rd generation cephalosporins) that are used in most other countries. This theory is supported by de Man *et. al* (1) who found in their prospective crossover intervention trial that newborns treated with the combination amoxicillin+ceftazidime had a much greater occurrence of enterobacterial isolates resistant to the treatment regimen than the children treated with the combination penicillin+tobramycin.
We did find some differences, however, between the two pediatric groups in our study. There was a significantly higher occurrence of enterobacteria resistant to 3rd generation cephalosporins in the children with cancer compared to the healthy controls, and we found a significant association between ≥ 10 days treatment with 3rd generation cephalosporins and increased resistance to these antibiotics. We also found significantly increased resistance to TMS in the children who had been treated with TMS long-term (pneumocystis prophylaxis).

E. coli was the most prevalent species, and the occurrence of resistance to various antibiotics correlated well with the NORM data both in the children with cancer and in the healthy controls, e.g. 30 % resistance to ampicillin in both study groups. However, we only found isolates resistant to 3rd generation cephalosporins in the cancer group (n=3). Also, we found a significant species shift to non-*E. coli* species (especially *Klebsiella* sp. and *Enterobacter* sp.) over time in the cancer group compared to the healthy controls.

Finally, among the healthy controls, almost 50 % had never received any antibiotic treatment, whereas all the children with cancer received at least one course of antibiotics, most often multiple courses, during the study period. When comparing the first and last fecal samples, we found that most of the children had different susceptibility patterns over time; only 12 % of the children with cancer and 33 % of the healthy children showed identical antibiotic susceptibility in both samples. This underscores the importance of longitudinal follow-ups in both patients and healthy individuals.
In conclusion, I hope more longitudinal studies following individual subjects (both patients and healthy controls) will be conducted in the future as I believe this can give us valuable insight regarding some of the mechanisms causing antibiotic resistance.

References:

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3. Gammelsrud KW, Lindstad BL, Gaustad P *et al*. Multilocus sequence typing of serial *Candida albicans* isolates from children with cancer, children with cystic fibrosis and healthy controls. Med Mycol 2012; 50: 619-626.
4. Gammelsrud KW Høiby EA, Sandvik L, Brandtzæg P. A longitudinal study of antibiotic resistance in fecal enterobacteria of pediatric cancer patients compared to healthy controls. Submitted 2013.

Karianne Wiger Gammelsrud, Department of Microbiology, Oslo University Hospital, Rikshospitalet.

Streptococcus pneumoniae in blood cultures and cerebrospinal fluids

TABLE 41. *Streptococcus pneumoniae* in blood cultures and cerebrospinal fluids (n=615). 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	≤ 0.064	> 2	93.7	6.3	0.0
Cefotaxime	≤ 0.5	> 2	99.2	0.8	0.0
Ceftriaxone	≤ 0.5	> 2	99.7	0.3	0.0
Erythromycin	≤ 0.25	> 0.5	94.0	0.2	5.8
Clindamycin	≤ 0.5	> 0.5	96.3	-	3.7
Tetracycline	≤ 1	> 2	93.5	0.5	6.0
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	93.5	1.1	5.4
Chloramphenicol	≤ 8	> 8	99.8	-	0.2
Oxacillin screen (mm)	≥ 20	< 20	91.2	-	8.8

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

TABLE 42. *Streptococcus pneumoniae* in blood cultures and cerebrospinal fluids (n=615). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G		0.7	47.8	42.3	2.9	1.3	2.6	0.7	1.8							
Cefotaxime		0.5	47.2	43.1	1.1	5.2	0.8	1.3	0.8							
Ceftriaxone		7.6	65.5	18.2	2.1	4.1	0.5	1.6	0.3							
Erythromycin					11.1	66.3	16.6	0.2	0.2	0.2	0.3	0.7	0.5			4.1
Clindamycin				0.2	11.7	68.9	15.4									3.7
Tetracycline					2.1	87.2	3.9	0.2	0.2	0.5	0.5	2.3	2.4	0.7	0.2	
TMS**					0.3	25.5	59.7	5.2	2.8	1.1	1.1	2.3	0.3	1.6		
Chloramph.										45.7	53.3	0.8	0.2			
Norfloxacin										7.5	66.3	25.4	0.3		0.2	0.3

	< 20	20	21	22	23	24	25	26	27	28	29	30	31	32	33	≥ 34
Oxacillin disk	8.8	0.2	0.2	0.7	0.2	1.1	4.2	6.5	16.4	22.2	16.7	15.6	5.9	0.8	0.5	

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

RESULTS AND COMMENTS

The results are summarized in Tables 41-42 and Figures 53-54. All systemic *S. pneumoniae* isolates submitted to the National Reference Laboratory for Pneumococci at the Norwegian Institute of Public Health during 2012 were included in the surveillance protocol. Thirteen isolates were recovered from cerebrospinal fluids, and seven 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 materials.

Norwegian breakpoints for pneumococci are in accordance with EUCAST, and these remained unchanged in 2012 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 6.3% (39/615) of *S. pneumoniae* isolates were intermediately susceptible to penicillin G. No fully resistant isolates were detected. Three of the non-susceptible isolates were recovered from cerebrospinal fluids and displayed MIC values of 0.25 mg/L. They were thus resistant according to meningitis breakpoints. The prevalence of non-susceptibility to penicillin G has gradually increased from 3.0% in 2010 to 4.3% in 2011 and now 6.3% in 2012. Six penicillin G non-susceptible isolates displayed intermediate susceptibility to cefotaxime and/or ceftriaxone (MIC 1mg/L). No penicillin G susceptible isolates displayed reduced susceptibility to cephalosporins. 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, 15/576 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 (21/39), trimethoprim-sulfamethoxazole (23/39) and tetracycline (20/39).

The prevalence of macrolide non-susceptibility increased to 6.0% in 2012 compared to 4.0% in the two previous years (Figure 54). The macrolide resistance phenotype was further characterised in 23/37 erythromycin non-susceptible isolates. Six (26% of erythromycin non-susceptible isolates, 1.6% of all isolates) displayed a phenotype compatible with efflux-based M-type resistance to erythromycin only. The remaining isolates were either constitutively (n=13, 57% of erythromycin non-susceptible isolates, 3.4% of all isolates) or inducibly (n=4, 17% of erythromycin non-susceptible isolates, 1.0% of all isolates) resistant to clindamycin, thus indicating the presence of *erm*-encoded MLS resistance to both macrolides and lincosamides. The distribution of MLS phenotypes was not significantly altered from 2011 to 2012. The low number of isolates analysed precludes any firm conclusions, but the results may suggest an *erm*-mediated resurgence of macrolide resistance as opposed to the *mef*-dominated peak 2002-2009 (Figure 54). Continuing surveillance should elucidate this issue further.

The 6.5% prevalence of non-suscpetibility to trimethoprim-sulfamethoxazole is a further increase from 3.8% in 2009, 4.3% in 2010 and 5.7% in 2011. Similarly, the prevalence of non-susceptibility to tetracycline has increased from 2.8% in 2010 to 4.2% in 2011 and now 6.5% in 2012 (Figure 53). The vast majority of isolates (99.8%) 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 42) reflects that levofloxacin, moxifloxacin and other "respiratory fluoroquinolones" are not marketed in Norway.

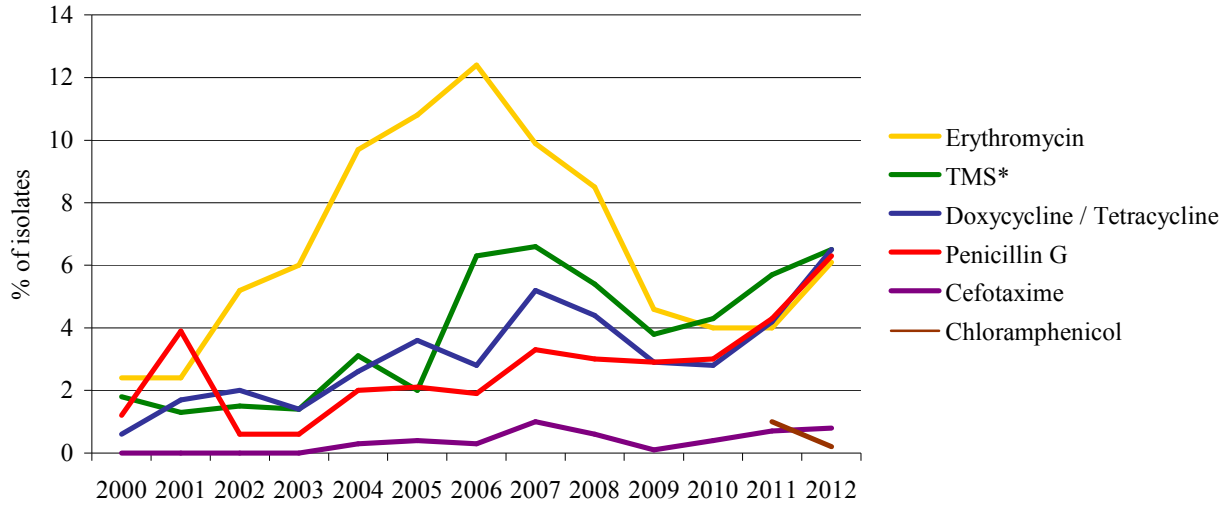

FIGURE 53. Prevalence (%) of non-susceptibility to various antimicrobial agents in *Streptococcus pneumoniae* blood culture isolates during 2000-2012. Doxycycline was substituted by tetracycline in 2005. All results are categorised according to the 2013 breakpoint protocol. *TMS=Trimethoprim-sulfamethoxazole.

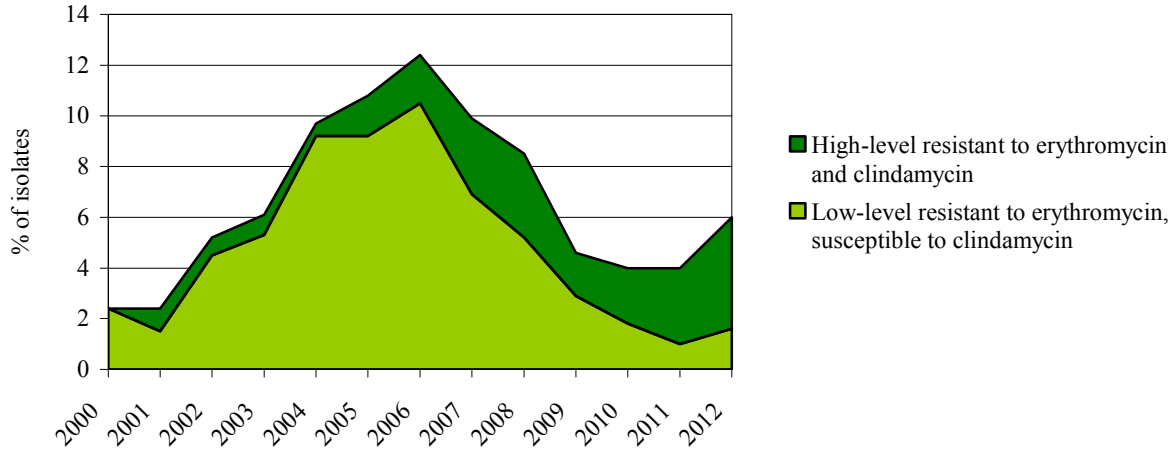

FIGURE 54. Prevalence (%) of non-susceptibility to various antimicrobial agents in *Streptococcus pneumoniae* blood culture isolates during 2000-2012. Doxycycline was substituted by tetracycline in 2005. All results are categorised according to the 2013 breakpoint protocol. *TMS=Trimethoprim-sulfamethoxazole.

Streptococcus pneumoniae in respiratory tract specimens

TABLE 43. *Streptococcus pneumoniae* in respiratory tract specimens (n=392). 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	≤ 0.064	> 2	93.8	5.3	0.9
Cefotaxime	≤ 0.5	> 2	99.2	0.8	0.0
Ceftriaxone	≤ 0.5	> 2	99.5	0.5	0.0
Erythromycin	≤ 0.25	> 0.5	93.8	0.8	5.4
Clindamycin	≤ 0.5	> 0.5	97.4	-	2.6
Tetracycline	≤ 1	> 2	93.4	0.0	6.6
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	93.8	3.1	3.1
Chloramphenicol	≤ 8	> 8	99.7	-	0.3
Oxacillin screen (mm)	≥ 20	< 20	92.3	-	7.7

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

TABLE 44. *Streptococcus pneumoniae* in respiratory tract specimens (n=392). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G	3.1	15.1	51.3	22.7	1.8	2.0	2.3		1.3	0.3	0.3					
Cefotaxime	2.1	11.2	49.5	28.3	3.3	2.3	1.5	1.0	0.5	0.3						
Ceftriaxone	2.3	16.3	57.7	15.1	3.8	1.5	1.5	1.3	0.3	0.3						
Erythromycin				2.8	25.5	52.6	13.0	0.8	0.3		1.0	1.0	0.3	0.3		2.6
Clindamycin			1.5	1.3	16.3	44.4	25.5	8.4	0.3						0.3	2.0
Tetracycline				0.3	2.8	50.8	38.0	1.5			0.5	0.8	1.5	2.8	1.0	
TMS**			0.3	0.5	5.4	36.7	43.9	3.1	4.1	3.1	1.8	0.3	0.5	0.5		
Chloramph.									4.6	79.1	16.1		0.3			
Norfloxacin										4.6	34.4	45.7	14.3	0.8		0.3

	< 20	20	21	22	23	24	25	26	27	28	29	30	31	32	33	≥ 34
Oxacillin disk	7.7	0.0	1.0	1.8	4.1	8.2	11.0	10.7	13.3	13.3	6.1	12.2	3.1	3.3	1.8	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. **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 2009. The prevalence rates of non-susceptibility to various antimicrobials are shown in Tables 43-44 and Figure 55. As for blood culture and cerebrospinal fluid isolates there was an increasing prevalence of penicillin non-susceptibility from 3.8% in 2009 to 6.2% in 2012. Conversely, non-susceptibility to erythromycin has declined from 8.0% in 2007 and 7.6% in 2009 to 6.2% in 2012. The prevalence rates of non-susceptibility to tetracycline (6.6%) and trimethoprim-sulfamethoxazole (6.2%) have remained essentially unchanged from 7.4% and 7.1% in 2009, respectively.

A total of 24/392 isolates (6.2%) were non-susceptible to penicillin G. One isolate was penicillin G resistant (MIC 4 mg/L) while the remaining 23 were intermediately susceptible (MIC 0.125-2 mg/L). The penicillin G resistant isolate as well as two of the penicillin intermediately susceptible isolates were non-susceptible to cefotaxime (MIC 1-2 mg/L) and/or ceftriaxone (MIC 0.5-2 mg/L). All penicillin G non-susceptible isolates were detected by the oxacillin screening test (sensitivity 100%), whereas six fully penicillin G susceptible isolates were classified as oxacillin resistant (specificity 98.4%). Beta-lactam resistant isolates were commonly cross-resistant to other antimicrobial agents such as trimethoprim-sulfamethoxazole (13/24), erythromycin (12/24) and tetracycline (14/24).

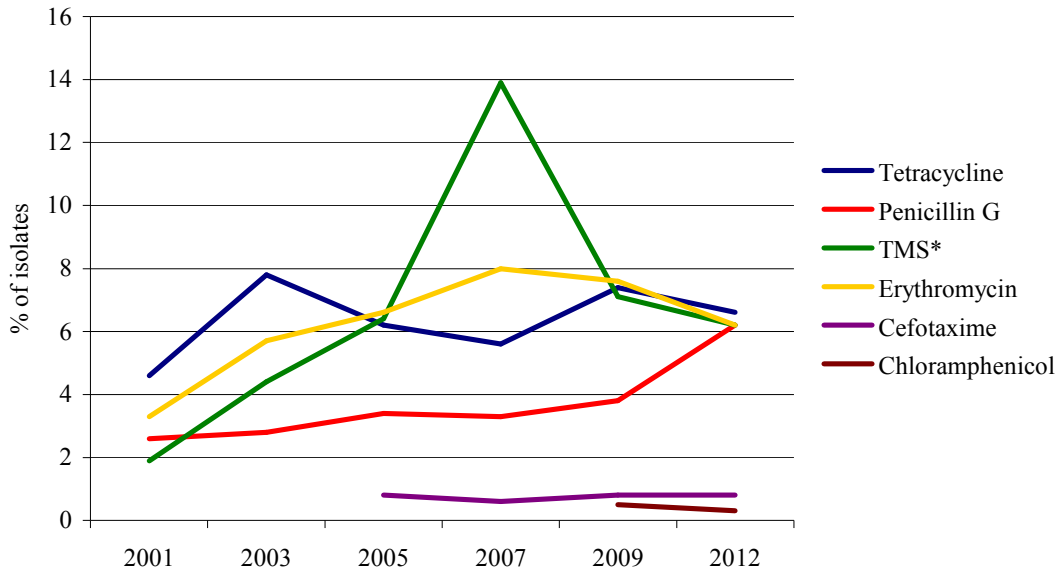

FIGURE 55. Prevalence of non-susceptibility to various antimicrobials in *S. pneumoniae* from respiratory tract samples 2001-2012. Doxycycline was replaced by tetracycline in 2005. *TMS=Trimethoprim-sulfamethoxazole. Please note that x-axis is not to scale.

The peak of macrolide resistant pneumococci observed among blood culture isolates in the last decade was less pronounced among respiratory tract isolates, probably due to differing clonal composition of invasive and respiratory tract isolates reflecting the invasive potential of pneumococcal serotypes. In 2012, 6.2% of respiratory tract isolates were non-susceptible to erythromycin compared to 6.0% in systemic isolates. The macrolide resistant systemic isolates 2002-2009 predominantly contained a *mef*-encoded efflux mechanism whereas *erm*-encoded ribosomal methylation was more common among respiratory tract isolates. The 24 erythromycin non-susceptible isolates in 2012 comprised six M-type isolates (25% of erythromycin non-susceptible isolates, 1.6% of all isolates) as opposed to eight constitutively resistant (33% of erythromycin non-susceptible isolates, 2.1% of all isolates) and ten inducibly resistant (42% of erythromycin non-susceptible isolates, 2.6% of all isolates) MLS_B isolates. Both the overall prevalence of macrolide resistance as well as the distribution of resistance mechanisms in systemic and localised infections are thus much better aligned than in previous years. A total of 12 isolates (3.1%) were concomitantly non-susceptible to penicillin G and erythromycin. This is a minor increase from 1.8% in 2009.

Streptococcus agalactiae in blood cultures and cerebrospinal fluids

TABLE 45. *Streptococcus agalactiae* isolates from blood cultures and cerebrospinal fluids (n=100). 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	88.0	0.0	12.0
Clindamycin	≤ 0.5	> 0.5	93.0	-	7.0
Tetracycline	≤ 1	> 2	24.0	0.0	76.0
Vancomycin	≤ 2	> 2	100.0	-	0.0

TABLE 46. *Streptococcus agalactiae* isolates from blood cultures and cerebrospinal fluids (n=100). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G				1.0	89.0	10.0										
Cefotaxime					43.0	57.0										
Erythromycin					8.0	74.0	6.0		1.0	1.0	1.0	4.0	1.0			4.0
Clindamycin					3.0	81.0	9.0		2.0	1.0						4.0
Tetracycline						21.0	3.0					1.0	5.0	23.0	47.0	
Vancomycin								70.0	30.0							

*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.

RESULTS AND COMMENTS

Streptococcus agalactiae (beta-haemolytic group B streptococci) has previously been included in NORM in 2006 and 2009. All systemic isolates in Norway were referred to the national reference laboratory at St. Olavs Hospital in Trondheim where confirmatory identification and susceptibility testing was performed. Relevant breakpoints have remained unchanged since 2009. A total of 100 consecutive strains were included in the survey in 2012. Twenty-one isolates originated from neonates and small children < 1 year of age. Most isolates were recovered from blood cultures, but there were also two isolates isolated from cerebrospinal fluids. Only one isolate was included from each patient.

As seen in Tables 45-46 there were no isolates with reduced susceptibility to penicillin G, cefotaxime or vancomycin. A total of 12 isolates (12.0%) were resistant to erythromycin compared to 7.7% in 2009. There were no intermediately susceptible strains in the 2012 survey while 10.7% of isolates displayed this phenotype in 2009. When looking at the erythromycin MIC distributions it is notable that the median value has decreased from 0.25 mg/L in 2009 to 0.125 mg/L in 2012. One may suspect that the intermediately susceptible isolates in 2009 represent the upper edge of the wild type distribution. It is therefore most likely that the intermediately susceptible isolates in the 2009 survey should be disregarded and that the prevalence of true macrolide resistance has increased from 7.7% to 12.0% over the three year period.

Five high-level erythromycin resistant isolates were concomitantly resistant to clindamycin, thus indicating constitutive MLS_B resistance. Four additional erythromycin resistant isolates displayed blunting towards clindamycin, thus indicating inducible MLS_B resistance. The remaining three low-level erythromycin non-susceptible isolates were fully clindamycin susceptible, which is compatible with an efflux-mediated M phenotype. A single isolate was recorded as clindamycin resistant (MIC 1 mg/L) in spite of a wild type erythromycin MIC level (MIC 0.094 mg/L). This isolate should be investigated further.

The prevalence of resistance to tetracycline (76.0%) was at the same level as in 2009 (75%) with the majority of isolates displaying MIC values of 16-64 mg/L (Table 46). A subgroup analysis of isolates from neonates and small children < 1 year did not reveal any differences from the overall population.

Mycobacterium tuberculosis

A total of 378 cases of infection with *M. tuberculosis* complex (not BCG) were reported to the Norwegian Surveillance System for Communicable Diseases (MSIS) in 2012. Twenty of the cases had been treated with anti-TB drugs previously. In 18 cases it was not known whether the cases had been previously treated. Four of the seven MDR-TB cases were treated for the first time, two had previously been treated, and for one case this was unknown.

Two hundred and eighty cases were confirmed by culture followed by susceptibility testing of the strain isolated. The results are presented in Table 47. The cases are registered in MSIS the year in which the first culture positive test was taken.

TABLE 47. Antimicrobial susceptibility of 261 isolates of *M. tuberculosis* complex (not *M. bovis* (BCG)) isolated from human infections in 2012. Figures from 2011 in parenthesis.

Origin of birth	No. of cases	No. of isolates	Resistance to antimicrobial agents (No. of isolates)					
			Isoniazid	Rifampicin	Ethambutol	Streptomycin	Pyrazinamid	MDR TB*
Norway	55 (43)	42 (22)	1 (0)	0 (0)	0 (1)	4 (3)	0 (1)	0 (0)
Europe excl. Norway	28 (30)	22 (22)	5 (2)	3 (1)	1 (1)	6 (1)	2 (4)	3 (1)
Asia	116 (112)	82 (86)	7 (11)	2 (0)	0 (0)	9 (15)	5 (2)	1 (0)
Africa	176 (172)	131 (131)	15 (19)	3 (3)	2 (1)	19 (19)	4 (9)	3 (3)
America	3 (2)	3 (1)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
Total	378 (359)	280 (262)	28 (33)	8 (4)	3 (3)	38 (39)	11 (16)	7 (4)
Proportion of resistant isolates (%)			10.0 (12.2)	2.9 (1.5)	1.2 (1.1)	13.6 (14.9)	3.9 (6.1)	2.5 (1.5)

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

Candida spp. in blood cultures

TABLE 48. Antimicrobial susceptibility of *Candida albicans* blood culture isolates (n=127). 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*	≤ 0.125	> 0.125	100.0	-	0.0
Anidulafungin*/**	≤ 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 and micafungin. Strains susceptible to anidulafungin are considered susceptible.

TABLE 49. *Candida albicans* blood culture isolates (n=127). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B							25.2	64.4	10.2								
Fluconazole					2.4	18.1	60.6	18.9									
Voriconazole	14.9	71.7	11.8	0.8		0.8											
Anidulafungin	74.0	22.8	2.4	0.8													
Caspofungin**				3.2	7.9	44.4	40.5	4.0									
Micafungin**	2.4	23.8	65.9	6.3	1.6												

*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 and micafungin. Strains susceptible to anidulafungin are considered susceptible (from 2013 micafungin S ≤ 0.016 mg/L and R > 0.016 mg/L).

TABLE 50. Antimicrobial susceptibility of *Candida glabrata* blood culture isolates (n=23). 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	82.6	17.4
Voriconazole**	≤ 0.125	> 0.125	34.8	-	65.2
Anidulafungin*/***	≤ 0.06	> 0.06	95.7	-	4.3

* Recommended breakpoints by the European Commitee on Antimicrobial Susceptibility Testing - EUCAST.
** There were no EUCAST breakpoints for fluconazol and voriconazol in 2012 as there was insufficient evidence for their use in treating *C. glabrata* infections. The breakpoints given are the new EUCAST breakpoints for fluconazol (2013), and for voriconazole the breakpoints made for *C. albicans*, *C. tropicalis* and *C. parapsilosis* are used.
*** There are no European breakpoints for caspofungin and micafungin. Strains susceptible to anidulafungin are considered susceptible.

TABLE 51. *Candida glabrata* blood culture isolates (n=23). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B							21.7	60.9	17.4								
Fluconazole**									8.7	13.0	34.8	17.4	4.3	4.3			17.4
Voriconazole**			4.3	4.3	8.7	17.4	30.4	13.0			4.3	8.7	8.7				
Anidulafungin		4.3	82.6	8.7				4.3									
Caspofungin***						4.3	78.3	13.0		4.3							
Micafungin***		4.3	74.0	13.0	4.3			4.3									

*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 are no EUCAST breakpoints for fluconazol and voriconazol as there is insufficient evidence for their use in treating *C. glabrata* infection. New fluconazol breakpoints in 2013 S ≤ 0.002 mg/L, R > 32 mg/L.
*** There are no European breakpoints for caspofungin and micafungin. Strains susceptible to anidulafungin are considered susceptible. From 2013 micafungin S ≤ 0.03 mg/L, R > 0.03 mg/L.

TABLE 52. Antimicrobial susceptibility of *Candida tropicalis* blood culture isolates (n=10). 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*	≤ 0.125	> 0.125	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 and micafungin. Strains susceptible to anidulafungin are considered susceptible.

TABLE 53. *Candida tropicalis* blood culture isolates (n=10). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B								40.0	60.0								
Fluconazole							10.0	90.0									
Voriconazole		10.0		40.0	50.0												
Anidulafungin		30.0	70.0														
Caspofungin**							88.9	11.1									
Micafungin**			33.3	55.6	11.1												

*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 and micafungin. Strains susceptible to anidulafungin are considered susceptible. One isolate only tested for anidulafungin. In 2013 EUCAST defined that there is insufficient evidence that *C. tropicalis* is a good target for therapy with micafungin and no breakpoints were defined.

TABLE 54. Antimicrobial susceptibility of *Candida parapsilosis* blood culture isolates (n=7). 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
Voriconazole*	≤ 0.125	> 0.125	100.0	-	0.0

* Recommended breakpoints by the European Commitee on antimicrobial susceptibility testing - EUCAST. Susceptibility testing for anidulafungin is not recommended as this species is a poor target for therapy with this drug. Isolates may be reported as resistant without prior testing. One must assume this also applies to other echinocandins (caspofungin and micafungin).

TABLE 55. *Candida parapsilosis* blood culture isolates (n=7). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B						14.3			85.7								
Fluconazole						14.4	28.6	42.9		14.3							
Voriconazole		57.1	28.6	14.3													
Anidulafungin**			7.7							42.9	28.6		14.3	14.3			
Caspofungin**								14.3	14.3	28.6	42.9						
Micafungin**								14.3	57.1	28.6							

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
** Susceptibility testing for anidulafungin is not recommended as this species is a poor target for therapy with this drug. One must assume this also applies to other echinocandins (caspofungin and micafungin). EUCAST determined breakpoints for anidulafungin (S ≤ 0.002 mg/L, R > 4 mg/L) and micafungin (S ≤ 0.002 mg/L, R > 2 mg/L) in 2013 that categorise the wild type as intermediately susceptible.

RESULTS AND COMMENTS

In 2012, 186 isolates of ten different *Candida* species were isolated from blood stream infections in 178 patients and received at the National Mycology Reference Laboratory, compared to 200 isolates of seven different *Candida* species in 2011. *Candida albicans* is still the most common *Candida* spp. observed (n=127, 68.3%; n=139, 69,5% in 2011) followed by *C. glabrata* (n=23, 12.4%; n=25, 12.4% in 2011), *C. tropicalis* (n=10, 5.4%; n=12, 6% in 2011) and *C. parapsilosis* (n=7, 3,8%; n=13, 6,5% in 2011).

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 results are shown in Tables 48-55.

The *C. albicans* and *C. tropicalis* isolates were susceptible to all drugs tested. Except one *C. kruzei* isolate all isolates were susceptible to amphotericin B (MIC of 4 mg/L).

No unexpected fluconazole resistance was detected and the species identification predicts the susceptibility pattern. There were only two isolates of *C. kruzei* and they were both fluconazole resistant. EUCAST has determined *C. glabrata* breakpoints for fluconazole (S $\leq$ 0.002 mg/L, R > 32 mg/L) valid from 2013, categorising the wild type as intermediately susceptible. When using these new breakpoints 82.5% of our *C. glabrata* isolates were categorised as intermediately susceptible and 17.4% as resistant. When testing voriconazole susceptibility, 18 of the isolates (78.3%) had MIC values $\leq$ 1.0 mg/L which is at the epidemiological cut-off value. Fifteen (65.2%) of the *C. glabrata* isolates had an MIC > 0,125 mg/L, which is the breakpoint of *C. albicans, C. tropicalis and C. parapsilosis.* There is still insufficient evidence that *C. glabrata* is a good target for therapy with voriconazole and no breakpoints have been set. The occurrence of heteroresistance in *C. glabrata* to fluconazole and voriconazole is low (n=3). All *C. glabrata* were susceptible to amphotericin B and, with one exception, also to anidulafungin.

In 2012 there were no European breakpoints for caspofungin and micafungin. Strains susceptible to anidulafungin were considered susceptible to other echinocandins. Cross resistance between the echinocandins is shown in isolates with hot-spot mutations in the target gene, but there may be differences depending on the specific genotype. The clinical significance is not established. EUCAST defined species-specific breakpoints for micafungin in 2013; *C. albicans* stains with MIC $\leq$ 0.016 mg/L are now categorised as susceptible, those with MIC > 0.016 mg/L as resistant, for *C. glabrata* S $\leq$ 0.03 mg/L and R > 0.03 mg/L and for *C. parapsilosis* S $\leq$ 0.002 mg/L and R > 2 mg/L. For other species there is insufficient evidence to indicate whether the wild type population of the pathogen can be considered susceptible to micafungin and there are still no breakpoints. For caspofungin, EUCAST breakpoints have not yet been established. When applying the new micafungin breakpoints to 2012 isolates, 7.9% (n=10) of *C. albicans* isolates considered susceptible had a micafungin MIC > 0.016 mg/L. Six of the isolates had MIC 0.032 mg/L and two isolates had MIC 0.064 mg/L. Among *C. glabrata* isolates, two strains (9%) had a micafungin MIC > 0.03 mg/L which was one isolate more than categorised anidulafungin resistant.

C. parapsilosis and *C. guilliermondii* are known to exhibit higher anidulafungin MIC values than other species. In January 2013 EUCAST modified species-specific breakpoints for *C. parapsilosis* (S $\leq$ 0.002 mg/L, R > 4 mg/L) and categorised the wild type as intermediately susceptible in order to accommodate use of anidulafungin in some clinical situations. Five out of the seven *C. parapsilosis* isolates in 2012 are then categorised as intermediately susceptible and two as resistant. There are new breakpoints for micafungin as well (S $\leq$ 0.002 mg/L, R > 2 mg/L) categorising all isolates in 2012 as intermediately susceptible. *C. parapsilosis* is still not considered as a good target for therapy with caspofungin.

The National Mycology Reference Laboratory uses Etest for MIC determinations and does not routinely perform the EUCAST standardised broth micro dilution method.

Resistance in influenza viruses

The Department of Virology at the Norwegian Institute of Public Health (NIPH) functions as a WHO National Influenza Centre (NIC) and is designated by the Ministry of Health as national reference laboratory for influenza. In the latter function lies also the obligation to monitor and assess the occurrence of resistance. In addition to national monitoring, a selection of influenza viruses that are shipped by European NICs to the WHO Collaborating Centre in the United Kingdom is also tested for antiviral susceptibility there.

Background

Two classes of antiviral drugs are being used against influenza virus infections. M2 blockers inhibit replication of influenza type A viruses, while the more recently developed neuraminidase inhibitors (NIs) inhibit the replication of both type A and B. Historically, resistance has been known to develop quite readily against the M2 blockers. Over the last decade, the prevalence of resistance in A(H3N2) viruses due to the S31N substitution has increased and during the last few years practically all circulating H3N2 viruses are resistant (1). Similarly, from its time of emergence the A(H1N1)pdm09 viruses have been uniformly resistant, also due to S31N.

The more recently developed NIs initially appeared to be much less affected by resistance development, and resistant mutants in general have seemed less viable. Nonetheless, an oseltamivir resistant A(H1N1) virus variant, carrying the neuraminidase mutation H274Y, emerged in 2007 (2,3) and within a year reached almost total predominance among seasonal viruses of this subtype. This global emergence of resistance was discovered first through analysis of viruses from Norwegian influenza surveillance, and it took place with no association to recorded usage of drug. However, as a consequence of the emergence in 2009 of the pandemic influenza A(H1N1)pdm09 virus, the previously circulating H1N1 viruses which were oseltamivir resistant appear to have become extinct.

A substantial peak in NI (primarily oseltamivir) usage during the 2009 A(H1N1)pdm09 pandemic did not lead to detectable emergence of resistant viruses. Also globally, very little oseltamivir resistance has been observed. Nonetheless, toward the end of the 2011 influenza season in Australia, local spread of oseltamivir resistant H1N1pdm09 viruses was observed (4). Apparently, these viruses did not spread beyond the initial area and ceased to circulate with the ending of the season there. No corresponding occurrence of circulating resistant viruses has subsequently been reported. Resistance to the other NI available in Norway, zanamivir, appears to be extremely rare. Community spread of oseltamivir resistant A(H1N1)pdm09 virus still remains a concern, given that data from animal studies suggest that the fitness of the H275Y variant is not significantly compromised and that there indeed was local spread of resistant virus in Australia in 2011.

Surveillance findings

In Norway the 2012/13 season was characterised by the co-circulation of influenza A(H1N1)pdm09, A(H3N2) and B (Yamagata lineage) viruses, with H1N1pdm09 being the most predominant. Influenza B (Victoria lineage) was encountered only sporadically. Findings from Norwegian surveillance are summarised in Table 56. All the A(H1N1)pdm09 and A(H3N2) viruses analysed in 2012/2013 have been susceptible to the neuraminidase inhibitors in the phenotypic assay (MUNANA) and in genotypic analysis. All tested viruses remain resistant to M2 blockers. All influenza B viruses that have been analysed are susceptible to both oseltamivir and zanamivir.

TABLE 56. Norwegian influenza viruses resistant to M2 blockers (adamantanes) and the NIs oseltamivir and zanamivir during the influenza since 2005/06 through 2012/13. Two screening tools were used to determine oseltamivir/zanamivir resistance: sequence analysis of viral genes or a neuraminidase inhibition assay.

	Adamantane resistance		Oseltamivir resistance			Zanamivir resistance		
	A(H1N1)	A(H3N2)	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B
2005/06/07	0% (n=6)	86% (n=14)	0% (n=11)	0% (n=23)	0% (n=21)	0% (n=11)	0% (n=23)	0% (n=21)
2007/08	0% (n=112)	100% (n=2)	68% (n=272)	0% (n=2)	0% (n=59)	0% (n=114)	0% (n=2)	0% (n=59)
2008/09	0% (n=5)	100% (n=65)	100% (n=33)	0% (n=13)	0% (n=1)	0% (n=5)	0% (n=12)	0% (n=1)
2009-pand*	100% (n=258)	100% (n=2)	0% (n=884)		0% (n=11)	0% (n=36)		0% (n=9)
2010/11*	100% (n=54)	100% (n=10)	1.6%** (n=244)	0% (n=1)	0% (n=30)	0% (n=2)	0% (n=1)	0% (n=24)
2011/12*	100% (n=19)	100% (n=56)	0% (n=27)	0% (n=71)	0% (n=5)		0% (n=59)	0% (n=4)
2012/13*	100% (n=5)	100% (n=5)	0% (n=267)	0% (n=15)	0% (n=23)	0% (n=29)	0% (n=15)	0% (n=21)

* Since the 2009 pandemic, all A(H1N1) have been pdm09. ** A(H1N1)pdm with the mutation 275Y commonly associated with oseltamivir resistance, in mixture with wild-type virus. We gratefully acknowledge the provision of antiviral susceptibility data on Norwegian influenza viruses provided by the WHO Collaborating Centre for Influenza Reference and Research, National Institute for Medical Research, London UK.

References:

1. Hungnes O, Dudman SG. Resistance in influenza viruses (Article in Norwegian) Tidsskr Nor Legeforen. 2008 Nov 20;128(22):2601-6.
2. Lackenby A, Hungnes O, Dudman SG, Meijer A, Paget WJ, Hay AJ, et al. Emergence of resistance to oseltamivir among influenza A(H1N1) viruses in Europe. Euro Surveill 2008;13.
3. Hauge SH, Dudman S, Borgen K, Lackenby A, Hungnes O. Oseltamivir-resistant influenza viruses A (H1N1), Norway, 2007-08. Emerg Infect Dis. 2009 Feb;15(2):155-62.
4. Hurt AC et al. Characteristics of a Widespread Community Cluster of H275Y Oseltamivir-Resistant A(H1N1)pdm09 Influenza in Australia. J Infect Dis. 2012 Jul;206(2):148-57.

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 authorised 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 penicillin 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 highly positive (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) has been separated from the total sales.

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

Data sources
In Norway, antibacterials are prescription only medicines, and only allowed sold through pharmacies. This 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 wholesales database covers total sales of antibacterials 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. The Norwegian Institute of Public Health collects the data. Data on drug use from wholesalers is available since the beginning of the seventies.

Data on antibacterial use in hospitals are retrieved from *Sykehusapotekenes Legemiddelstatistikk* (the hospital pharmacies drug statistics database) which is a cooperation of LIS, Legemiddel Innkjøp Samarbeid (Drug Purchasing 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. For 2012, there have been some problems collecting data for a few small hospitals in North Norway and they are, for 2012, not included in the total. The estimated use in these hospitals represents approximately 1.3% of total sales in hospitals.

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

Drug Classification
The data is categorised according to the ATC classification system (1). Defined Daily Doses (DDDs) are employed as units of measurement. The ATC/DDD index of 2013 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 the 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. Of the antimycobacterials, only rifampicin is included (plain and in combinations) and data are presented as total amount 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 (2012). ATC index with DDDs 2013. WHO Collaborating Centre, Oslo.

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

Sampling strategy

Altogether, 53 clinical isolates of *Staphylococcus schleiferi* (of both spp. *coagulans*/*schleiferi* with species identification done by PCR (Sasaki et al 2010)) from dogs obtained at the Norwegian Veterinary Institute between 2009 and 2012 were included for susceptibility testing. *Staphylococcus aureus* from 117 cases of clinical mastitis in cattle were tested for methicillin resistance (one isolate per herd). The isolates were obtained from the laboratories of the Norwegian Veterinary Institute and from the TINE Mastitis Laboratory in Molde. Samples from 175 randomly selected swine holdings were obtained at the farm by staff of the Norwegian Food Safety Authority, and examined for methicillin resistant *Staphylococcus aureus* (MRSA). From each herd, animals were sampled by rubbing a five cm^2 area behind the ear of a total of ten animals with a cloth moistened with sterile water (one sample per herd). The study was anonymous. In addition, faecal boot swab samples were collected from the randomly selected swine holdings. Altogether 169 herds were included (one sample per herd). The boot swab samples were examined for *E. coli* producing extended-spectrum beta-lactamase (ESBL). The indicator *E. coli* from poultry breeders (i.e. parent animals from different poultry production lines) were collected from samples obtained by the Norwegian *Salmonella* control programme for live animals. From each flock a piece of a boot swab was analysed. A total of 165 samples were collected. The samples were also used for selective isolation of ESBL producing *E. coli*. In addition, *E. coli* isolates were obtained from broiler meat samples bought at retail. The broiler meat was from the three biggest Norwegian broiler meat producers. Altogether 205 samples were collected, and the samples were also used for selective isolation of ESBL producing *E. coli*. *E. coli* was collected from 134 faecal samples from three wild reindeer populations, and the samples were also used for selective isolation of ESBL producing *E. coli*. The samples were obtained from a research project.

Indicator isolates of *E. coli*

Sample material, i.e. boot swabs from poultry breeders and swine holdings was mixed with sterile distilled water prior to plating onto the surface of lactose-bromthymol blue agar and incubated at 37°C for 24h. *E. coli* from poultry meat was isolated by plating out on lactose-bromthymol blue agar plates after enrichment in MacConkey broth (see description under ESBL producing *E. coli*). Faecal samples from reindeer was plated directly onto lactose-bromthymol blue agar and incubated at 37°C for 24h. Typical colonies were subcultured on blood agar (Heart infusion agar, Difco) containing 5% bovine blood and incubated at 37°C for 24h. Colonies were identified as *E. coli* by typical colony appearance, lactose fermentation, positive indole reaction, and negative citrate and oxidase.

ESBL producing *E. coli*

From the suspension of sample material (poultry breeders and swine) and sterile distilled water used for *E. coli* isolation, 0.1 ml was plated onto MacConkey agar (Difco) containing 1 mg/L cefotaxime and MacConkey agar containing 2 mg/L ceftazidime. Meat samples from poultry of approximately 5 g were cultured in MacConkey broth for 24 h at 41°C before plating out on MacConkey agar plates with 1 mg/L cefotaxime and MacConkey agar plates with 2 mg/L ceftazidime. Sample material from reindeer was plated directly onto the selective plates. The agar plates were incubated at 37°C for 24-48h. Positive colonies were selected, and the isolates confirmed as *E. coli* using API 20 E (bioMérieux) or PCR (Heininger 1999). Presumptive ESBL positive *E. coli* were further investigated by disk diffusion (Beckton Dickinson), PCR (Pérez-Pérez et al, 2002) and DNA sequencing.

Methicillin resistant *Staphylococcus aureus* (MRSA)

Screening for MRSA from swine was performed by incubation of sample material in Mueller-Hinton broth with 6.5% NaCl. After incubation 1 mL was transferred to 9 mL Tryptone-Soya broth with 75 mg/L aztreonam and 3.5 mg/L cefoxitin and incubated at 35°C for 16-20h, followed by plating on Brilliance MRSA agar plates (Oxoid) (Technical specifications on the harmonized monitoring and reporting of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* in food-producing animals and food (EFSA journal 2012: 10 (10):2897). Suspected colonies were subjected to further identification including PCR for detection of the *mec*A/*nuc* genes (Poulsen 2003, Stegger 2012).

Susceptibility testing

Isolates were tested for antimicrobial susceptibility at NVI, Oslo. MIC values were obtained using the VetMIC™ microdilution method (Dep. of Antibiotics, National Veterinary Institute, Sweden). Epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 05.06.2012) were used, except for ciprofloxacin for *E. coli* and trimethoprim for *S. schleiferi*. For these exceptions, and for additional antimicrobial agents not defined in EUCAST recommendations, cut-off values were defined on the basis of MIC distributions obtained in the NORM-VET programme (see also Appendix 6).

Quality assurance systems

The following susceptible bacteria were included as quality controls on a regular basis: *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213. 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, *S. aureus* CCUG 35603. 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 (EU CRL for Antimicrobial Resistance in Denmark).

Data processing

Susceptibility data were recorded and stored in the sample registration system at NVI as discrete values (MIC). The data management and analysis were performed in SAS v 9.3 (SAS Institute Inc., Cary, NC, USA) and the 95% confidence intervals were calculated by the exact binomial test using R v. 2.7.1 (R Development Core Team, 2008).

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

Sampling strategy - animals

Salmonella
Samples from animals were collected according to the Norwegian *Salmonella* control programme for live animals. Additional isolates were obtained from animals from clinical submissions or necropsies at the Norwegian Veterinary Institute (NVI). One isolate of each serovar per incident was included for susceptibility testing.
In addition, isolates of *Salmonella* spp. from reptiles collected during the period 2010-2012 were included.

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.

A total of 269 human *Campylobacter* isolates were obtained from clinical specimens. Five regional laboratories submitted the first five independent isolates each month to the National Reference Laboratory for Enteropathogenic Bacteria at the Norwegian Institute of Public Health.

Isolation and identification of bacteria
Isolation and identification of *Salmonella* spp. from animals was carried out at the NVI according to ISO 6579:2002/Amd.1:2007: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.
The reference analyses on human 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
Isolates from animals were tested for antimicrobial susceptibility at NVI, Oslo. MIC values were obtained using the VetMIC™ microdilution method (Dep. of Antibiotics, National Veterinary Institute, Sweden).

Salmonella spp., *Yersinia* spp. and *Shigella* spp. isolates from humans were susceptibility tested at the NRL for Enteropathogenic Bacteria at 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), by the EUCAST protocol.

For animal isolates, epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 05.06.2012) 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 (see also Appendix 6).
For human isolates EUCAST clinical breakpoints version 3.1 2013 were used if established, otherwise epidemiological cut-off values were used.

Quality assurance systems
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 two external quality assurance programmes: For veterinary pathogens (Veterinary Laboratories Agency Quality Assurance Unit Loughborough, UK) and for resistance monitoring (EU CRL for Antimicrobial Resistance in Denmark).

The NRL for Enteropathogenic Bacteria at 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
Susceptibility data were recorded and stored in the sample registration system at NVI. The susceptibility data were stored as discrete values (MIC). The data management and analysis were performed in SAS v 9.1 (SAS Institute Inc., Cary, NC, USA) and the 95% confidence intervals were calculated by the exact binomial test using R v. 2.7.1 (R Development Core Team, 2008).

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

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 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. 2012 was the thirteenth year of surveillance, and all 21 diagnostic laboratories in Norway participated in the surveillance system in addition to seven 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. 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 2012 were as follows: *E. coli* in blood cultures (6 months); *Klebsiella* spp., *Staphylococcus aureus* and *Enterococcus* spp. in blood cultures (9 months); *Streptococcus agalactiae* from sterile sites (100 first consecutive isolates); *Streptococcus pneumonia* from blood cultures and cerebrospinal fluids, and *Candida* spp. in blood cultures (12 months); S*treptococcus pneumoniae* 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* from all samples (12 months); influenza viruses from respiratory tract samples (throughout season). Influenza viruses and *S. pneumoniae* from blood cultures and cerebrospinal fluids were further analysed at the the Norwegian Institute of Public Health in Oslo. *Candida* spp. from blood cultures were further analysed at Oslo University Hospital, Rikshospitalet. *Streptococcus agalactiae* and MRSA were further analysed at St. Olav University Hospital in Trondheim. ESBL-producing *Enterobacteriaceae* were genetically characterised at University Hospital of North Norway in Tromsø. *M. tuberculosis* was further 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 breakpoints with few exceptions as explained in the text. All *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 the clover leaf test. All *Enterococcus* spp. isolates were screened for glycopeptide resistance using a vancomycin 6 mg/L BHI agar. *S. pneumoniae* and *S. agalactiae* isolates were susceptibility tested using MIC gradient tests (bioMerieux) on MH II agar supplemented with 5% lysed horse blood. Susceptibility testing of *Candida* spp. isolates was performed by MIC gradient tests using RPMI agar containing 2% glucose and MOPS. All resistance values were recorded either as mm inhibition zone sizes or MIC values in order to monitor trends in the occurrence of resistance.

For *M. tuberculosis*, all isolates were tested using the BACTEC 460 or BACTEC MGIT 960 systems. All three test laboratories participate in the WHO external DST quality control programme. The same three laboratories and Haukeland University Hospital also perform tests for mutations in *rpoB* gene to detect resistance to rifampicin.

Confirmation of resistance phenotypes

E. coli and *Klebsiella* spp. with reduced susceptibility to third generation cephalosporins were examined for ESBL production using ESBL combination MIC gradient tests according to the instructions of the manufacturer (bioMerieux). 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 for confirmation of VRE. Erythromycin resistant *S. pneumonia*, *S. agalactiae* 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), *C. albicans* ATCC 10231.

Data processing

The specially designed eNORM computer programme was used for the registration and storage of patient data, sample data and resistance data. The results were further analysed by WHONET 5.3 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:
Cut-off values NORM-VET

Epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 13.05.2013) 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*	*Staphylococcus schleiferi**
Ampicillin	> 8	■	■	
Cefotaxime	> 0.25		■	
	> 0.5	■		
Ceftazidime	> 0.5		■	
Cefalotin	> 1			■
Chloramphenicol	> 16	■	■	■
Ciprofloxacin	> 0.06	■	■	
	> 1			■
Clindamycin	> 0.25			■
Colistin	> 2		■	
Erythromycin	> 1			■
Florfenicol	> 16	■	■	
Fusidic acid	> 0.5			■
Gentamicin	> 2	■	■	■

Antimicrobials	Resistant MIC (mg/L)	*Salmonella* spp.	*Escherichia coli*	*Staphylococcus schleiferi**
Kanamycin	> 8		■	■
	> 16	●		
Nalidixic acid	> 16	■	■	
Oxacillin	> 2			■
Sulfamethoxazole	> 64		■	
	> 256	●		
Streptomycin	> 16	■	■	
Tetracycline	> 1			■
	> 8	■	■	
Trimethoprim	> 2	■	■	
	> 8			●

Squares: Cut-off values recommended by EUCAST
Filled circles: Cut-off values not defined by EUCAST - defined on the basis of the actual MIC distributions obtained in the NORM-VET programme.
*For *Staphylococcus schleiferi* the cut-off values for *Staphylococcus aureus* was used.

Appendix 7: 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.

	MIC values mg/L																
Antimicrobials	S	R	*Escherichia coli*	*Klebsiella* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp.	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumonia*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
Amphotericin B	≤ 1	> 1											■	■	■	■
Ampicillin	≤ 4	> 8								■						
	≤ 8	> 8	■		■		■									
						■#										
Amoxi-Clav*	≤ 8	> 8	■													
Anidulafungin	≤ 0.03	> 0.03											■			
	≤ 0.06	> 0.06												■	■	
Cefepime	≤ 1	> 4	■	■												
Cefoxitin									■#							
Cefotaxime	≤ 0.5	> 0.5										■				
	≤ 0.5	> 2									■					
	≤ 1	> 2	■	■												
Ceftazidime	≤ 1	> 4	■	■												
Ceftriaxone	≤ 0.5	> 2									■					
Cefuroxime	≤ 0.5	> 8	■	■												
Chloramphenicol	≤ 8	> 8			■		■				■					
						■#										
Ciprofloxacin	≤ 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											■		■	■
Fusidic acid	≤ 1	> 1							■							
Gentamicin	≤ 1	> 1							■							
	≤ 2	> 2						■#								
	≤ 2	> 4	■	■												
	≤ 128	> 128								■						

Antimicrobials	MIC values mg/L S	MIC values mg/L R	*Escherichia coli*	*Klebsiella* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp.	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumoniae*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
Imipenem	≤ 4	> 8								■						
Linezolid	≤ 4	> 4							■	■						
Mecillinam	≤ 8	> 8	■	■												
Meropenem	≤ 2	> 8	■	■												
Mupirocin	≤ 1	> 256							■#							
Nalidixic acid					■#	■#	■#	■#								
Nitrofurantoin	≤ 64	> 64	■													
Norfloxacin	≤ 4	> 4							■#							
Oxacillin											■#					
Penicillin G	≤ 0.064	> 2									■					
	≤ 0.25	> 0.25										■				
Piperacillin-Tazo**	≤ 8	> 16	■	■												
Quinupristin-dalfopristin	≤ 1	> 2							■							
Rifampicin	≤ 0.06	> 0.5							■							
Tetracycline	≤ 1	> 2							■		■	■				
	≤ 2	> 2			■#	■#	■#	■								
Tigecycline	≤ 0.25	> 0.5								■						
	≤ 0.5	> 0.5							■							
	≤ 1	> 2	■													
Trimethoprim	≤ 2	> 4	■	■												
TMS***	≤ 1	> 2									■					
	≤ 2	> 4	■	■	■	■#	■		■							
Vancomycin	≤ 2	> 2										■				
	≤ 4	> 4								■						
Voriconazole	≤ 0.125	> 0.125											■	■	■	■

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

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