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Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway

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The NORM and NORM-VET programmes are part of the
Norwegian Government's Action Plan against Antimicrobial Resistance issued in 2000.

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 by antimicrobial usage 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 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 antimicrobial usage 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 in Sweden, Belgium, Luxembourg and Italy. 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 usage and resistance in both human and veterinary medicine and have 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 emphasized. The action plan recognized 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 surveillance of both resistance and drug usage was again emphasized. A combined action plan for antimicrobial resistance and infection control 2007 – 2011 will be issued later in 2007.

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 National Veterinary Institute. The usage of antimicrobial agents in humans and animals is based on wholesalers' data reported to the Norwegian Institute of Public Health. This reporting was made mandatory from January 1, 2002. Data on the usage of feed additives, including antimicrobial and coccidiostatic growth promoters, are collated at the Norwegian Food Safety Authority.

This report, which is the seventh annual joint report from NORM and NORM-VET, presents data for 2006. 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 antimicrobial usage and occurrence of resistance in Norway. The NORM and NORM-VET programmes are valuable tools for setting policies, assessing risks and evaluating interventions.

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Tromsø / Oslo, September 2007

II. SAMMENDRAG

Dette er den syvende felles rapporten fra Norsk overvåkingssystem for antibiotikaresistens hos mikrober (NORM) og Norsk overvåkingsprogram for antibiotika-resistens i mikrober fra fôr, dyr og næringsmidler (NORM-VET). Rapporten presenterer data over forekomst av antibiotika-resistens og forbruk av antibiotika til mennesker og dyr i 2006. Data fra relevante prosjekter som ikke er med i de kontinuerlige overvåkings-programmene, 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 Zoonose-senteret 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 antibiotika til terapeutisk bruk på landdyr var 6 448 kg i 2006. Fra 1995 til 2001 ble salget av veterinære antibiotika til landdyr redusert med ca 40%. Etter dette har forbruket holdt seg konstant på noenlunde samme nivå. Forbruksmønsteret har i løpet av perioden utviklet seg i gunstig retning, det vil si at andelen penicillinbruk har økt. Rene penicillin-preparater utgjorde 41% av salget av veterinære antibiotika til landdyr i 2006, og av dette var 77% beta-laktamase følsomme penicilliner. Forbruket av tetracykliner utgjorde kun 4%. Nedgangen i antibiotikaforbruket og endringene i forskrivnings-mønsteret skyldes først og fremst at husdyrnæringen i andre halvdel av 1990-årene gjennomførte systematiske kampanjer for å redusere forbruket av antibiotika til dyr samt kampanjer for riktig bruk av antibiotika.
Totalsalget av veterinære antibiotika til terapeutisk bruk til oppdrettsfisk i Norge var på 1 428 kg aktiv substans i 2006. Kinoloner utgjorde 79% av salget i 2006. Forbruket av antibiotika i oppdrettsnæringen har blitt redusert med 97% siden 1987. Denne betydelige reduksjonen kan tilskrives innføringen av effektive vaksiner til laks og ørret samt sykdomsforebyggende tiltak, herunder bedrede miljøforhold. I 2006 ble 40 % av all antibiotika rekvirert til oppdrettsfisk brukt til torsk, 25% til ørret, 22% til laks og og 13% til andre arter. Det årlige forbruket av antibiotika til torsk i perioden 2000-2006 er godt korrelert med produksjonen av oppdrettstorsk i samme periode.
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. Salgstallene, i kg aktiv substans, er noe høyere enn før forbudet, noe som kan forklares ved økt produksjon av broilere. Forbruks-mønstret for koksidiostatika er vesentlig endret etter 1996, fra monensin til narasin, som nå utgjør hovedparten av forbruket av de ionofore koksidiostatika.

Forbruk av antibiotika hos mennesker

Totalsalget av antibiotika til systemisk bruk hos mennesker var 19,0 DDD/1000 innbyggere/dag i 2006. Det samlede forbruket har vært forholdsvis stabilt gjennom mange år, men fra 2005 har vi sett en økning, hovedsakelig grunnet penicillingruppen og metenamin. Også salget av tetracykliner, makrolider og kinoloner øker, mens salget av sulfonamider og trimetoprim er synkende.
I 2006 utgjorde penicilliner 42% av det totale antibiotika-forbruket i Norge. Det har skjedd en forskyvning mot bredspektrede penicilliner, men samtidig var forbruket av penicillinase følsomme penicilliner høyere i 2006 enn i de foregående år. Tetracykliner utgjorde 17% av total-forbruket. Salget av denne medikamentgruppen har vært synkende siden 1993, men har økt i 2005 og 2006. Makrolider, linkosamider og streptogramin utgjorde 12% av totalforbruket i 2006. Forbruket var forholdsvis stabilt gjennom 1990-tallet, men har økt siden 2000. Erytromycin utgjorde 55% av salget i denne gruppen. Salget av kefalosporiner, monobaktamer og karbapenemer har økt over de siste år, men er fortsatt på et relativt lavt nivå. Denne medikamentgruppen utgjorde 3% av totalsalget i 2006. Kinoloner utgjorde kun 3% av total-forbruket, men salget har økt med 88% siden 1999. Det urinveisantiseptiske middelet metenamin utgjorde 14% av totalforbruket, og salget har økt med 39% siden 1999.

Bruken av antibakterielle midler varierer mellom de ulike fylkene, og fordelingen av områder med høyt og lavt forbruk er relativt stabil over tid. Salget av antibakterielle midler til sykehus og allmennpraksis utgjorde henholdsvis 8% og 92%. Penicilliner sto for 48% av salget i sykehus og 41% i allmennpraksis. Andre viktige medikament-grupper utenfor sykehus var tetracykliner (18%) og makrolider/linkosamider (12%). På sykehus var kefalosporiner (22%), kinoloner (7%) og metronidazol (6%) mest brukt etter penicilliner.

Resistens hos indikatorbakterier fra dyr og mat

Forekomsten av ervervet antibiotikaresistens blant bakterier som utgjør den normale tarmfloraen, kan tjene som en indikator på selektivt antibiotikapress i ulike populasjoner. I NORM-VET benyttes *Escherichia coli* og *Enterococcus* spp. som indikatorbakterier. I 2006 ble indikatorbakterier fra broiler (faeces og kjøtt) inkludert. Forekomsten av resistens var moderat og i et internasjonalt perspektiv lav. Forekomsten av resistens blant isolater fra faeces og kjøtt var på samme nivå. Totalt var 81,2% av alle *E. coli* isolatene følsomme for alle antibiotika som ble undersøkt. Det ble hyppigst påvist resistens mot ampicillin, etterfulgt av resistens mot sulfamethoxazol og tetracyklin/streptomycin. Et enkelt isolat viste nedsatt følsomhet for ceftiofur og cefotaxim. Ytterligere undersøkelser viste at dette isolatet innholdt et ESBL kodende gen. Dette er første gangen ESBL er påvist i norsk dyrehold. Funnet viser at gener som koder for ESBL, kan forekomme i normalfloraen hos friske dyr som ikke har vært eksponert for bredspektrede betalaktamer.

Totalt var 22,7% av enterokokkisolatene følsomme for alle antibiotika som ble undersøkt. *E. faecium* utgjorde flertallet av isolatene. *E faecium* var hyppigst resistent mot narasin og i mindre grad mot bacitracin, erytromycin og tetracyklin, mens forekomsten av vankomycinresistens var

svært lav. Seleksjonspresset som oppstår ved bruk av narasin som fôrtilsetning i broilerproduksjonen er sannsynligvis årsaken til at narasinresistens forkommer hyppig blant enterokokker fra broiler. Det var en signifikant reduksjon i forekomsten av vancomycin-resistens ved bruk av selektiv isolasjonsmetode sammenlignet med tidligere rapporter. Dette vil bli fulgt opp i fremtiden.

Resistens hos zoonosebakterier og andre enteropatogene bakterier

I 2006 ble det gjort resistensbestemmelse av 13 *Salmonella* spp. isolater fra norske dyr. Syv av isolatene var *S*. Typhimurium fra storfe, fjørfe, hund, katt, hest, og en villfugl. De øvrige seks isolatene var *S*. Kedugo, *S. diarizonae* (38:r:z) og *S*. Mikawasima fra hester, *S*. Montevideo fra hund, *S*. Anatum fra fjørfe, og *S*. Dublin fra svin. Kun et enkelt isolat fra fjørfe var multi-resistent. Videre var et *S*. Anatum isolat fra fjørfe resistent mot tetracykliner, de øvrige isolatene var følsomme for alle de undersøkte antibiotika.

I tillegg ble 97 isolater av *Salmonella* spp. fra diagnostiske innsendelser fra årene 1997-2006 fra reptiler i dyreparker etc. resistensbestemt. Forekomsten av resistens var lav blant disse isolatene. Resultatene indikerer at resistens ikke er utbredt blant *Salmonella* som av og til blir isolert fra norske dyr.

Av de humane salmonellose-tilfellene som ble rapportert i 2006, var 79,1% oppgitt å ha blitt smittet i utlandet. Andelen *S*. Typhimurium isolater som var følsomme for alle antibiotika, var høyere for kategorien "smittet i Norge" (63,7%) enn for kategorien "smittet i utlandet" (34,7%). Multiresistens definert som resistens mot to eller flere antibiotika, ble hyppigere påvist hos de utenlands-smittede (43,4%) enn hos de innenlandssmittede (26,6%).

Resultatene for *S*. Typhimurium 2001-2006 indikerer en økende forekomst av resistens mot tetracykliner og ampicillin. De to siste åren har imidlertid situasjonen vært stabil.

Forekomsten av antibiotikaresistens var betydelig lavere blant *S*. Enteritidis enn blant *S*. Typhimurium med unntak av nalidiksinsyre. Til sammen 18,7% av *S*. Enteritidis isolatene var resistente mot nalidiksinsyre. Forekomsten av resistens blant *S*. Enteritidis var på samme nivå i 2006 som i tidligere rapporter fra NORM/NORM-VET.

Resultatene fra 2006 viser at forekomsten av resistens hos *Campylobacter jejuni* fra norske broilere fremdeles er lav og stabil. Hele 91,6% av isolatene var følsomme for alle undersøkte antibiotika, 6,5% var resistente mot ett antibiotikum (ampicillin) og 1,9% mot både nalidiksinsyre og enrofloxacin.

Forekomsten av resistens og resistensmønstrene hos *C. jejuni* fra norske broilere samsvarer med *C. jejuni* fra mennesker smittet i Norge med unntak av høyere forekomst av kinolonresistens blant isolatene fra mennesker. Dette forholdet ble også påvist i tidligere rapporter. Resistens var betydelig mer utbredt blant *C. jejuni* fra pasienter smittet i utlandet (68,9% resistente mot minst ett antibiotikum) enn hos pasienter smittet i Norge (12,9%). Forskjellen kan forklares med høyere forekomst av resistens mot ciprofloxacin/nalidiksinsyre (58.8% / 62.2% versus 0% / 6.5% og mot tetracyklin 43.7% versus 0%), for henholdsvis utenlandssmittede og innelands-smittede.

Forekomsten av resistens hos *C. jejuni* fra pasienter smittet i Norge så vel som utenlandssmittede var stabilt for perioden 2001-2006 med unntak av gentamicinresistens som viste en signifikant økning blant de få infeksjoner som var smittet i Norge i 2006. De aller fleste *Shigella*-isolatene var fra pasienter smittet utenfor Norge. Antibiotikaresistens var utbredt i *Shigella* slik det også rapporteres fra andre land.

Resistens hos kliniske isolater fra mennesker

Forekomsten av antibiotikaresistente kliniske bakterie-isolater fra mennesker var fortsatt meget lav i 2006. Det ble påvist 2 tilfeller av meticillinresistente *Staphylococcus aureus* (MRSA) blant 750 blodkulturisolater (0,3%) som ble inkludert i NORM-protokollen, og kun 3 av 1261 (0,2%) *S. aureus* isolater i laboratorienes datasystemer ble rapportert som MRSA. Fire av 1277 (0,3%) *S. aureus*-isolater fra blodkultur og spinalvæske var MRSA i 2006. Denne andelen er stabil fra tidligere år. Meldesystemet for infeksjonssykdommer (MSIS) registrerte imidlertid 333 tilfeller av MRSA-infeksjon i 2006 hvilket er en økning fra 260 i 2005. Hele 271 av disse tilfellene var pasienter med sårinfeksjoner og abscesser. MRSA utgjør fortsatt en svært liten andel av *S. aureus* isolatene fra slike prøver (4/1135, 0,4%). MSIS registrerte videre 270 tilfeller av MRSA-kolonisering i 2006. Det totale antallet MRSA-meldinger på 603 var en 32% økning fra 2005. Det er således en vedvarende risiko for videre MRSA-spredning i Norge.

Blodkulturisolater av *E. coli* og *Klebsiella* spp. var som tidligere stort sett følsomme for bredspektrede antibiotika. Forekomsten av resistens/nedsatt følsomhet for gentamicin var 2,3% hvilket er uendret fra 2005 (2,5%). Økningen av resistens/nedsatt følsomhet for ciprofloxacin fortsatte fra 3,3% i 2004 og 5,0% i 2005 til 6,4% i 2006. Det er en klar samvariasjon mellom forbruket av fluorokinoloner og nedsatt følsomhet for denne medikamentgruppen. *Klebsiella* spp. hadde lavere forekomst av resistens mot aminoglykosider og fluorokinoloner enn *E. coli*. Produksjon av bredspektrede beta-laktamaser (ESBL) er blitt et utbredt problem i mange land, og enkelttilfeller er også blitt rapportert fra Norge. Til sammen 11/1043 *E. coli* (1,1%) og 2/396 (0,5%) av *Klebsiell*a spp. fra blod-kulturer hadde denne fenotypen. For *E. coli* er dette en økning sammenlignet med 0,7% i 2004 og 0,5% i 2005. Andelen ESBL positive isolater var høyere blant *E. coli* fra blodkulturer (1,1%) enn fra urinprøver (0,3%). Forekomsten av resistens hos *Proteus mirabilis* fra urinprøver var på samme nivå som hos tilsvarende *E. coli* isolater bortsett fra at *P. mirabilis* er iboende resistent mot nitrofurantoin.

Det ble ikke påvist klinisk signifikant vankomycin-resistens i enterokokker i 2006. Forekomsten av nedsatt følsomhet for ampicillin i *Enterococcus faecium* ligger nå stabilt over 80 % (82,5% i 2005 og 81,0% i 2006), og høygradig gentamicinresistens ble påvist i 27,9% av *E. faecalis* og 46,6% av *E. faecium*. De aller fleste (26 av 27) *E. faecium*-isolater med høygradig gentamicinresistens hadde samtidig nedsatt følsomhet for ampicillin. Alle enterokokkisolater var følsomme for linezolid.

Streptococcus pneumoniae fra blodkulturer var generelt følsomme for alle relevante antibiotika. Tretten av 688 isolater (1,9%) hadde nedsatt følsomhet for penicillin G, og to av disse hadde også redusert følsomhet for cefotaxim. Forekomsten av makrolidresistens fortsatte å øke fra 9,7% i 2004 og 10,8% i 2005 til 12,4% i 2006.

Forekomsten av makrolidresistens er spesielt høy blant isolater med nedsatt følsomhet for penicillin G (5 av 13). *Streptococcus pyogenes* (betahemolytiske streptokokker gruppe A) fra blodkulturer, halsprøver og sår var uten unntak følsomme for penicillin G. Forekomsten av makrolidresistens har holdt seg stabil fra tidligere år og var på 1,6% i 2006. Systemiske isolater av *Streptococcus agalactiae* (betahemolytiske streptokokker gruppe B) var følsomme for penicillin G, cefotaxim og vankomycin. Det ble imidlertid påvist en opphopning av makrolidresistente GBS i aldersgruppen under 1 år. Dette blir undersøkt videre ved referanselaboratoriet for GBS på St. Olavs Hospital.

I alt 294 tilfeller av tuberkulose ble meldt til MSIS i 2006. Det ble utført resistensbestemmelse av 216 *Mycobacterium tuberculosis* isolater fra pasienter som ikke hadde blitt behandlet for tuberkulose tidligere. Kun et enkelt isolat fra Europa utenom Norge ble klassifisert som multiresistent. Det ble også gjort resistensbestemmelse av ni isolater fra pasienter som tidligere var blitt behandlet for tuberkulose. To isolater fra Europa utenom Norge var multiresistente, og ett isolat fra en afrikansk pasient var resistent mot isoniazid.

Det ble utført resistensbestemmelse av 161 blodkulturisolater av *Candida albicans* (112), *C. glabrata* (34) og *C. tropicals* (15). Alle isolater av *C. albicans* og *C. tropicalis* var følsomme for fluconazole og voriconazole. Til sammen 44,1% av *C. glabrata* var intermediært følsomme for eller resistente mot fluconazole. Alle gjærsoppisolatene var følsomme for amphotericin B og caspofungin. Resultatene er i overensstemmelse med tidligere studier fra Norge.

Overvåking av HIV-resistens ble rapportert for første gang i 2006. Det ble gjort resistensbestemmelse av virusisolater fra 118/275 nydiagnostiserte HIV positive (42,9%), og 14/118 (11,9%) hadde én eller flere mutasjoner som kan redusere effekten av antiretroviral behandling.

Konklusjon

Antibiotikaresistens er fortsatt et begrenset problem i Norge både når det gjelder 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 når det gjelder 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 resistens fra andre land. Det er derfor nødvendig med fortsatt aktiv innsats for å sikre at vi også i fremtiden kan gi tilfredsstillende 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 seventh joint report from the NORM surveillance programme for antimicrobial resistance in human pathogens and the NORM-VET monitoring programme for antimicrobial resistance in the veterinary and 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 2006. 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, National 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 antimicrobials in Norwegian animal production and aquaculture is low. In 2006, the total sales of antimicrobial drugs approved for therapeutic use in animals in Norway were 6,448 kg (fish not included). The annual usage of veterinary antimicrobial drugs decreased gradually by approximately 40% from 1995 to 2001, and has thereafter remained relatively stable. The patterns of use have gradually become more favourable as the proportion of penicillin use has increased. The proportion accounted for by pure penicillin preparations rose from 24% in 1995 to 41% in 2005. Altogether, 77% of the veterinary penicillin preparations sold in 2006 were beta-lactamase sensitive penicillins. The sales of sulfonamides decreased from 14% in 1995 to 0.4% in 2006. The proportion accounted for by tetracyclines declined slightly, from 5% to 4%, during the same period. The reduced antimicrobial drug use as well as the favourable prescribing patterns is mainly explained by a successful campaign on prudent use of antimicrobials conducted by the Norwegian husbandry organizations during the second part of the 1990s.

In 2006, the total sales of antimicrobial drugs for therapeutic use in farmed fish were 1,428 kg of active substance. Quinolones accounted for 79% of this amount. The usage of antimicrobials in Norwegian aquaculture declined by approximately 97% from 1987 to 1996 and has thereafter remained relatively constant. This reduction is mainly attributed to the introduction of effective vaccines in salmonids as well as improved health management. In 2006, 40% of the prescribed amount of antimicrobial agents in aquaculture was for Atlantic cod, 25% for rainbow trout, 22% for Atlantic salmon, while 13 % were for other (new) fish species. The increased usage in cod in the period 2000-2006 correlates well with the increased production of this species.

In 2006, the total sales of coccidiostatic feed additives, in kilograms of active substance, were slightly higher than before the use of antimicrobial growth promoters was abandoned 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 is now dominated by narasin.

Usage of antimicrobial agents in humans

In 2006, the overall sales of antibacterials for systemic use in humans were 19.0 DDD/1,000 inhabitants/day. Total sales of antibacterials have remained relatively unchanged for many years. However, in 2005 and 2006 an increase has been observed. This change is mainly due to increased use of the penicillin group and methenamine. Furthermore, the subgroups of tetracyclins, macrolides and quinolones are increasing, while the subgroup of sulfonamides and trimethoprim is decreasing.

Penicillins represented 42% of total antimicrobial sales in 2006. Within the subgroup of penicillins the beta-lactamase sensitive penicillins are most used. This has been the case for many years, but there has been a shift towards use of more broad-spectrum penicillins. Tetracyclines represented 17% of total use. The sales have gradually decreased since 1993, but in 2005 and 2006 a small increase was registered. Macrolides, lincosamides and streptogramins represented 12% of total use in 2006. The sales were fairly stable throughout the nineties, but since 2000 the use has steadily increased. Erythromycin accounts for 55% of total sales within this group. Sales of cephalosporins, monobactams and carbapenems, although limited, have been increasing over the last years. The group presently represents 3% of the total sales of antibacterials. Quinolones use has been steadily increasing. Although it accounts for only a minor fraction (3%) of total antibacterial sales, this is still an 88% increase since 1999. Finally, the urinary prophylactic agent methenamine represents 14% of total antibacterial use, and the sales have increased by 39% since 1999.

The usage of antibacterials varies between counties, and the pattern has been relatively stable with the same high- and low-use counties. Antibacterial sales to hospitals and ambulatory care represented 8% and 92% of total sales, respectively. Penicillins accounted for around 48% in hospitals and 41% in ambulatory care. The other important groups in ambulatory care were tetracyclins (18%) and macrolides/lincosamides (12%) whereas cephalosporins (22%), quinolones (7%) and metronidazole (6%) were most widely used in hospitals after penicillins.

Resistance in indicator bacteria from animals and food

The prevalence of antimicrobial resistance among certain bacteria of the normal enteric microflora can serve as an indicator of the selective antimicrobial pressure in the various populations. In NORM-VET 2006, *Escherichia coli* and *Enterococcus* spp. from broilers (faeces and meat) were included as indicator bacteria. The occurrences of resistance in *E. coli* and *Enterococcus* spp. in 2006 were moderate, and relatively low in an international perspective. The prevalence of resistance in the isolates obtained from faecal samples and from meat samples were similar.

In total, 81.2 % of *E. coli* isolates were susceptible to all antimicrobial agents included. Resistance to ampicillin was most commonly observed, followed by resistance to sulfamethoxazole and streptomycin/tetracycline. One isolate showed resistance to ceftiofur and cefotaxime. This isolate was further investigated and was found to contain

an ESBL encoding gene. This is the first ESBL ever detected in Norwegian livestock, and the finding demonstrates that an ESBL encoding gene can occur in *E. coli* of the normal flora of healthy domestic animals not previously exposed to broad-spectrum β-lactams.

In total, 22.7% of the *Enterococcus* isolates were susceptible to all antimicrobial agents included. The *E. faecium* strains were frequently resistant to narasin and to a lesser extent bacitracin, erythromycin and tetracycline, while resistance towards vancomycin was negligible. The selection pressure exerted by the common use of narasin as a feed additive in broiler production is probably the reason why narasin resistance is frequently observed among enterococci from broilers.
By using a selective isolation method, a significant decrease of vancomycin resistance compared to previous reports was found. This will be further investigated in the future.

Resistance in zoonotic and non-zoonotic enteropathogenic bacteria

In 2006, a total of 13 *Salmonella* spp. isolates were susceptibility tested. Seven of the isolates were *S.* Typhimurium from cattle, poultry, dog, cat, horse, duck and a wild bird. The remaining six *Salmonella* spp. isolates were *S.* Kedugo, *S. diarizonae* (38:r:z) and *S.* Mikawasima from horses, *S.* Montevideo from a dog, *S.* Anatum from poultry, and *S.* Dublin from swine. Only a single isolate from poultry was multiresistant. Except for a tetracycline resistant *S.* Anatum strain from poultry, the remaining isolates were susceptible to all antimicrobial agents included in the survey.
Additionally, 97 isolates of *Salmonella* spp. from diagnostic submissions (1997-2006) from reptiles in zoo facilities etc. were susceptibility tested. The occurrence of resistance among these isolates was low. The data, although very limited, indicate that antimicrobial resistance is not widespread among *Salmonella* occasionally isolated from animals in Norway.

In 2006, 79.1% of the human cases of salmonellosis were reported as being infected abroad. The proportion of *S.* Typhimurium isolates susceptible to all antimicrobial agents was higher for the category "infected in Norway" (63.7%) than for the "infected abroad" category (34.7%). Multiresistant strains defined as resistant to two or more antimicrobial agents, were more common in the category "infected abroad" (43.4%) than in the category "infected in Norway" (26.6%). The data from 2001-2006 indicate that the prevalence of resistance to tetracyclines and ampicillin in *S.* Typhimurium may be increasing. However, the situation has been stable over the last two years.
The prevalence of resistance was considerably lower in *S.* Enteritidis isolates than in *S.* Typhimurium except for nalidixic acid. In total, 18.7% of *S.* Enteritidis isolates were resistant to nalidixic acid. The resistance frequencies observed for *S.* Enteritidis in 2006 are similar to those reported in previous reports.

The results obtained in 2006 show that the prevalence of resistance in *Campylobacter jejuni* from Norwegian broilers is still low and stable. A total of 91.6 % of the isolates were susceptible to all antimicrobial agents. Only 6.5% were resistant to one antimicrobial agent (ampicillin) and 1.9% to both nalidixic acid and enrofloxacin. The prevalence of resistance and the resistance patterns for *C. jejuni* isolated from Norwegian broilers correspond quite well with what was observed for *C. jejuni* isolated from humans infected in Norway, except for a higher prevalence of resistance to quinolones among isolates of human origin. This relationship was also observed in previous reports. Resistance was significantly more widespread in *C. jejuni* isolates derived from patients infected abroad (68.9% resistant to at least one antimicrobial) than patients infected in Norway (12.9%). The discrepancies are explained by the widespread occurrence of resistance to ciprofloxacin/nalidixic acid (58.8% / 62.2% versus 0% / 6.5%) and to tetracycline (43.7% versus 0%) in isolates acquired abroad as opposed to isolates from patients infected in Norway, respectively.
The occurrence of resistance in *C. jejuni* from both humans infected in Norway and those infected abroad is relatively stable, except for a significant ($p<0.05$) increase in gentamicin resistance among the few isolates from infections aquired in Norway in 2006. The vast majority of the *Shigella* isolates tested originated from patients infected abroad. Resistance was widespread in this species as previously reported from other countries.

Resistance in human clinical isolates

The prevalence of resistance in human clinical isolates was still very low in Norway in 2006. Only two methicillin resistant *Staphylococcus aureus* (MRSA) isolates were detected among 750 strains included in the NORM protocol (0.3%), and a total of three out of 1,261 (0.2%) *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 1,277 including four MRSA strains (0.3%). This prevalence has remained stable over the last years. However, the Norwegian Surveillance System for Communicable Diseases (MSIS) registered 333 cases of MRSA infections in 2006 which is an increase from 260 cases in 2005. A majority of 271 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 (0.4%). Furthermore, MSIS registered 270 cases of MRSA colonization giving a total of 603 MRSA notifications in 2006. This is an increase of 32% from 2005, thus demonstrating the continuing threat of MRSA dissemination in Norway.
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 2.3% which is unchanged from 2.5% in 2005. *E. coli* non-suscpetibility to fluoroquinolones continued to increase from 5.0% in 2005 to 6.4% in 2006. There is a clear correlation between the total usage of fluoroquinolones and non-susceptibility to these agents. The prevalence of resistance to aminoglycosides and fluoroquinolones was lower in *Klebsiella* spp. isolates than in *E. coli*. Extended spectrum beta-lactamases (ESBL) have emerged as a significant clinical problem in many countries, and occasional cases have also been reported from Norway. A total of 11/1,043 (1.1%) *E. coli* and 2/396 (0.5%) *Klebsiella* spp. isolates from blood cultures displayed this phenotype. For *E. coli*, this is a significant increase from to 0.7% in 2004 and 0.5% in

2005. The proportion of ESBL positive isolates was higher among *E. coli* from blood cultures (1.1%) than among urinary tract isolates (0.3%). The prevalence of resistance among urinary tract *Proteus mirabilis* isolates was comparable to the findings in corresponding *E. coli* isolates except for the inherent resistance to nitrofurantoin in *P. mirabilis*.

Clinically significant vancomcyin resistance was not detected in enterococci in 2006. The prevalence of non-susceptibility to ampicillin in *E. faecium* has stabilized above 80% (82.5% in 2005 and 81.0% in 2006), and high-level gentamicin resistance (HLGR) was detected in 27.9% of *E. faecalis* and 46.6% of *E. faecium*. Virtually all (26 out of 27) HLGR *E. faecium* isolates were also non-susceptible to ampicillin. All enterococcal isolates were suscpetible to linezolid.

Streptococcus pneumoniae from blood cultures were generally susceptible to all relevant antimicrobials. Thirteen out of 688 isolates (1.9%) displayed reduced susceptibility to penicillin G, and two isolates were also non-susceptible to cefotaxime. The prevalence of macrolide resistance continued to increase from 9.7% in 2004 and 10.8% in 2005 to 12.4% in 2006. Macrolide resistance was especially widespread among penicillin non-susceptible isolates (5/13). *Streptococcus pyogenes* (beta-haemolytic group A streptococci) from blood cultures, throat swabs and wounds were universally susceptible to penicillin G. The prevalence of macrolide resistance of 1.6% in 2006 has remained stable from earlier surveys. Systemic *Streptococcus agalactiae* isolates (beta-haemolytic group B streptococci) were susceptible to penicillin G, cefotaxime and vancomycin. However, a cluster of macrolide resistant isolates was noted among strains from neonates and children < 1year. These findings will be further investigated at the national reference center for GBS at St. Olavs Hospital.

A total of 294 cases of tuberculosis were reported to MSIS in 2006. Susceptibility tests were performed on 216 *Mycobacterium tuberculosis* primary isolates. Only a single isolate originating from Europe outside Norway was classified as multidrug resistant (MDR). Susceptibility tests were also performed on *Mycobacterium tuberculosis* isolates from nine previously treated patients. Two isolates originating from Europe outside Norway were MDR, and one isolate from an African patient was monoresistant to isoniazid.

Susceptibility testing was performed on 161 blood culture isolates of *Candida albicans* (112), *C. glabrata* (34) and *C. tropicals* (15). All *C. albicans* and *C. tropicalis* isolates were susceptible to fluconazole and voriconazole. A total of 44.1% of *C. glabrata* isolates displayed reduced susceptibility to fluconazole. All yeast isolates were fully susceptible to amphotericin B and caspofungin. The results are in accordance with previous studies from Norway.

Surveillance data on HIV resistance to antiretroviral drugs was reported for the first time in 2006. A total of 118 viral isolates from 275 newly diagnosed HIV positive patients were susceptibility tested (42.9%), and 14/118 (11.9%) harboured one or more mutations that may reduce the efficacy of antiretroviral treatment.

Conclusion

Antimicrobial resistance is still a limited problem in Norway. The relatively low usage of antimicrobial agents as well as the advantageous 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 healthcare have succeeded. 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 1, 2007.
Data provided by Statistics Norway.

Age group	All	Males	Females
0 to 4 years	289,785	147,973	141,812
5 to 14 years	616,146	316,206	299,940
15 to 24 years	586,767	299,979	286,788
25 to 44 years	1 318,449	669,287	649,162
45 to 64 years	1 184,390	600,549	583,841
65 years and older	685,597	291,794	393,803
All age groups	4 681,134	2 325,788	2 355,346

TABLE 2. Livestock population in Norway and the number of slaughtered animals in 2006.

		Number of *	
Animal category	Herds	Animals	Slaughtered animals
Cattle	20,500[1]	918,200[1]	332,100[2]
Dairy cow**	13,500[1]	233,700[1]	-
Suckling cow**	4,100[1]	50,800[1]	-
Combined production (cow)**	1,300[1]	33,300[1]	-
Goat	1,300[1]	72,100[1]	21,100[2]
Dairy goat**	510[1]	42,500[1]	-
Sheep	16,000[1]	2,334,200[1]	1,211,300[2]
Breeding sheep > 1 year**	15,800[1]	894,100[1]	-
Swine	3,000[1]	813,800[1]	1,527,500[2]
Breeding animal > 6 months**	1,800[1]	61,200[1]	-
Fattening pigs for slaughter	2,700[1]	432,000[1]	-
Poultry			-
Egg laying hen (> 20 weeks of age)	2,000[1]	3,262,700[1]	1,764,300[2]
Flocks > 250 birds**	740[1]	3,235,800[1]	-
Broiler	520[2]	-	49,167,500[2]
Turkey, ducks and geese for slaughter	100[1]	250,700[1]	1,025,200[2]
Flocks > 25 birds**	51[1]	250,400[1]	-
Ostrich	9[1]	81[1]	-

Data from: [1)] Register of Production Subsidies as of July 31, 2006; [2)] Register of Slaughtered Animals.
* Numbers > 100 rounded to the nearest ten, numbers > 1000 rounded to the nearest hundred.
** Included in above total.

TABLE 3. Animals (excluding fish) imported to Norway in 2006. *Data provided by Norwegian Livestock Industry´s Biosecurity Unit - KOORIMP.*

	Live animals*		Semen	Embryos
Animal species	Individuals	Consignments	Doses	
Cattle			35,404	50
Swine			170	
Goat	20	1		
Sheep	71	4	24	
Reindeer live animals for slaughter	150	2		
Fur animals	16,361	42		
Poultry – day old chicks	97,499	10		
Turkey – day old chicks	8,050	4		
Ducks and geese	1,345	2		

TABLE 4. Production volume of the most important species in Norwegian aquaculture during the time period 1992-2006. *Data provided by the Norwegian Directorate of Fisheries.*

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	157	498	451	662	67.1	40.6
2000	440,861	48,778	169	129	548	851	37.6	7.6
2001	436,103	71,764	864	318	377	920	22.3	2.5
2002	462,495	83,560	1,258	319	424	2,557	5.0	1.7
2003	509,544	68,931	2,185	272	426	1,829	1.2	1.6
2004	563,815	63,401	3,165	350	649	3,747	45.5	3.3
2005	586,512	58,875	7,409	352	1,197	4,885	3.0	2.0
2006	626,382	62,707	11,087	881	1,185	3,705	4.0	1.0

[1] From the wild population

V. USAGE OF ANTIMICROBIAL AGENTS

USAGE IN ANIMALS

Kari Grave

Therapeutic usage of veterinary antimicrobials

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/or dogs and cats. Thus, the figures represent national sales data for veterinary antimicrobial agents. Antimicrobial agents authorized for human use, but prescribed for animals, are not included. Such drugs are primarily used in small animal practices (see Appendix 1 for inclusion criteria).
Table 5 summarizes the sales in 2006 of veterinary antimicrobial agents for therapeutic use in domestic animals in Norway. The data are organized according to therapeutic substance groups (ATCvet groups) and show the total usage for the various routes of administration. The total annual sale of veterinary antimicrobial agents for terrestrial animals for the period 1995-2006 is given in Figure 1. Figure 2 illustrates the proportion of the total sale of the various groups of antimicrobial substances. In 2006, the sales of veterinary antimicrobial agents approved for therapeutic use in animals in Norway amounted to 6,448 kg of active substance (Table 5). The annual usage of veterinary antimicrobial agents decreased gradually by 40% from 1995 to 2001; thereafter this usage has remained on a relatively constant level although a slight increase was observed for 2005-2006 (Figure 1).

TABLE 5. Sales in 2006, calculated as kilograms of active substance, of veterinary antimicrobial agents approved in Norway for therapeutic use in animals (farmed fish not included, see Table 6). Number of sold items in 2006 for the various veterinary drug formulations, strengths and package sizes were obtained from the Norwegian Institute of Public Health and represent sales from drug wholesalers to Norwegian pharmacies.

Groups of substances	ATCvet code	Active substance or combinations of substances	Gastro-intestinal (QA07)	Uterine (QG01)	Systemic indiv. (QJ01)	Systemic herds (QJ01)	Intra-mammary (QJ51)
Tetracyclines	QG01AA07	Oxytetracycline		3			
	QJ01AA02	Doxycycline			< 0.1		
	QJ01AA06	Oxytetracycline			122	153	
Beta-lactams	QJ01CA04	Amoxicillin			127	176	
	QJ01CE09/QJ51CE09	Benzylpenicillinprocain[1,2]			2,058		7
	QJ01CE90	Penethamate hydroiodide[1]			5		
	QJ01CR02/QJ51RV01	Amoxicillin+clavulanic acid			282		8
Sulfonamides	QJ01EQ06	Sulfanilamid[3]			20		
Sulfonamides and	QJ01EQ10	Sulfadiazine+trimethoprim[4]			1,223	324	
trimethoprim[3]	QJ01EQ13	Sulfadoxine+trimethoprim			105		
Lincosamides	QJ01FF01	Clindamycin			16		
	QJ01FF02	Lincomycin			3		
Aminoglycosides	QA07AA01	Neomycin	29				
	QA07AA90	Dihydrostreptomycin (DHS)	128				
Quinolones	QJ01MA90	Enrofloxacin			28		
	QJ01MA96	Ibafloxacin			1		
Others	QJ01XX92	Tiamulin			13	141	
Combinations	QG01AE99	Sulfadimidine+procaine penicillin[1]+DHS		197			
	QJ01RA01/QJ51RC23	Benzylpenicillinprocain[1] +DHS			529		736
	QJ51RC25	Penethamate hydroiodide[1] + DHS					14
Total per route of administration			**157**	**200**	**4,532**	**794**	**765**
Total (kg)							**6,448**

[1]Calculated as benzylpenicillin; [2]Includes one preparation used on exemption from market authorization; [3]Represents an extemporaneously prepared preparation; [4]Includes a premix approved for farmed fish that are used solely in terrestrial animals such as pigs and calves (Kari Grave, unpublished data)

The proportion accounted for by pure penicillin preparations rose from 24% in 1995 to 41% in 2006. Altogether 77% of the veterinary penicillin preparations sold in 2006 were beta-lactamase sensitive penicillins. From 1995 to 2006, the sale of sulfonamides in combination with trimethoprim (or baquiloprim 1995-2000) increased from 11% to 26% of the total sales. The proportion of sale of the combination preparations of penicillins and aminoglycosides decreased from 34% to 20% from 1995 to 2006. The corresponding figures for the sulfonamides were 14% in 1995 and 0.3% in 2006. The proportion accounted for by tetracyclines declined from 5% to 4% during the same period. The reduced use as well as the favourable prescribing patterns is mainly explained by a successful campaign on prudent use of antimicrobials conducted by the Norwegian husbandry organizations during the second part of the 1990s.

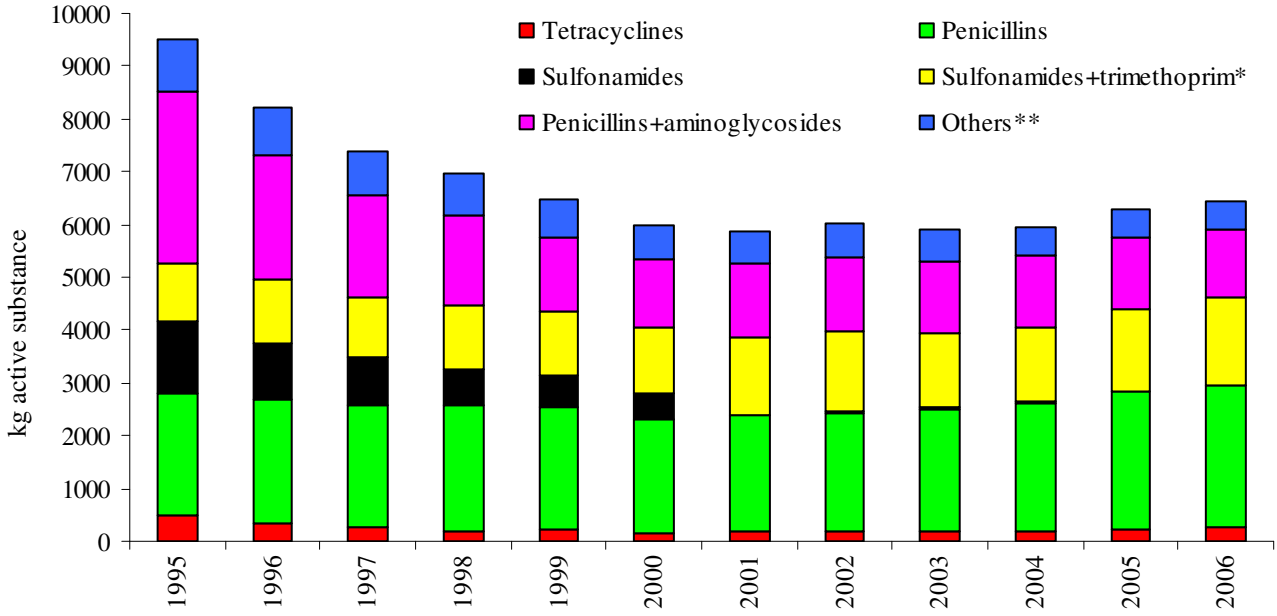

FIGURE 1. Sales (in kilograms of active substance) of veterinary antimicrobial agents (QA07AA, QG01AA, QG01AE, QJ01, QJ51) for therapeutic use in Norway 1995–2006, fish not included. Number of sold items in 2006 for the various veterinary drug formulations, strengths and package sizes were obtained from the Norwegian Institute of Public Health and represent sales from drug wholesalers to Norwegian pharmacies.
*Includes small amounts of baquiloprim (1995-2000); includes a premix approved for farmed fish used solely in terrestrial animals such as pigs, horse and calves (Kari Grave, unpublished data). **Includes ATCvet codes: QAA7AA01; QA07AA90; QG01AE99; QJ01EQ06; QJ01FA01; QJ01FF01; QJ01FF02; QJO1MA90; QJ01XX92.

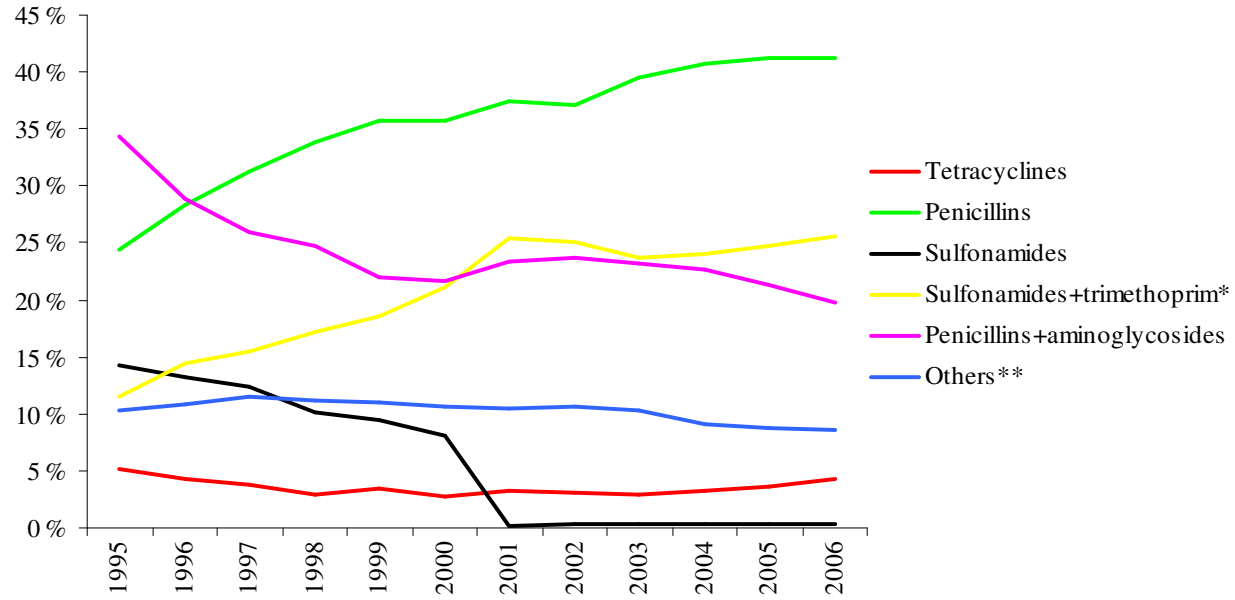

FIGURE 2. Sales (as percentage of total sales) of veterinary antimicrobial agents (QA07AA, QG01AA, QG01AE, QJ01, QJ51) in Norway 1995–2006, fish not included. Number of sold items in 2006 for the various veterinary drug formulations, strengths and package sizes were obtained from the Norwegian Institute of Public Health and represent sales from drug wholesalers to Norwegian pharmacies. Sulfonamides were not sold during 2001-2003.
*Includes small amounts of baquiloprim. **Includes ATCvet codes: QAA7AA01; QA07AA90; QG01AE99; QJ01EQ06; QJ01FA01; QJ01FF01; QJ01FF02; QJO1MA90; QJ01XX92.

TABLE 6. Total sales (in kilograms of active substance) of veterinary antimicrobial agents for therapeutic use in farmed fish in Norway in the period 1995-2006. The data were obtained from the Norwegian Institute of Public Health and represent sales from drug wholesalers to Norwegian pharmacies and sales by feed mills.

Groups of substances/active substance	ATCvet code	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Tetracyclines													
Oxytetracycline	QJ01AA06	70	27	42	55	25	15	12	11	45	9	8	0
Amphenicols													
Florfenicol	QJ01BA90	64	64	123	135	65	148	109	205	154	111	202	302
Quinolones													
Flumequine	QJ01MB07	182	105	74	53	7	52	7	5	60	4	28	7
Oxolinic acid	QJ01MB91	2,800	841	507	436	494	470	517	998	546	1,035	977	1,119
Total		**3,116**	**1,037**	**746**	**679**	**591**	**685**	**645**	**1,219**	**805**	**1,159**	**1,215**	**1,428**

In 2006, the sales of veterinary antimicrobial agents for use in farmed fish were 1,428 kg active substance, of which 79% were quinolones. The annual usage of antimicrobial agents in Norwegian fish farming peaked in 1987 when the reported sales figures amounted to approximately 48 tonnes. This implies that the usage of antimicrobials in Norwegian aquaculture declined by approximately 98% from 1987 to 1996 (Table 6) and has thereafter remained relatively constant although a slight increase is observed for the last three years. From 1987 the total production of farmed fish increased more than ten times. This significant decrease in the usage of antimicrobial agents in Norwegian aquaculture in the period 1987 to 1996 was mainly attributed to the introduction of effective vaccines against bacterial diseases in Atlantic salmon and rainbow trout as well as to improved health management.

In 2006, 40 % of the prescribed amounts of antimicrobial agents in aquaculture were for Atlantic cod, 25% for rainbow trout and 22% for Atlantic salmon (Figure 3). The increased usage in Atlantic cod in the period 2000-2006 is positively correlated with the increased production of this species (Table 4).

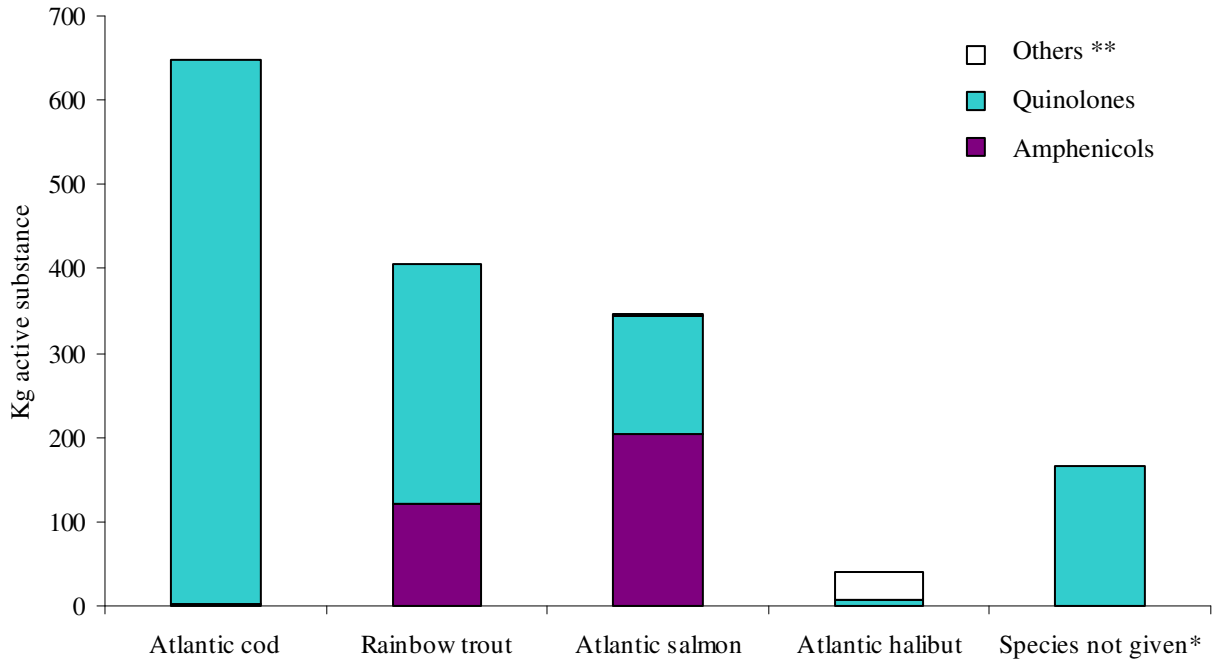

FIGURE 3. Prescribed amounts (in kilograms of active substance) of veterinary antimicrobial agents in Norwegian aquaculture in 2006 split into various fish species. Prescription data were obtained from the Norwegian Food Safety Authority (Trygve Helle, data on file). Additionally, 0.5 kg amphenicols were prescribed for turbot and 0.15 kg tetracyclines for perch.
*Species not given on the prescriptions (7), but prescribed for use in fish farms cultivating coalfish. **Others: Procainpenicillin+dihydrostreptomycin (4 prescriptions for Atlantic cod fry), tetracylines (1 prescription for perch fry and 1 for Atlantic halibut hatchery); lincomycin+spectinomycin (1 prescription for Atlantic halibut fry).

Antimicrobial and coccidiostatic feed additives

Data on the usage of various substances and categories of feed additives were obtained through annual reports from the Norwegian Agricultural Inspection Service (1995-2002) and The Norwegian Food Safety Authority (2003-2006). Table 7 summarizes total sales of antimicrobial growth promoters and coccidiostat feed additives in Norway in the period 1995–2006.

The glycopeptide avoparcin was licensed for the Norwegian market as a growth promoter in broilers and turkeys in 1986. It was prohibited in 1995 due to a reported association between its use and the occurrence of vancomycin resistant enterococci in animal husbandry. The same year, the Norwegian food animal production industry voluntarily abandoned the use of all antimicrobial growth promoters. These measures resulted in an immediate reduction in the usage of these substances (Table 7). In 1998, the streptogramin virginiamycin was officially prohibited due to reports from other countries of an association between its use and the occurrence of enterococci that were resistant to quinupristin-dalfopristin, a streptogramin combination preparation used in human medicine. No antimicrobial growth promoters have been used in animals in Norway since 1998.

Coccidiostats as feed additives are still used in Norwegian poultry production. The total sales of coccidiostats, in kilograms of active substance, are slightly higher than before the ban on antimicrobial growth promoters. During the same time the production of broilers has increased. However, the pattern of usage has changed (Table 7). While monensin was the most frequently used ionophore in the poultry industry in 1995, the usage of coccidiostats is now almost totally dominated by narasin.

TABLE 7. Total sales, in kilograms of active substance, of antimicrobial growth promoters and of coccidiostats as feed additives in Norway 1995-2006. Data were obtained through annual reports from the Norwegian Agricultural Inspection Service (1995-2002) and the Norwegian Food Safety Authority (2003-2006).

	Total sales in kg active substance											
Active substance	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Avoparcin[1]	419	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.
Zincbacitracin	129	64	27	0	0	0	0	0	0	0	0	0
Virginiamycin[2]	0	0	0	0	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.	Prohib.
Total antimicrobial growth promoters	**548**	**64**	**27**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Lasalocid	996	480	471	193	208	80	96	514	108	173	37	13
Monensin	3,422	891	561	485	557	776	629	521	717	817	852	889
Salinomycin	214	27	0	0	27	233	12	0	0	0	0	0
Narasin	24	3,508	3,343	3,530	4,062	4,486	4,195	4,470	5,067	5,270	5,318	5,615
Total ionophore coccidiostats	**4,656**	**4,906**	**4,375**	**4,208**	**4,854**	**5,575**	**4,932**	**5,505**	**5,892**	**6,260**	**6,207**	**6,517**
Amprolium/etopabat	156	116	582	174	201	135	159	74	42	0.8	0	0
Total other	**156**	**116**	**582**	**174**	**201**	**135**	**159**	**74**	**42**	**0.8**	**0**	**0**

[1]Prohibited since May 31, 1995. [2]Prohibited since 1999.

USAGE IN HUMANS

Hege Salvesen Blix

In 2006, the overall sales of antibacterials for systemic use in humans represented 19.0 DDD/1000 inhabitants/day (16.3 DDD/1000 inhabitants/day exclusive of methenamine). Total sales of antibacterials have remained relatively unchanged for many years. However, in 2005 and 2006 an increase has been observed. This change is mainly due to increased use of the penicillin group and methenamine. Furthermore, the subgroups of macrolides and quinolones are steadily increasing, while the subgroup of sulfonamides and trimethoprim is decreasing (Tables 8-9, Figure 4).

TABLE 8. Human usage of antibacterial agents in Norway 1999-2006 by ATC groups. The usage is presented as DDD (Defined Daily Doses)/1000 inhabitants/day and in % change 2005-2006. Methods for data collection on human usage of antimicrobial agents are presented in Appendix 2

ATC	Groups of substances	1999	2000	2001	2002	2003	2004	2005	2006	Change (%) 2005-2006
J01A	Tetracyclines	3.19	3.17	3.11	3.13	3.03	2.97	3.11	3.24	+ 4
J01B	Amphenicols	0.005	0.004	0.003	0.002	0.002	0.001	0.001	0.002	
J01CA	Penicillins with extended spectrum	1.96	2.01	2.1	2.23	2.29	2.37	2.53	2.74	+ 8
J01CE	β-lactamase sensitive penicillins	5.01	4.66	4.68	4.48	4.38	4.23	4.55	4.63	+ 2
J01CF	β-lactamase resistant penicillins	0.32	0.35	0.41	0.50	0.59	0.63	0.56	0.66	+ 18
J01CR	Combination of penicillins	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
J01D	Cephalosporins, monobactams, carbapenems	0.47	0.52	0.55	0.58	0.62	0.61	0.57	0.60	+ 5
J01E	Sulfonamides and trimethoprim	1.26	1.17	1.16	1.15	1.08	1.09	1.06	1.04	- 2
J01F	Macrolides, lincosamides and streptogramins	1.59	1.59	1.8	1.98	1.92	1.89	2.12	2.24	+ 6
J01G	Aminoglycosides	0.05	0.04	0.06	0.06	0.07	0.06	0.07	0.07	
J01M	Quinolones	0.33	0.35	0.40	0.44	0.48	0.52	0.57	0.62	+ 9
J01X	Other antibacterials	2.34	2.39	2.55	2.57	2.63	2.83	3.05	3.18	+ 4
	Total exclusive of methenamine	**14.7**	**14.3**	**14.7**	**15.0**	**14.9**	**14.8**	**15.6**	**16.3**	**+ 4**
	Total all antibacterials	**16.6**	**16.3**	**16.8**	**17.1**	**17.1**	**17.2**	**18.2**	**19.0**	**+ 4**

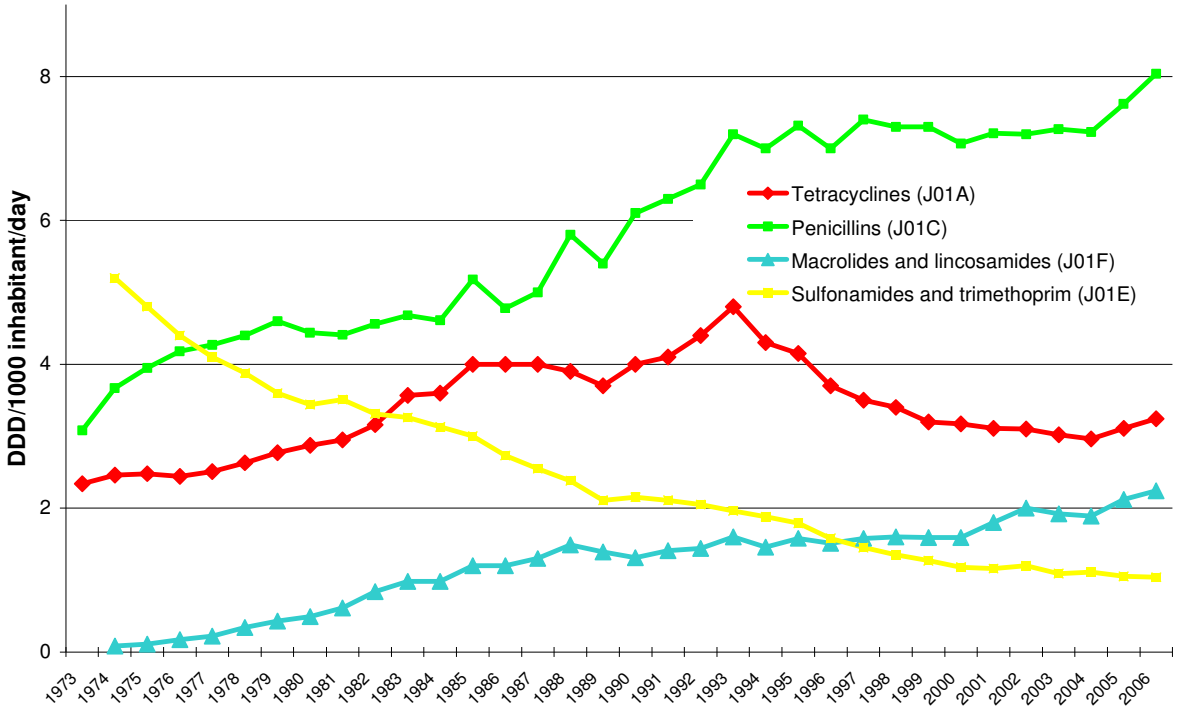

FIGURE 4. Sales of penicillins (J01C), tetracyclines (J01A), macrolides, lincosamides and streptogramines (J01F), and sulfonamides and trimethoprim (J01E) in Norway 1973-2006.

In 2006, the penicillins (ATC group J01C) represented 42% of the total antimicrobial use in Norway (Figure 5). Within the subgroup of penicillins the β-lactamase sensitive penicillins (J01CE) are most commonly prescribed. This has been the case over many years. There has been a shift towards use of more broad-spectrum penicillins. Penicillins with extended spectrum (J01CA) now represent 33% of the penicillin group compared to 25% in 1996, and the subgroup of β-lactamase-resistant penicillins represents 8% today compared to 5% in 1996 (Figure 6). Use of β-lactamase sensitive penicillins (J01CE) decreased during the last decade, but the use has again increased in 2005 and 2006.

Tetracyclines (J01A) represent 17% of total use. The sales have increased since 2005, but the proportion of total sales is the same as last year.

Macrolides, lincosamides and streptogramins (J01F) represented 12% of total use in 2006. The sales were fairly stable in the nineties. However, since 2000 the use has steadily increased. The pattern within the group J01F has remained relatively unchanged over the years. Erythromycin is most frequently used, representing 55% of the subgroup (Figure 7).

Over the latest years, sales of cephalosporins, monobactams and carbapenems (J01D), although little, have been increasing. This group now represents 3% of the total sales of antibacterials. The internal subgroup pattern has changed since 1996 (Figure 8). First generation cephalosporins i.e. cefalexin and cefalotin, represent 54% of ATC group J01D.

The use of quinolones (J01M) has also been increasing. Still, it represents only a minor fraction (3%) of total antibacterial sales, but the increase has been 88% since 1999.

The increase of ATC group J01X is mainly due to the urinary prophylactic agent methenamine with 2.7 DDD/1000 inhabitants/day in 2006, representing 14% of total antibacterial use. The sales have increased by 42% since 1999.

The usage of antibacterials varies between the 19 Norwegian counties with the same high-use and low-use counties over time (Figure 9).

The antibacterial sales to hospitals represented 8% of total sals in the country measured in DDDs in 2006. The therapy pattern of antibacterials in hospitals differs from ambulatory care (Figure 10). Antibacterial use in nursing homes which is around 6% of total sales, is included in ambulatory care.

Penicillins (J01C) represent around 48% and 41% of the use in hospitals and in ambulatory care, respectively. The most important other groups in ambulatory care are tetracyclins (18%) and macrolides and lincosamides (12%). In hospitals, cephalosporins (22%) are the most commonly used group after the penicillins, followed by quinolones (7%) and metronidazole - oral and parenteral (6%).

The slow, but steady shift towards use of more “broad-spectrum” antibacterials in Norway is of concern and deserves close surveillance. The pertussis epidemic observed in Norway since 1997 may have contributed to the increased prescription of non-betalactam drugs. Therapy traditions in ambulatory care have a large impact on the total burden of antimicrobials and consequently to the development of bacterial resistance. Hence, surveillance of antimicrobial use and guidance of appropriate prescription of antibacterials in ambulatory care are important priority areas.

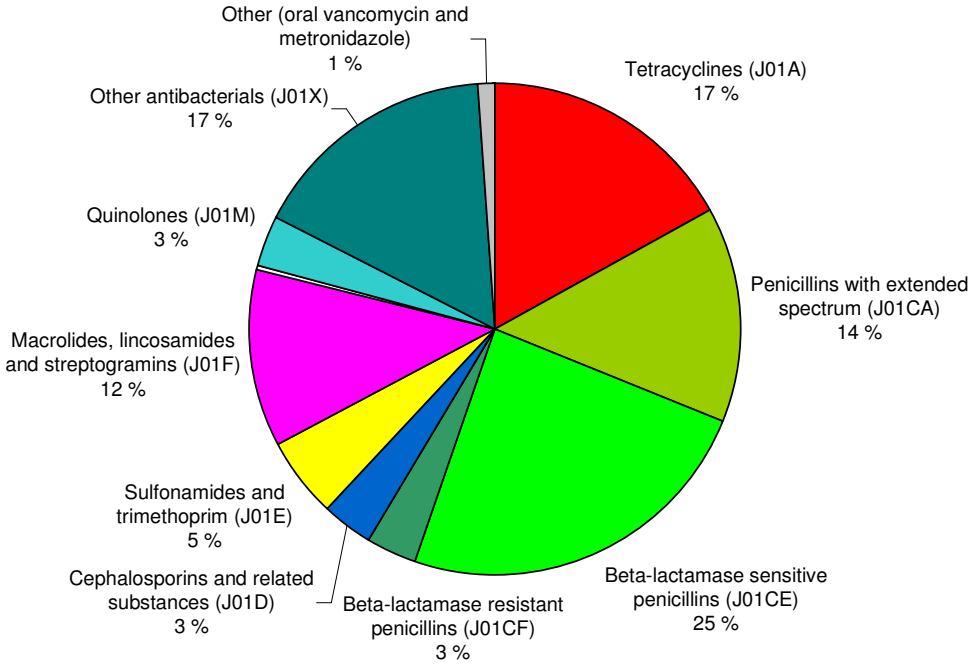

FIGURE 5. Relative amount of antibacterial agents for systemic use in 2006 in Defined Daily Doses (DDD) (total sale in Norway).

TABLE 9. Human usage of single antibacterial 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	1999	2000	2001	2002	2003	2004	2005	2006
A07A A09	Vancomycin	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
J01A A02	Doxycycline	2.20	2.10	2.1	2.03	1.93	1.80	1.89	1.97
J01A A04	Lymecycline	0.09	0.14	0.19	0.26	0.30	0.34	0.39	0.45
J01A A06	Oxytetracycline	0.25	0.24	0.22	0.21	0.19	0.20	0.20	0.19
J01A A07	Tetracycline	0.65	0.69	0.64	0.62	0.60	0.62	0.64	0.63
J01AA07*	Minocycline							0.0003	0.0003
J01AA12	Tigecycline								0.0001
J01B A01	Chloramphenicol	0.005	0.004	0.003	0.002	0.002	0.001	0.002	0.002
J01C A01	Ampicillin	0.09	0.09	0.08	0.09	0.1	0.1	0.1	0.1
J01C A02	Pivampicillin	0.14	0.13	0.11	0.11	0.09	0.08	0.07	0.06
J01C A04	Amoxicillin	0.87	0.83	0.89	0.94	0.95	0.94	1.06	1.11
J01C A08	Pivmecillinam	0.86	0.96	1	1.09	1.14	1.25	1.29	1.46
J01C A11	Mecillinam	0.004	0.004	0.005	0.005	0.005	0.005	0.006	0.006
J01C E01	Benzylpenicillin	0.23	0.21	0.23	0.24	0.25	0.24	0.26	0.26
J01C E02	Phenoxymethylpenicillin	4.78	4.45	4.45	4.24	4.13	3.99	4.29	4.37
J01C E08*	Benzathine benzylpenicillin	<0.0001	0.0001	<0.0001	0.0001	0.0001	0.0002	0.0001	0.0002
J01C F01	Dicloxacillin	0.22	0.25	0.31	0.39	0.48	0.51	0.41	0.54
J01C F02	Cloxacillin	0.10	0.10	0.09	0.11	0.11	0.11	0.15	0.12
J01C F05*	Flucloxacillin				0.0001	0.0002	0.0002	0.0001	0.0001
J01C R02*	Amoxicillin and enzyme inhibitor	0.01	0.01	0.01	0.01	0.01	0.0003	0.0000	0.0001
J01C R05	Piperacillin and enzyme inhibitor		0.0001	0.0006	0.0014	0.0024	0.005	0.01	0.01
J01D B01	Cefalexin	0.22	0.26	0.27	0.29	0.3	0.29	0.24	0.26
J01D B03	Cefalotin	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06
J01D B04*	Cefazolin							0.002	0.002
J01D C01	Cefoxitin	0.0004	0.0004	0.0003	0.0002	0.0001			
J01D C02	Cefuroxim	0.13	0.13	0.14	0.15	0.15	0.14	0.13	0.12
J01D D01	Cefotaxim	0.04	0.04	0.05	0.05	0.07	0.07	0.08	0.09
J01D D02	Ceftazidim	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01D D04	Ceftriaxone	0.008	0.011	0.01	0.01	0.01	0.02	0.02	0.02
J01D F01	Aztreonam	0.0008	0.001	0.001	0.001	0.001	0.001	0.0005	0.0008
J01D H02	Meropenem	0.008	0.012	0.014	0.017	0.02	0.02	0.026	0.031
J01D H03	Ertapenem								0.000
J01D H51	Imipenem and enzyme inhibitor	0.006	0.006	0.005	0.005	0.006	0.005	0.005	0.004
J01E A01	Trimethoprim	0.84	0.79	0.8	0.8	0.74	0.76	0.73	0.70
J01E B02	Sulfamethizole	0.001	0.002	0.002	0.0001				
J01E C20	Sulfonamides, combinations	0.0004							
J01E E01	Sulfamethoxazol and trimethoprim	0.42	0.38	0.36	0.36	0.34	0.34	0.33	0.34
J01F A01	Erythromycin	1.01	1.00	1.13	1.2	1.09	1.03	1.16	1.24
J01F A02	Spiramycin	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01
J01F A09	Clarithromycin	0.26	0.26	0.3	0.36	0.37	0.37	0.39	0.40
J01F A10	Azithromycin	0.18	0.19	0.21	0.24	0.26	0.28	0.32	0.34
J01FA15	Telithromycin				0.0001	0.0003	0.0003		
J01F F01	Clindamycin	0.11	0.12	0.14	0.16	0.19	0.20	0.23	0.25
J01GA01*	Streptomycin				0.0015	0.0004	0.0004	0.0002	0.0003
J01G B01	Tobramycin	0.03	0.02	0.03	0.04	0.04	0.03	0.03	0.03
J01G B03	Gentamicin	0.006	0.006	0.008	0.02	0.03	0.03	0.03	0.04
J01G B06*	Amikacin				0.0009	0.0008	0.0003	0.0004	0.0009

ATC	Substance	1999	2000	2001	2002	2003	2004	2005	2006
J01G B07	Netilmicin	0.02	0.02	0.02	0.007				0.0001
J01M A01	Ofloxacin	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.04
J01M A02	Ciprofloxacin	0.26	0.29	0.34	0.38	0.42	0.47	0.52	0.57
J01MA12*	Levofloxacin				0.001	0.0003		0.0003	0.0003
J01M B02	Nalidixic acid	0.01	0.01	0.01					
J01X A01	Vancomycin	0.004	0.005	0.005	0.006	0.006	0.007	0.007	0.008
J01X A02	Teicoplanin	0.0007	0.0012	0.0013	0.0013	0.0009	0.0007	0.0008	0.0008
J01X B01	Colistin	0.003	0.003	0.003	0.003	0.002	0.002	0.002	0.002
J01X C01	Fusidic acid	0.003	0.003	0.01	0.01	0.007	0.008	0.006	0.006
J01X D01	Metronidazole	0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.07
J01X E01	Nitrofurantoin	0.37	0.37	0.36	0.35	0.35	0.36	0.36	0.37
J01X X05	Methenamin	1.91	1.95	2.08	2.13	2.18	2.37	2.59	2.71
J01XX08	Linezolid				0.002	0.004	0.006	0.007	0.006
P01AB01	Metronidazole	0.18	0.18	0.18	0.19	0.19	0.20	0.20	0.20
J04AB**	Rifampicin	0.052	0.046	0.054	0.043	0.049	0.068	0.077	0.082

* Drugs not licensed for the Norwegian marked but prescribed off-licence.
** Given as the amount of rifampicin in plain and combination products.

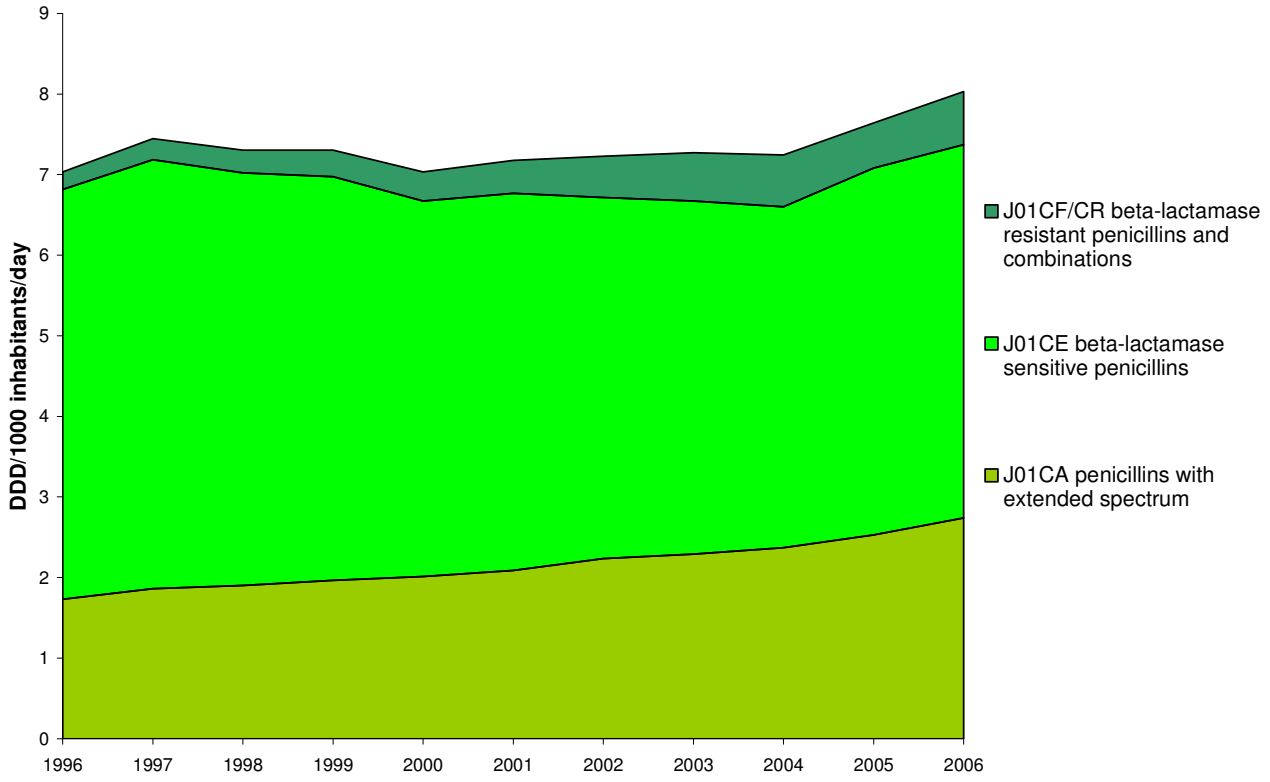

FIGURE 6. Sales of penicillins (J01C) in Norway 1996-2006 and changes between groups of penicillins.

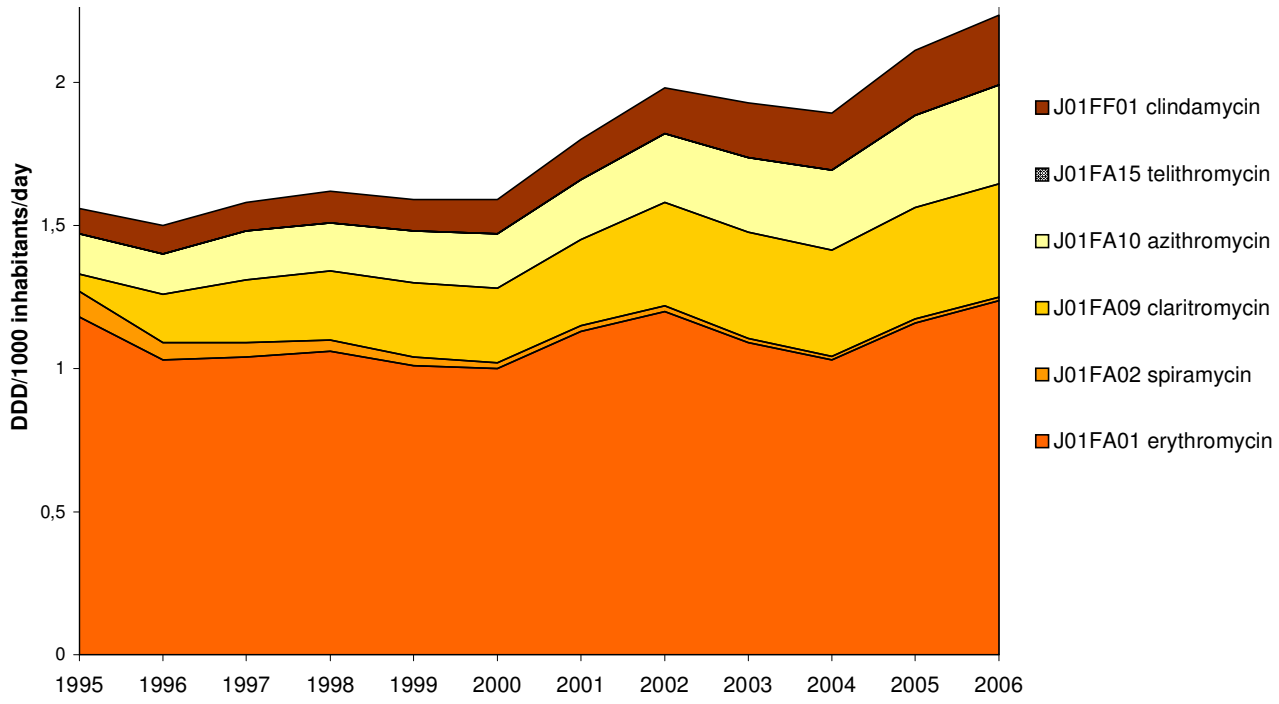

FIGURE 7. Sales of macrolides, lincosamides and streptogramins (J01F) in Norway 1996-2006.

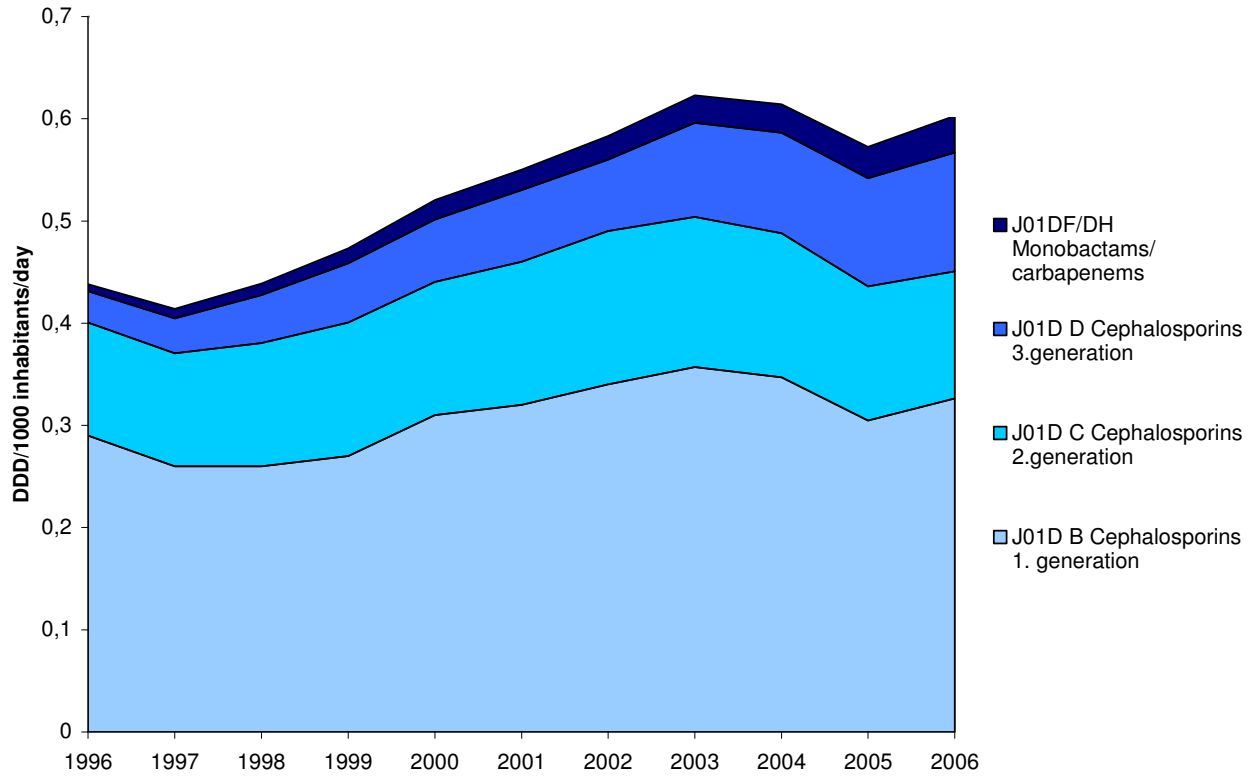

FIGURE 8. Sales of cephalosporins, monobactams and carbapenems (J01D) in Norway 1996-2006 and changes between generations of cephalosporins and monobactams/carbapenems.

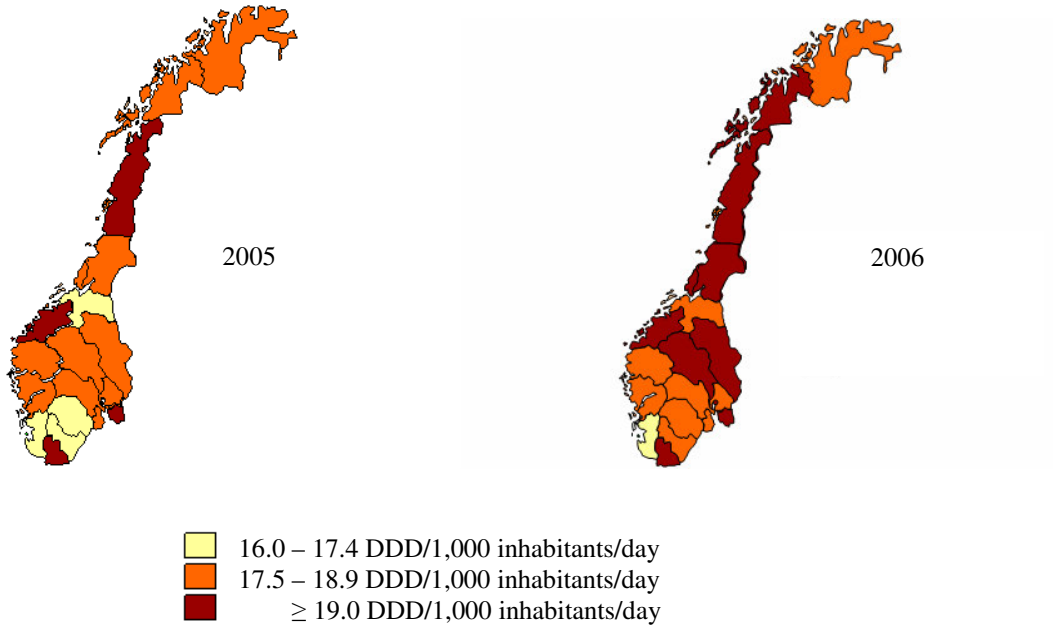

FIGURE 9. Sales of antibacterial agents for systemic use (ATC group J01) in the different counties of Norway in 2005 and 2006.

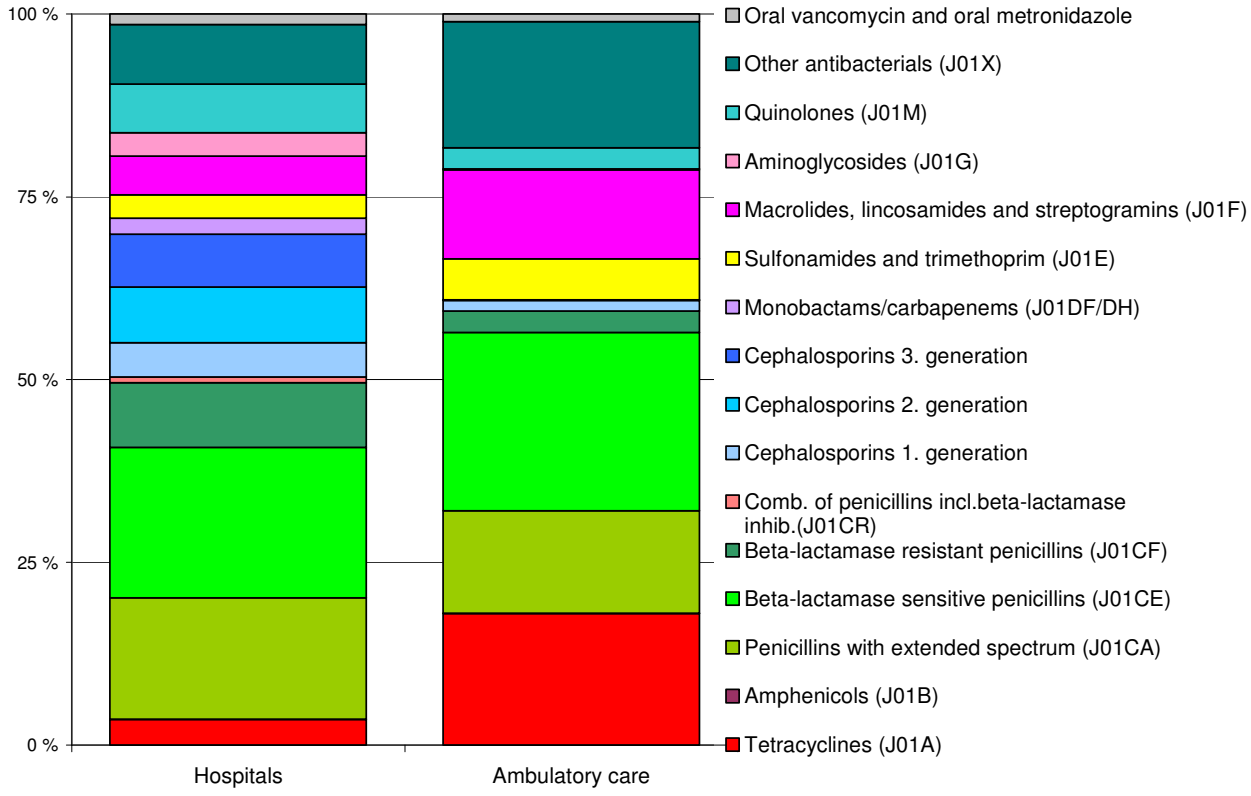

FIGURE 10. Proportions of antibacterial agents prescribed for systemic use in Norway 2006 measured in DDD shown for hospitals and ambulatory care.

Use of antibacterials in ambulatory care

Although surveillance of antibacterial use has been carried out on an aggregated level since the early nineteen seventies it is only recently that it has been possible to follow the use in individual patients. Data collection for the Norwegian Prescription Database (NorPD) started in January 2004. This database covers all inhabitants, and every drug prescription in Norway is included in the database. The database includes information on age, gender, residence, prescriber, pharmacy, dispensing date and relevant information on the prescribed drug. The data as such, are unique since we are able to follow individuals in the population over time. A limitation – at the moment – is that indication for the prescription is not included.
Population prevalences for antibacterials were extracted from the database for 2005 and 2006 (January to December). The population prevalences were 200 and 203 per 1,000 men and 285 and 288 per 1,000 women for 2005 and 2006, respectively. This means that among Norwegian inhabitants, 20% of the men and 29% of the women use antibacterials in a year. Antibacterial use is here defined as antibacterials for systemic use (ATC J01, excluding methenamine J01XX05), oral vancomycin A07AA09 and oral metronidazole P01AB01.
Antibacterial use differs by age groups. Children between 8 and 15 years are the least frequent users. From the age of 16-17 years we find a rapid increase in antibacterial use. The most frequent users are the youngest children and the elderly (Figure 11). The database gives information on the residence of discrete individuals i.e. regardless of where the antibacterial is purchased. Residents in North Norway use less antibacterials than those in other regions, but the gender difference is the same all over the country (Figure 12).

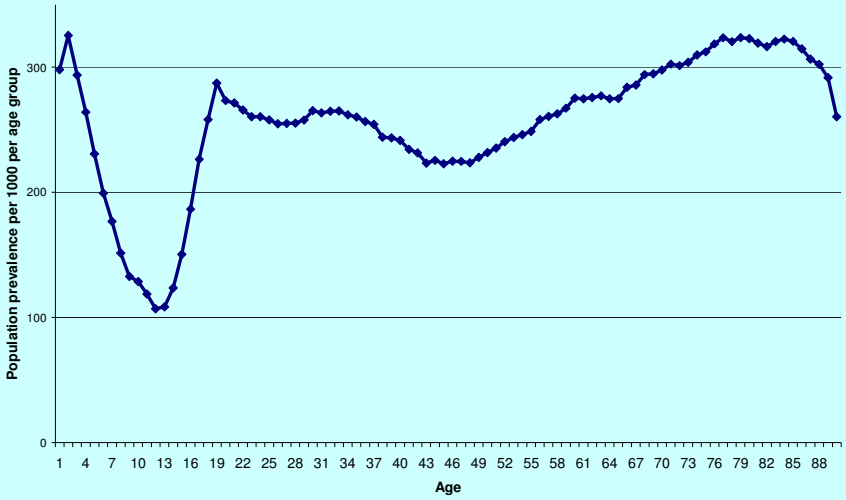

FIGURE 11. Prevalence of systemic antibacterial use by age in Norway during 2006. Antibacterials for systemic use include ATC group J01 (excl. methenamine), vancomycin (A07AA09) and metronidazole (P01AB01).

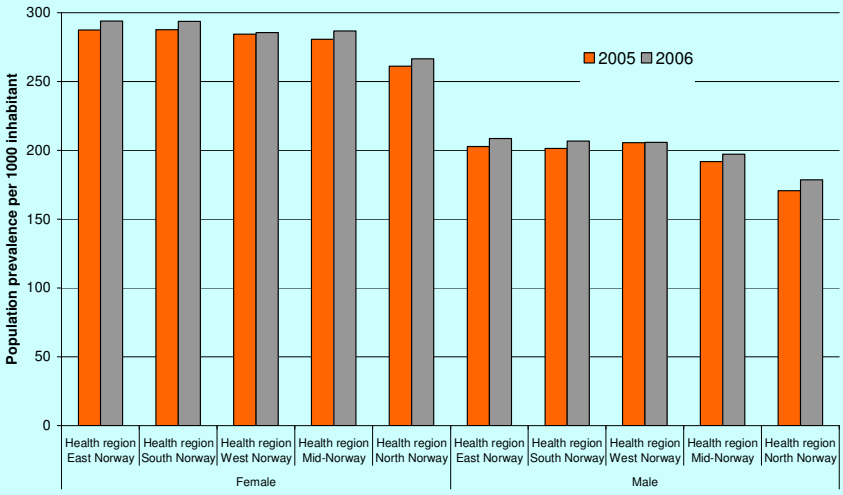

FIGURE 12. Prevalence of systemic antibacterial use by gender and residence in Norway during 2006. Antibacterials for systemic use include ATC group J01 (excl. methenamine), vancomycin (A07AA09) and metronidazole (P01AB01)

Blix HS, Engeland A, Litleskare I, Ronning M. Age- and gender-specific antibacterial prescribing in Norway. J Antimicrob Chemother. 2007; 59(5):971-6.

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New national centre for antibiotic use and resistance in primary care (ASP)

The centre was officially opened during a conference in Oslo November 2006 after an initiative by the National Committee for Prudent Antibiotic Use. It is given governmental support for 5 years as a starting period. The centre is located at the Institute of General Practice and Community Medicine, University of Oslo. The centre has a goal to facilitate prudent and restricted use of antibiotics in primary care, and thereby reduce the development of antibacterial resistance in Norway. This is an important arena of action as more than 90% of antibiotics are prescribed in primary care in Norway. The centre will focus on 3 major tasks in the coming years:

1. Initiate and support relevant research in primary care.

We have given financial and scientific support to two research programmes which were started in 2006. One is a large pedagogic randomised intervention trial including 450 Norwegian GP's focusing on their antibiotic prescription practices for respiratory tract infections. The pedagogic intervention consists of lectures on prudent antibiotic use and resistance. In addition it is based on feedback reports on the participants´ own prescribing practices using peer groups with a peer academic detailer as group leader (the Rx-PAD-study).

A second PhD project will also be based on this study. It will focus on the use of delayed prescription as a tool to reduce unnecessary antibiotic prescription. It is based on data from the Rx-PAD-study in addition to a qualitative study on the GP's attitudes to delayed prescription and a questionnaire study among patients.

Furthermore the centre is supporting 5 smaller studies:

a) Mastitis in primary care.
b) The use of alcohol based desinfection in hand washing in kindergartens to reduce spread of infectious diseases.
c) The use of antibiotics in emergency rooms.
d) The use of CRP and Strep-tests among GP practices.
e) A study of antibiotic resistance in urinary tract infections.

2. Support development of guidelines and postgraduate activities for GPs.

We have received funding for revision of the Norwegian guidelines for antibiotic treatment in primary care, published in 2000. The work started out at a conference in May 2007, and 30 specialists and academic GPs are involved in the work. The revision is planned to be finished in May 2008, and the plan is to produce a printed version and an electronic version which will be available on each GP's computer and on the Internet.

The centre will furthermore participate in specialisation and postgraduate CME-activities for Norwegian GPs by arranging courses and workshops. The Rx-PAD will possibly be followed up by regular courses for GPs where they can critically discuss their own practice based on data extraction from their own data files.

Our goal is also to harmonize all relevant existing guidelines on antibiotic use in Norway.

3. Information to the public.

The centre will open a website that will give information both to health-care professionals and the public about important topics related to antibiotic use and antibacterial resistance in Norway. We will contribute to development of suitable written information and brochures to be given to the public and patients that consult in primary care for common infections.

We will participate in the public debate on the topic and contribute to stimulation of an active public opinion concerning antibiotic use and antibacterial resistance. We will inform the public of the development of resistance among the most common bacteriae and the consequences for clinical practice.

Morten Lindbæk, University of Oslo

VI. OCCURRENCE OF ANTIMICROBIAL RESISTANCE

INDICATOR BACTERIA FROM ANIMALS AND FOOD

Madelaine Norström, Kari Grave, Marianne Sunde

The prevalence of acquired antimicrobial resistance among certain bacteria of the normal enteric microflora can be used as an indicator of the selective antimicrobial pressure in the 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 so-called indicator bacteria of the normal enteric microflora from healthy animals as well as indicator bacteria from feed and food is important to get a better 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 2006, indicator bacteria from broilers were included in the monitoring. The substances included in the test panels might not always be substances used in the veterinary medicine, but are included because of their importance for human health. Some cut-off values defining resistance (breakpoints) used in NORM-VET 2000-2005 have been changed over the years. To facilitate comparisons, data on prevalence of resistance from earlier reports have been recalculated using current cut-off values. Sampling, laboratory methods and data processing are described in Appendix 3.

Escherichia coli from broilers

A total of 219 faecal and 129 meat samples from broilers were collected. *E. coli* was detected in 190 (86.8%) of the faecal samples and 119 (92.2%) of the meat samples. One isolate per sample positive for *E. coli* was tested for susceptibility. The results are presented in Table 10 and Figures 13-14, and in the text.

TABLE 10. Antimicrobial resistance in *Escherichia coli* from faecal (n=190) and (n=119) meat samples from broilers.

		Resistance (%)		Distribution (%) of MIC-values (mg/L)													
Substance	Sample	[95% CI*]		0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	Faeces	3.7	[1.6-7.8]					31.6	60.0	4.7			1.6	1.6	0.5		
	Meat	5.0	[2.0-11.1]				0.8	27.7	64.7	1.7			0.8	3.4	0.8		
Chloramphenicol	Faeces	0.0	[0.0-2.5]						10.0	56.8	33.2						
	Meat	0.0	[0.0-3.9]						13.4	48.7	37.8						
Florfenicol	Faeces	0.0	[0.0-2.5]							42.6	51.6	5.8					
	Meat	0.0	[0.0-3.9]							47.1	44.5	8.4					
Ampicillin	Faeces	13.2	[8.9-19.0]				1.1	10.0	48.4	25.8	1.6		0.5	12.6			
	Meat	7.6	[3.8-14.3]					12.6	51.3	27.7	0.8			7.6			
Ceftiofur	Faeces	0.5	[0.0-3.3]		5.3	38.9	49.4	6.3		0.5							
	Meat	0.0	[0.0-3.9]		2.5	41.2	47.1	9.2									
Cefotaxime	Faeces	1.1	[0.2-4.2]	63.7	30.5	4.7	0.5	0.5									
	Meat	0.0	[0.0-3.9]	60.5	37.0	2.5											
Trimethoprim	Meat	3.1	[1.3-7.1]			30.5	54.2	11.1	1.1	0.5				2.6			
	Meat	0.8	[0.0-5.2]			26.1	59.7	11.8	1.7					0.8			
Sulfamethoxazole	Faeces	8.9	[5.4-14.1]									85.3	5.3	0.5			8.9
	Meat	6.7	[3.1-13.2]									83.2	8.4	1.7			6.7
Streptomycin	Faeces	2.1	[0.7-5.6]						2.1	46.8	46.3	2.6	0.5		0.5		1.1
	Meat	7.6	[3.8-14.3]						1.7	49.6	35.3	5.9	0.8	3.4	2.5	0.8	
Gentamicin	Faeces	1.1	[0.2-4.2]				50.0	42.1	6.8	1.1							
	Meat	1.7	[0.3-6.6]				48.4	44.5	5.0	1.7							
Kanamycin	Faeces	0.0	[0.0-2.5]						46.3	44.7	7.9	1.1					
	Meat	0.0	[0.0-3.9]						44.5	47.9	5.0	2.5					
Nalidixic acid	Faeces	1.1	[0.2-4.2]					1.1	41.1	49.5	6.8	0.5		1.1			
	Meat	0.0	[0.0-3.9]					0.8	34.5	63.0	1.7						

		Resistance (%)		Distribution (%) of MIC values (mg/L)													
Substance	Sample	[95% CI*]		0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64
Ciprofloxacin	Faeces	1.1	[0.2-4.2]		20.5	59.5	18.9	1.1									
	Meat	0.0	[0.0-3.9]	0.8	20.2	60.5	18.5										

Bold vertical lines denote microbiological 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.
* CI= Confidence interval.

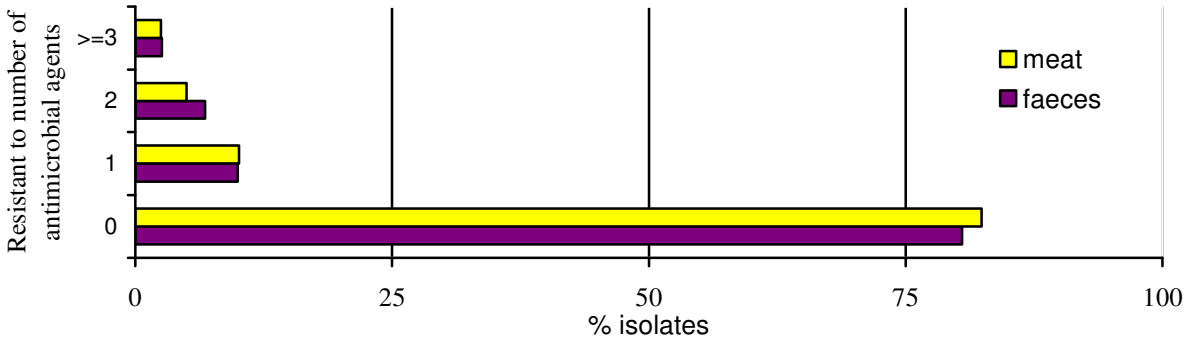

FIGURE 13. Antimicrobial resistance profile for *E. coli* from faecal (n=190) and meat (n=119) samples from broilers. Proportions of isolates susceptible to all or resistant to one, two and three or more antimicrobial agents are illustrated.

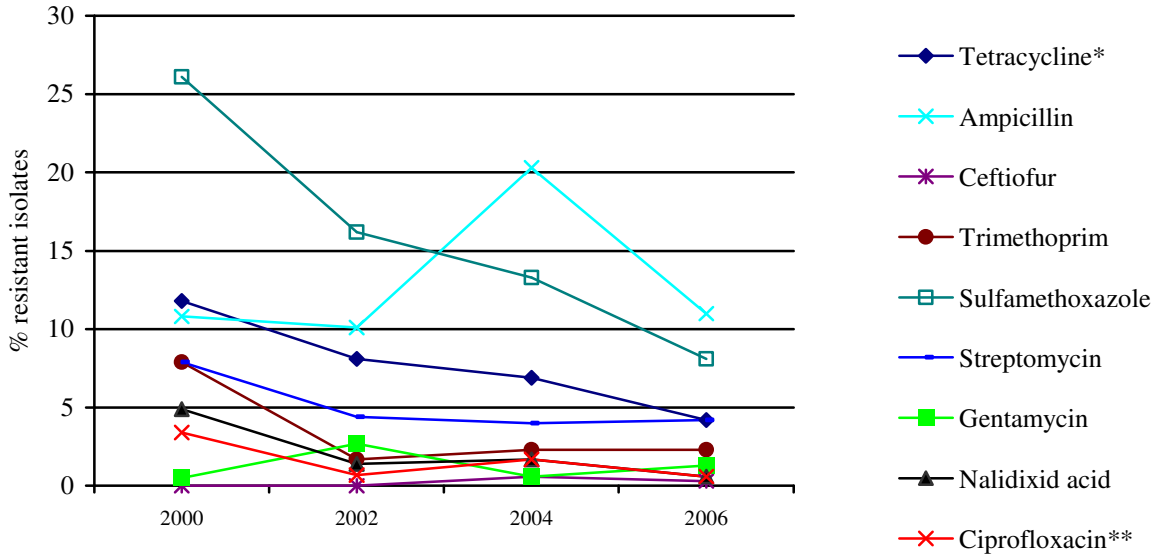

FIGURE 14. Prevalence of resistance to various antimicrobials in *E. coli* from broiler isolates (meat and faecal samples) 2000-2006. The breakpoints in NORM-VET 2006 were applied.
*Oxytetracycline in 2002 and 2004.
**Enrofloxacin before 2006.

RESULTS AND COMMENTS

The occurrence of resistance among *E. coli* from faecal and meat samples from broilers was, in an international perspective, low. The prevalence of resistance in the isolates obtained from faecal samples and those obtained from meat samples were similar. In total, 80.5% and 82.4% of the faecal and meat isolates, respectively, were susceptible to all antimicrobial agents included, 10.0% and 10.1% were resistant to one antimicrobial drug (predominantly ampicillin), 6.8% and 5.0%, to two (mainly ampicillin and sulfamethoxazole) and 2.6% and 2.5% to three or more antimicrobial agents (Figure 13). Resistance to ampicillin was most commonly observed, followed by resistance to sulfamethoxazole and tetracycline/streptomycin. Amoxicillin (cross-resistance with ampicillin) but also some oxytetracycline is used for clinical purposes in broilers, while streptomycines and trimethoprim are not used in Norwegian broiler production. Fluoroquinolone resistance was observed in 1.1% of the faecal isolates which is the same level as previously reported (NORM/ NORM-VET 2000, 2002 and 2004). One isolate showed resistance to ceftiofur and cefotaxime even though cephalosporines are not used in food animals in Norway. This isolate was investigated further and was found to contain an ESBL encoding gene, see textbox on page 30 for further details. This is the first ESBL positive isolate ever detected in Norwegian livestock.

The prevalence of resistance to various antimicrobials in *E. coli* for the years 2000-2006 (Fig. 14) indicates that the occurrence of resistance in *E. coli* from broiler has decreased significantly ($p<0.05$) for tetracyclines, trimethoprim, sulfamethoxazole and nalidixic acid since 2000 and resistance to streptomycines is significantly lower ($p<0.05\%$) than observed in 2004. Resistance to ampicillin (cross-resistance with amoxicillin) has remained at the same low level with the exception of 2004, when the prevalence was doubled.

Formerly, sulfonamides were the most commonly used antimicrobials in poultry in Norway. Since the early 1990s antimicrobials belonging to this drug group have not been in use. This may explain why, since 2000, resistance towards the sulfonamides (Figure 14) has declined significantly.

First description of an extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* from Norwegian livestock

The extended spectrum beta-lactamase (ESBL) enzymes are able to hydrolyze broad-spectrum beta-lactams such as third- and fourth-generation cephalosporins as well as monobactams. ESBL producing bacteria is an emerging problem worldwide and have particularly been associated with hospital infections. ESBL producing bacteria have now also been detected from a variety of sources outside the hospitals (Aarestrup 2005, Liebana 2006, Hasman 2005, Mesa et al 2006, Paterson 2006). So far, no ESBL producing bacteria have been detected from Norwegian livestock. However, in 2006 an isolate of *Escherichia coli* from a faecal sample of a healthy broiler showed reduced susceptibility to cephalosporins. The minimum inhibitory concentration (MIC) values to ceftiofur and cefotaxime were 4 mg/L and 1 mg/L, respectively. This is the first time an isolate of animal origin from a Norwegian livestock has shown reduced susceptibility to cephalosporins.

The isolate was investigated for clavulanic acid (CLA) synergy using a disk test. Extension of zones of inhibition (synergism) towards a tablet containing amoxycillin+CLA was observed. A confirmation test using disks with cefepime (30 µg) and ceftazidime (30 µg) with and without CLA was also positive. MICs to cephalothin, ceftazidime and cefepime were determined and the values are listed in Table 11.

Polymerase chain reaction (PCR) was performed for detection of the most common ESBL encoding genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}). PCR and subsequent DNA sequencing showed the presence of a bla_{TEM-20} gene. The strain expressed low-level resistance to cefotaxime (MIC = 1 mg/L), a value close to the clinical breakpoint recommended by EUCAST (S ≤ 1 mg/L, R > 2 mg/L, www.eucast.org). However, the MIC was above the susceptible wild-type distribution (WT ≤ 0.25 mg/L).

Conjugation experiments were carried out with *E. coli* DH5α, a *Salmonella* Typhimurium strain and an intestinal *E. coli* from a dog as recipient strains. The conjugation experiments showed that diminished susceptibility to cephalosporins was transfered to the three different recipient strains indicating that bla_{TEM-20} was located on a mobile DNA element. The MICs did not change noteworthy when bla_{TEM-20} was transfered into the other recipient strains. However, the *Salmonella* transconjugant had a cefotaxime MIC of 2 mg/L, which is close to a resistant phenotype.

The finding of an *E. coli* containing an ESBL encoding gene from a domestic animal in Norway is surprising as there is no selection pressure from cephalosporin usage. There have been no commercial veterinary preparations containing cephaloporins available on the market in Norway until summer 2007, and to our knowledge such preparations have never been licensed for therapeutic use to production animals in Norway. Interview with the farmer confirmed that there had been no use of broad-spectrum β-lactams to any animals or humans at the farm.

The origin of the detected ESBL producing strain is unknown. One explanation might be that the strain, or the transferable plasmid harboured by the strain, could be part of the bacterial flora of imported animals taken into Norway for breeding purposes. Our findings show that an ESBL encoding gene occurs in an *E. coli* present in the normal flora of a healthy domestic animal not exposed to broad–spectrum β-lactams.

TABLE 11. Minimum inhibitory concentrations (MICs) of strains in the study.

Strain	Ampicillin* S ≤ 0,5 R > 8	Cefalothin** WT ≤ 32	Ceftiofur** WT ≤ 1	Cefotaxime*** S ≤ 1 R > 2	Cefepime*** S ≤ 1 R >8	Ceftazidime*** S ≤ 1 R> 8
E. coli, broiler	>256	64	4	1	0.25	0.5
Transconjugant (DH5α)	>256	64	1	1	0.25	0.5
Transconjugant (*E. coli*, dog)	>256	64	1	1	0.5	1.5
Transconjugant (*Salmonella*)	>256	≥ 256	2	2	0.5	1

* Breakpoints recommended by NWGA. ** Clinical breakpoint not given, WT distribution given.
*** Clinical breakpoints recommended by EUCAST

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Enterococcus spp. from broilers

A total of 219 faecal and 126 meat samples from broilers were collected. *E. faecium* or *E. faecalis* was identified in 205 (93.6 %) of the faecal samples and 103 (81.7%) of the meat samples from broilers. One isolate per positive sample was susceptibility tested. The results are presented in Tables 12-13, Figures 15-16, and in the text.

TABLE 12. Antimicrobial resistance in *Enterococcus faecalis* from faeces (n=5) and meat (n=14) samples from broilers.

		Resistant	Distribution (n) of MIC values (mg/L)														
Substance	Sample	(n)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥ 2048
Tetracycline	Faeces	0			2	3											
	Meat	2			7	5						1	1				
Chloramphenicol	Faeces	0							5								
	Meat	1					1	1	11			1					
Ampicillin	Faeces	0				4	1										
	Meat	0			1	12	1										
Erythromycin	Faeces	1			1	1	2						1				
	Meat	2			3	6		3					2				
Streptomycin	Faeces	1										3	1				1
	Meat	1									2	7	4				1
Gentamicin	Faeces	0							2	3							
	Meat	0						1	8	3	2						
Kanamycin	Faeces	0									3	2					
	Meat	0								1	9	2	2				
Vancomycin	Faeces	0					3	2									
	Meat	0					12	2									
Bacitracin#	Faeces	1						1	2	1				1			
	Meat	0						2	4	6	2						
Linezolide	Faeces	0				1	4										
	Meat	0			1	1	11	1									
Virginiamycin	Faeces	NR								3	2						
	Meat	NR						1		6	5	2					
Narasin	Faeces	1				1	2	1		1							
	Meat	1		6		6	1			1							

Bold vertical lines denote microbiological 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. #Measured in U/ml.

TABLE 13. Antimicrobial resistance in *Enterococcus faecium* from faeces (n=200) and meat (n=89) samples from broiler.

		Resistance (%)		Distribution (%) of MIC values (mg/L)													
Substance	Sample		[95%CI*]	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥ 2048
Tetracycline	Faeces	8.5	[5.2-13.5]		66.0	25.0	0.5		0.5	1.5	0.5	4.5	1.5				
	Meat	11.2	[5.8-20.1]		75.3	12.4	1.1			2.2	3.4	2.2	3.4				
Chloramph.	Faeces	0	[0.0-2.3]			1.0	8.5	35.5	48.0	5.0	2.0						
	Meat	0	[0.0-5.2]				5.6	40.4	49.4	4.5							
Ampicillin	Faeces	1.5	[0.4-4.7]	3.5	9.0	28.0	36.5	15.0	6.5		1.0	0.5					
	Meat	0	[0.0-5.2]	5.6	5.6	24.7	43.8	14.6	5.6								
Erythromycin	Faeces	18.5	[13.5-24.7]		14.0	34.0	19.5	14.0	8.0	6.5	1.5		2.5				
	Meat	21.3	[13.6-31.5]		6.7	43.8	18.0	10.1	4.5	9.0	3.4		4.5				
Streptomycin	Faeces	0.5	[0.0-3.2]							4.0	50.0	42.5	3.0				0.5
	Meat	0	[0.0-5.2]							10.1	46.1	40.4	3.4				
Gentamicin	Faeces	0	[0.0-2.3]				1.0	19.5	62.0	15.5	2.0						
	Meat	0	[0.0-5.2]				4.5	25.8	52.8	15.7	1.1						
Kanamycin	Faeces	2.0	[0.6-5.4]									8.0	35.5	30.5	17.5	6.5	2.0
	Meat	1.1	[0.1-6.9]								1.1	14.6	27.0	43.8	9.0	3.4	1.1
Vancomycin	Faeces	0	[0.0-2.3]			68.5	10.5	21.0									
	Meat	1.1	[0.1-6.9]			66.3	18.0	14.6						1.1			
Bacitracin#	Faeces	41.0	[34.2-48.2]			11.5	12.0	1.0	2.0	15.5	17.0	13.0	8.5	19.5			
	Meat	37.1	[27.6-48.5]			15.7	6.7	1.1	11.2	20.2	7.9	12.4	13.5	11.2			
Linezolide	Faeces	0	[0.0-2.3		1.0	11.0	59.0	29.0									
	Meat	0	[0.0-5.2]		11.2	60.7	28.1										
Virginiamycin	Faeces	3.5	[1.5-7.4]		5.5	37.0	19.0	35.0	2.5	0.5		0.5					
	Meat	3.4	[0.9-10.3]		5.6	61.8	9.0	20.2	2.2	1.1							
Narasin	Faeces	72.5	[65.7-78.4]	16.5	5.5	3.5	11.5	47.0	8.5	5.5							
	Meat	68.5	[57.7-77.7]	11.2	14.6	5.6		12.4	52.8	3.4							

Bold vertical lines denote microbiological 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. * CI= Confidence interval. #Measured in U/ml.

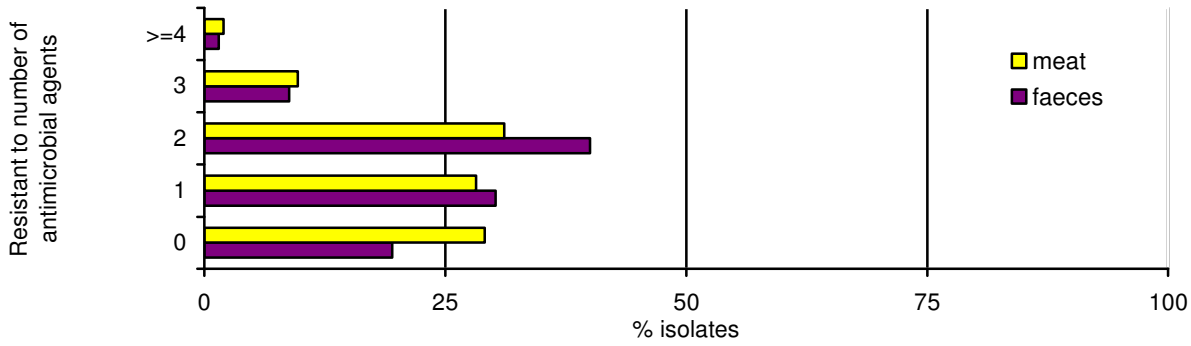

FIGURE 15. Antimicrobial resistance profile for *Enterococcus* spp. (205 meat and 103 faecal samples). Proportions of isolates susceptible to all or resistant to one, two, three and four or more antimicrobial agents are included.

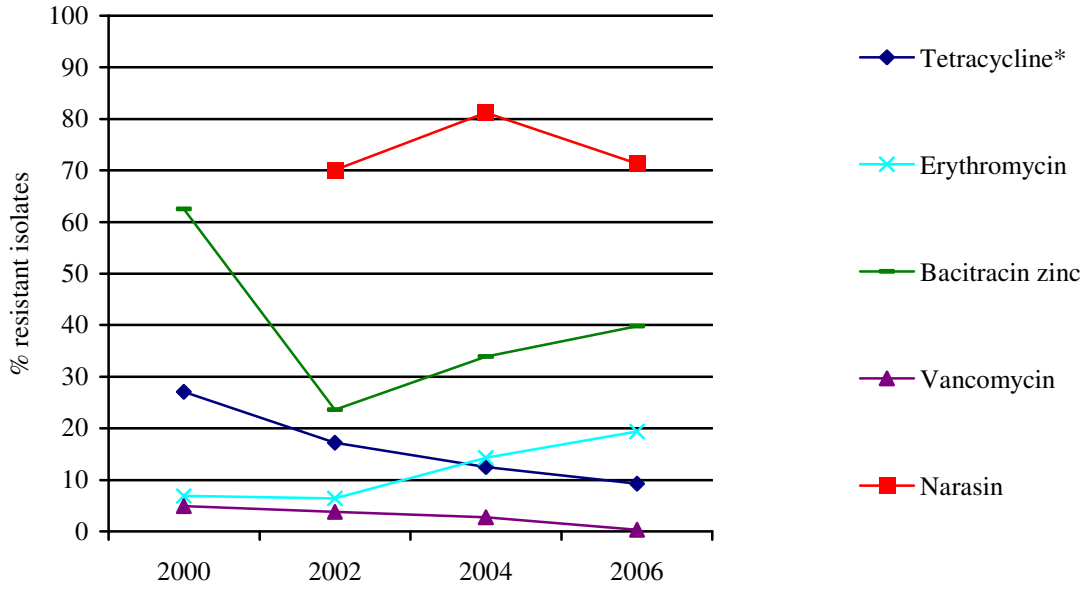

FIGURE 16. Prevalence of resistance to various antimicrobials in *E. faecium* from broiler isolates (meat and faecal samples) in the years 2000-2006. The breakpoints in NORM-VET 2006 were applied.
* Oxytetracycline in 2002 and 2004

RESULTS AND COMMENTS

E. faecalis is known to be inherently resistant to the streptogramin virginiamycin, while *E. faecium* is known to be susceptible to this antimicrobial agent. Resistance to virginiamycin is therefore not included in the following comments.

Coccidiostats are routinely used in Norwegian broiler production, and since 1996 such use has been dominated by the ionophore narasin. The selection pressure exerted by the common use of narasin in broiler production is probably the reason why narasin resistance is frequently observed among enterococci from broilers, *E. faecium* in particular. The prevalence of resistance to the other antimicrobial agents among *E. faecalis* and *E. faecium* from healthy broilers was moderate.

In 2006, a low number of *E. faecalis* isolates was obtained both from the faecal and the meat samples collected. Therefore no statistical comparisons or conclusions could be made for this species alone. However, a high number of *E. faecium* isolates were obtained from the collected samples. The results for this species are therefore more robust than in previous years.

The resistance profiles presented in Figure 15 include all the *Enterococcus* spp. isolated in 2006 divided by origin of sample; faecal or meat. The resistance in faecal and meat samples was similar. In total 19.5% and 29.1% of the faecal and the meat isolates, respectively, were susceptible to all antimicrobial agents included, 30.2% and 28.2% were resistant to one antimicrobial agent and 50.3% and 42.7% were resistant to two or more antimicrobial agents..

The *E. faecium* strains tested in 2006 were frequently resistant to narasin and to a lesser extent to bacitracin, erythromycin and tetracycline while the resistance towards vancomycin was negligible (Table 13 and Figure 16). Resistance to narasin is explained by its use as a feed additive in most of the broiler production in Norway while the low frequency of tetracycline resistance is explained by insignificant use of oxytetracycline for clinical purposes in Norwegian broiler production. Erythromycin has never been used in broilers in Norway. However, resistance to erythromycin acquired by *E. faecalis* may be explained by former use of spiramycin in broilers as cross-resistance between erythromycin and spiramycin is common. Spiramycin was licensed for use in poultry until 1998 when it was withdrawn due to limited sales.

Bacitracin was formerly used as a growth promoter, but such use was negligible during the 1990s. No use of bacitracin has been recorded in animal production in Norway after 1997 (Table 7). Thus, the observed resistance towards bacitracin in *E. faecalis* may indicate that such resistance to some extent persists.

Only a single *E. faecium* isolate from poultry obtained by a random selection was vancomycin resistant. The isolate was recovered from meat and the MIC-value was >128 mg/L.

Avoparcin, which induces cross-resistance to vancomycin, was routinely used as a growth promoter in Norwegian broiler and turkey production from 1986 until it was banned in 1995. Studies have shown that this use has selected for an extensive reservoir of vancomycin resistant enterococci (VRE) in Norwegian broiler production. The reservoir has persisted at a high prevalence level for at least eight years after the ban was implemented.

Eight (2.4%) of the strains from poultry obtained by a selective isolation procedure were *vanA* positive. All of these isolates were *E. faecium* from faecal samples. This is a significant decrease compared to previous reports and will be followed up in the future.

The prevalence of resistance to various antimicrobials in *Enterococcus* spp. in the period 2000-2006 indicates possible trends in the occurrence of resistance in *Enterococcus* spp. from broilers (Figure 16). While resistance to tetracycline and vancomycin has declined significantly ($p<0.05\%$), resistance to erythromycin has increased significantly ($p<0.05\%$). In recent years, minor amounts of a tylosin premix have been sold in Norway on exemption from market authorization, and this premix may have been used in slaughter chicken as well as in pigs.

ZOONOTIC AND NON-ZOONOTIC ENTEROPATHOGENIC BACTERIA

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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, all *Salmonella* isolates from control programmes concerning feed samples, animals and food products, as well as a representative number of *Campylobacter* isolates from broiler and broiler meat samples 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.

SALMONELLA SPP.

Salmonella from animals

The situation regarding occurrence of *Salmonella* spp. in food producing animals in Norway is very good. Such animals are virtually free from *Salmonella* spp. except for the endemic occurrence of *S. enterica* subsp. *diarizonae* in sheep. To document this favourable situation, Norway runs an extensive surveillance programme that covers both live animals (cattle, pigs and poultry) and meat samples (cattle, pigs, sheep and poultry). The *Salmonella* isolates examined in NORM-VET include those that are detected in this programme, in addition to selected isolates from other relevant projects, as well as clinical submissions to the National Veterinary Institute. The data are presented in Table 14-15 and in the text.

TABLE 14. Antimicrobial resistance in *S.* Typhimurium (n=7) and other *Salmonella* spp. (n=6) isolates from animals. Distribution (n) of MICs (mg/L).

		Distribution (n) of MIC values (mg/L)															
Substance	Resistance (n)	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	1							2	10				1				
Chloramphenicol	1								3	6	3					1	
Florfenicol	0									10	3						
Ampicillin	1						5	5	2					1			
Ceftiofur	0					2	6	5									
Cefotaxime	0			6	7	1											
Trimethoprim	1					5	6	1						1			
Sulfamethoxazole	1											2	8	2			1
Streptomycin	1									1	8	3					1
Gentamicin	0						9	4									
Kanamycin	0								9	4							
Ciprofloxacin	0	2		1	10												
Nalidixic acid	0								1	10	2						

Bold vertical lines denote microbiological 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 15. Antimicrobial resistance in *Salmonella* spp. (n=97) from diagnostic submissions of reptiles during the years 1997-2006. Distribution (%) of MICs (mg/L).

	Resistance (%)	Distribution (%) of MIC values (mg/L)															
Substance	[95% CI*]	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	2.1 [0.4-8.0]							61.9	36.1			1.0			1.0		
Chloramphenicol	0.0 [0.0-4.7]							1.0	15.5	67.0	16.5						
Florfenicol	0.0 [0.0-4.7]									82.5	17.5						
Ampicillin	0.0 [0.0-4.7]						43.3	52.6	4.1								
Ceftiofur	0.0 [0.0-4.7]					10.3	64.9	24.7									
Cefotaxime	0.0 [0.0-4.7]			54.6	42.3	3.1											
Trimethoprim	0.0 [0.0-4.7]					72.2	27.8										
Sulfamethoxazole	1.0 [0.0-6.4]											42.3	47.4	9.3			1.0
Streptomycin	1.0 [0.0-6.4]								3.1	15.5	41.2	25.8	13.4	1.0			
Gentamicin	0.0 [0.0-4.7]						90.7	9.3									
Kanamycin	0.0 [0.0-4.7]								94.8	3.1	1.0		1.0				
Ciprofloxacin	0.0 [0.0-4.7]	16.5		41.2	41.2	1.0											
Nalidixic acid	1.0 [0.0-6.4]								16.5	82.5					1.0		

Bold vertical lines denote microbiological 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.
*CI = Confidence interval.

RESULTS AND COMMENTS

In 2006, a total of 13 isolates of *Salmonella* spp. were detected and susceptibility tested. The seven isolates of *S.* Typhimurium were from cattle, poultry, dog, cat, horse, duck and a wild bird. The other six *Salmonella* spp. were *S.* Kedugo, *S. diarizonae* (38:r:z) and *S.* Mikawasima from horses, *S.* Montevideo from a dog, *S.* Anatum from poultry, and *S.* Dublin from swine. Only one isolate from poultry was multiresistant. The other isolates were susceptible to all antimicrobial agents included except one *S.* Anatum isolate from poultry which was resistant to tetracyclines.
In addition, 97 isolates of *Salmonella* spp. from diagnostic submissions (1997-2006) from reptiles in zoo facilities etc. were susceptibility tested (Table 15). The occurrence of resistance among these isolates was low. One isolate was resistant to tetracycline, sulfa-methoxazole and streptomycin, while two other isolates were monoresistant to tetracycline and nalidixic acid, respectively. The remaining 94 isolates were susceptible to all the antibiotics included in the survey.
The data, although very limited, indicate that antimicrobial resistance is not very widespread among those *Salmonella* that occasionally are isolated from animals in Norway.

Salmonella from human clinical specimens

In 2006, a total of 1,813 cases of human salmonellosis were reported of which 384 (21%) were infected in Norway. The incidence rate was 39.4 per 100,000. Altogether 895 (49%) of the cases were due to *S.* Enteritidis, of which 85 (9%) were infected in Norway, while 295 (16%) of the cases were due to *S.* Typhimurium, of which 135 (46%) were infected in Norway. The latter is partly explained by the endemic occurrence of specific clones of this serovar in Norwegian wildlife.
Data from surveillance programmes show that domestically produced food is not an important source of human salmonellosis acquired in Norway. The most likely sources of salmonellosis acquired in Norway are wild birds and hedgehogs, imported food products and patients infected abroad. Thus, the isolates categorized as "infected in Norway" also partly reflect the *Salmonella* situation outside Norway.
The proportion of multiresistant *S.* Typhimurium DT104 from domestically acquired cases of *S.* Typhimurium infections was 13.3%. This is lower than in 2005 (22.2%) but still higher than in 2003 and 2004, with a proportion of 3.8% and 0%, respectively. The proportion of multi-resistant *S.* Typhimurium DT104 from *S.* Typhimurium infections acquired abroad was 18.7%. In total, 275 isolates of *S.* Typhimurium, 834 isolates of *S.* Enteritidis, 19 isolates of *S.* Typhi, eight isolates of *S.* Paratyphi A, six isolates of *S.* Paratyphi B, and 508 isolates of other *Salmonella* spp. were susceptibility tested. The results are presented in Tables 16-19, Figures 17-20, and in the text.

TABLE 16. *Salmonella* Typhimurium isolates (n=113), including multiresistant DT104 (n=15), from patients infected in Norway. Distribution (%) of antimicrobial susceptibility groups. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 0.5	> 8	2.7	68.1	29.2
Chloramphenicol	≤ 8	> 8	85.0	-	15.0
Tetracycline	≤ 4	> 8	66.4	0.9	32.7
Nalidixic acid	≤ 16	> 16	89.4	-	10.6
Ciprofloxacin	≤ 0.5	> 1	99.1	0.0	0.9
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	95.6	0.9	3.5

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

TABLE 17. *Salmonella* Typhimurium isolates (n=150), including multiresistant DT104 (n=28), from patients infected outside Norway. Distribution (%) of antimicrobial susceptibility groups. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 0.5	> 8	2.0	56.0	42.0
Chloramphenicol	≤ 8	> 8	77.3	-	22.7
Tetracycline	≤ 4	> 8	42.0	1.3	56.7
Nalidixic acid	≤ 16	> 16	78.0	-	22.0
Ciprofloxacin	≤ 0.5	> 1	96.7	3.3	0.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	89.3	0.0	10.7

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

TABLE 18. *Salmonella* Enteritidis isolates from patients (n=834[#]). Distribution (%) of antimicrobial susceptibility groups. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 0.5	> 8	0.9	92.1	7.0
Chloramphenicol	≤ 8	> 8	99.5	-	0.5
Tetracycline	≤ 4	> 8	97.0	0.0	3.0
Nalidixic acid	≤ 16	> 16	81.3	-	18.7
Ciprofloxacin	≤ 0.5	> 1	99.8	0.2	0.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	98.7	0.1	1.2

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.
Place of infection; Norway (n=62), abroad (n=746), unknown (n=26).

TABLE 19. *Salmonella* spp. (excluding *S*. Typhimurium, *S*. Enteritidis, *S*. Typhi and *S*. Paratyphi) (n=508[#]). Distribution (%) of antimicrobial susceptibility groups. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 0.5	> 8	1.6	86.0	12.4
Chloramphenicol	≤ 8	> 8	94.5	-	5.5
Tetracycline	≤ 4	> 8	74.2	0.2	25.6
Nalidixic acid	≤ 16	> 16	86.0	-	14.0
Ciprofloxacin	≤ 0.5	> 1	97.6	1.4	1.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	92.5	0.2	7.3

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.
Place of infection; Norway (n=83), abroad (n=398), unknown (n=27).

FIGURE 17. Prevalence of resistance to various antimicrobials in *Salmonella* Typhimurium including multiresistant DT104. from humans infected in Norway 2001-2006. The breakpoints in NORM 2006 were applied.
*TMS=Trimethoprim-sulfamethoxazole.

FIGURE 18. Prevalence of resistance to various antimicrobials in *Salmonella* Typhimurium including multiresistant DT104. from humans infected outside Norway 2001-2006. The breakpoints in NORM 2006 were applied.
*TMS=Trimethoprim-sulfamethoxazole.

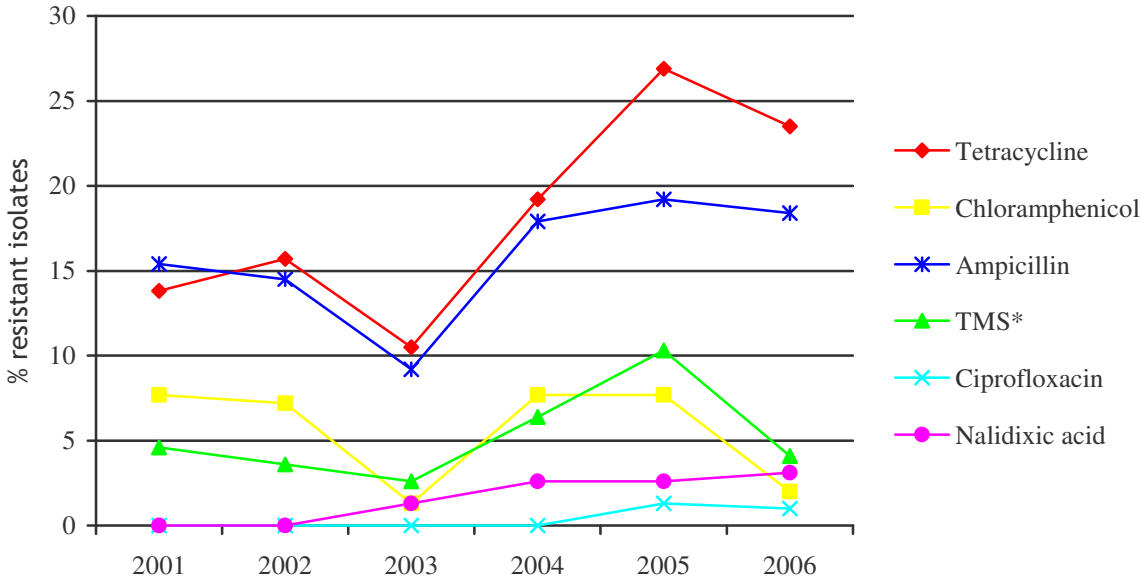

FIGURE 19. Prevalence of resistance to various antimicrobials in *Salmonella* Typhimurium excluding multiresistant DT104. from humans infected in Norway 2001-2006. The breakpoints in NORM 2006 were applied.
*TMS=Trimethoprim-sulfamethoxazole.

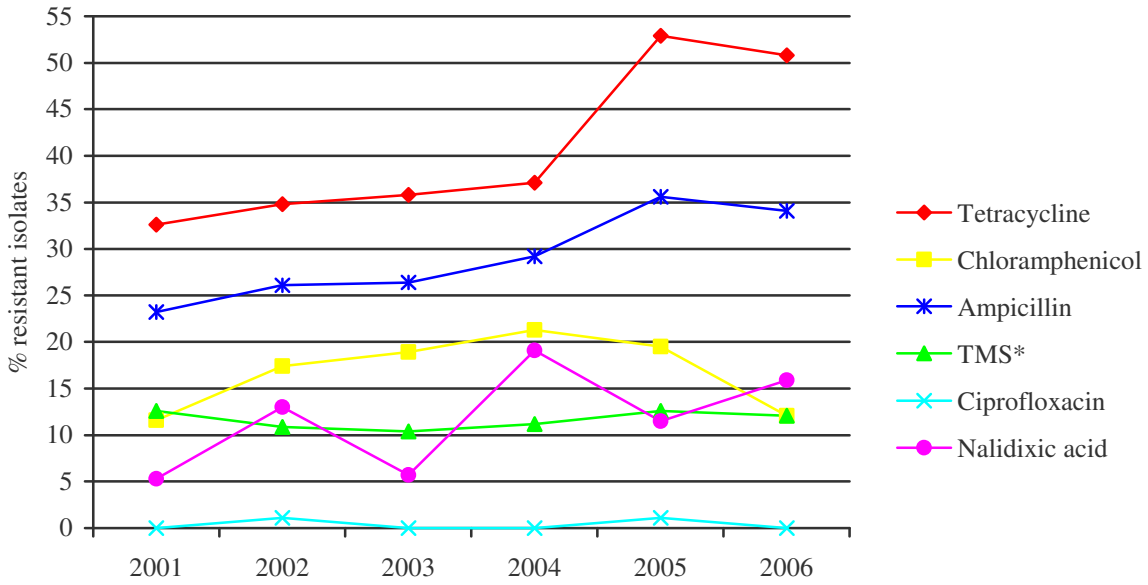

FIGURE 20. Prevalence of resistance to various antimicrobials in *Salmonella* Typhimurium excluding multiresistant DT104. from humans infected outside Norway 2001-2006. The breakpoints in NORM 2006 were applied.
*TMS=Trimethoprim-sulfamethoxazole.

RESULTS AND COMMENTS

For *S.* Typhimurium, resistance to tetracycline was most commonly observed followed by resistance to ampicillin, nalidixic acid, chloramphenicol and trimethoprim-sulfamethoxazole (Figure 20).

The proportion of *S.* Typhimurium isolates susceptible to all antimicrobial agents tested was higher for the category "infected in Norway" (63.7%) than for the "infected abroad" category (34.7%) (Figure 21). Multiresistant strains defined as resistant to two or more antimicrobial agents, were more common in the category "infected abroad" (43.4%) than in the category "infected in Norway" (26.6%). The prevalence of resistance for the years 2001-2006 to various antimicrobials in *S.* Typhimurium from both humans infected in Norway (Figures 17 and 19) and abroad (Figures 18 and 20) indicates that the occurrence of resistance to tetracycline and ampicillin might be increasing. However, the situation has been stable over the last two years.

The vast majority of *S.* Enteritidis isolates had been acquired abroad (Table 18). The proportion of *S.* Enteritidis isolates resistant to the different antimicrobial agents included, except for nalidixic acid, was considerably lower than for *S.* Typhimurium. In total, 18.7% of the isolates of *S.* Enteritidis were resistant to nalidixic acid. There were no isolates fully resistant to ciprofloxacin, but 0.2% of the isolates were only intermediately susceptible to this agent. The resistance frequencies observed for *S.* Enteritidis in NORM/NORM-VET 2006 are similar to those reported in previous reports.

With regard to *Salmonella* spp. isolates other than *S.* Typhimurium and *S.* enteritidis, most infections had been acquired abroad and antimicrobial resistance was frequently detected (Table 19). Resistance to tetracycline was most common, followed by resistance to nalidixic acid and ampicillin. Similar to what was observed for *S.* Enteritidis isolates, ciprofloxacin resistance was observed in 1.0%, while 1.4% showed reduced susceptibility to ciprofloxacin. It is emphasized that the use of fluoroquinolones in Norway is very limited in both human and veterinary medicine.

The few isolates of *S.* Typhi (n=19), *S.* Paratyphi A (n=8) and *S.* Paratyphi B (n=6) in 2006 indicate that multiresistance is common including resistance to nalidixic acid. With the exception of one case of unknown origin, all these infections had been acquired abroad. Four and three isolates of *S.* Typhi and *S.* Paratyphi A, respectively, were resistant to one or more of the antimicrobial agents included in the survey. No resistance was observed in the six isolates of *S.* Paratyphi B.

In 2006, the detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefpodoxime. All isolates with reduced susceptibility to cefpodoxime were further characterized by combination Etests and/or molecular examination. A total of eight isolates displayed reduced susceptibility to cefpodoxime. One isolate of *S.* Typhimurium and two isolates of *S.* Enteritidis were identified as ESBL producers.

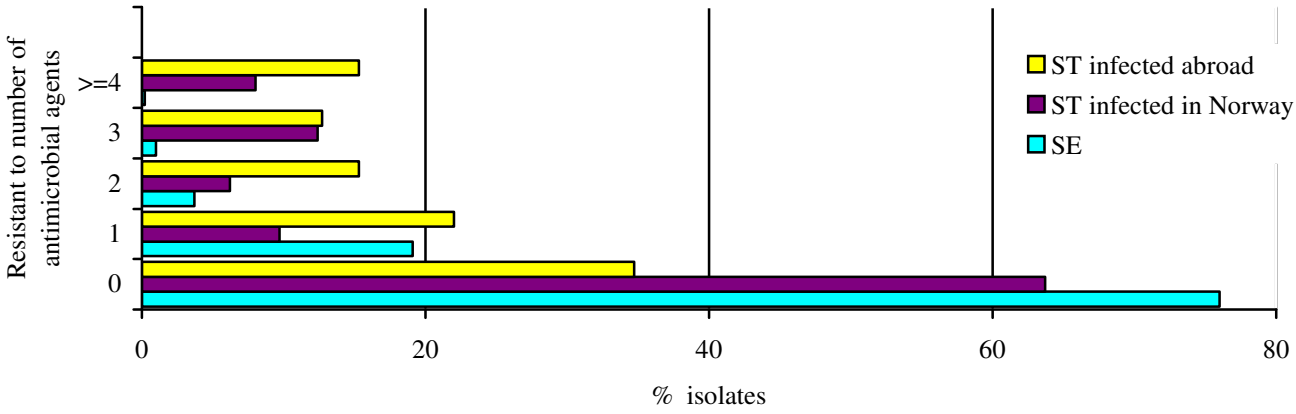

FIGURE 21. Antimicrobial resistance profiles for all *Salmonella* Enteritidis (SE) from humans (n=834) and for *Salmonella* Typhimurium (ST) from humans infected in Norway (n=31) and abroad (n=150), respectively. Proportion of isolates susceptible to all or resistant to one, two, three, or four or more antimicrobial agents are illustrated.

CAMPYLOBACTER SPP.

Campylobacter jejuni from broilers

The isolates of *Campylobacter jejuni* in broilers originate from the Norwegian action plan against *Campylobacter* spp. in broiler meat production. All broiler flocks slaughtered before 50 days of age are tested for the presence of *Campylobacter* spp. In 2006, one isolate per positive farm identified was submitted for susceptibility testing. A total of 108 isolates from broiler flocks (cloacal samples) were susceptibility tested. The results are presented in Table 20, Figure 22, and in the text.

TABLE 20. Antimicrobial resistance in *Campylobacter jejuni* (n=108) from broiler flocks. Distribution (%) of MICs (mg/L).

	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	≥ 512
Oxytetracycline	0.0	[0.0-4.3]				94.4	5.6										
Ampicillin	6.5	[2.9-13.4]						2.8	8.3	63.0	19.4	1.9	2.8	0.9	0.9		
Erythromycin	0.0	[0.0-4.3]				12.0	57.4	30.6									
Gentamicin	0.0	[0.0-4.3]				10.2	73.1	16.7									
Enrofloxacin	1.9	[0.3-7.3]		5.6	80.6	12.0					1.9						
Nalidixic acid	1.9	[0.3-7.3]							1.9	31.5	64.8			0.9		0.9	

Bold vertical lines denote microbiological cut-off values. 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.
*CI = Confidence interval.

RESULTS AND COMMENTS

The results show that the occurrence of antimicrobial resistance among *C. jejuni* isolates from Norwegian broilers is low. A total of 91.6 % of the included isolates were susceptible to all antimicrobial agents tested. Altogether, 6.5% were resistant to one antimicrobial agent (ampicillin) and 1.9% to both nalidixic acid and enrofloxacin. The results reflect the usage of antimicrobial agents in poultry production. Antimicrobials except coccidiostatic agents are rarely used, and only for therapeutic purposes. If used, the aminopenicillin amoxicillin or the tetracycline oxytetracycline are the drugs of choice. Nalidixic acid is not used in poultry, but a minor amount of enrofloxacin has been used on exemption from marked authorization in recent years (K. Grave, unpublished data).

The results are similar to those presented in previous NORM/NORM-VET reports (2001, 2002, 2003, 2004 and 2005) as seen in Figure 22. However, the data indicate an increasing trend of resistance to ampicillin. This may be explained by a slight increase in the use of amoxicillin for the treatment of necrotic enteritis in broilers (K. Grave, unpublished data).

The prevalence of resistance and the resistance patterns for *C. jejuni* isolated from Norwegian broilers are similar to what was observed for *C. jejuni* isolated from humans infected within Norway, except for a higher prevalence of resistance to quinolones (nalidixic acid and ciprofloxacin) among the isolates of human origin. This relationship was also observed in previous NORM/NORM-VET reports.

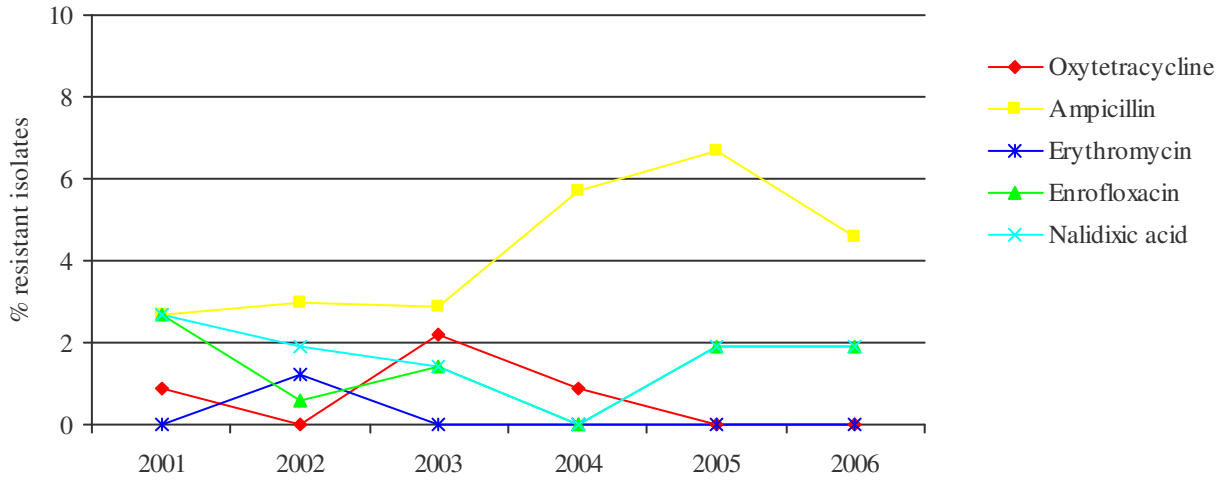

FIGURE 22. Prevalence of resistance to various antimicrobials in *Campylobacter jejuni* from Norwegian broilers 2001-2006. The breakpoints for resistance defined in NORM 2006 were applied.

Campylobacter spp. from human clinical specimens

Among the 2,593 cases of human campylobacteriosis recorded in Norway in 2006, 48% were reported as acquired abroad. The incidence rate was 56.3 per 100,000. The vast majority of cases were sporadic. Case-control studies in Norway have revealed that consumption of broiler meat purchased fresh and drinking of untreated water are important risk factors for domestically acquired campylobacteriosis. A total of 248 isolates of *C. jejuni*, 31 from patients infected in Norway, 119 from patients infected abroad and 98 from patients where the origin of infection was unknown, as well as eleven isolates of *C. coli*, six isolates of *C. lari* and three isolates of *C. upsaliensis* were susceptibility tested. The results are presented in Tables 21-24, Figures 23-25, and in the text.

TABLE 21. *Campylobacter jejuni* isolates from patients infected in Norway (n=31). Distribution (%) of antimicrobial susceptibility groups.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline	≤ 2	> 2	100.0	-	0.0
Erythromycin	≤ 0.5	> 4	3.2	96.8	0.0
Gentamicin	≤ 4	> 4	87.1	-	12.9
Nalidixic acid	≤ 16	> 16	93.5	-	6.5
Ciprofloxacin	≤ 1	> 2	100.0	0.0	0.0

TABLE 22. *Campylobacter jejuni* isolates from patients infected in Norway (n=31). Distribution (%) of MICs (mg/L).

	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Tetracycline		3.2	25.8	38.7	19.3	9.7	3.2							
Erythromycin					3.2	32.2	51.6	12.9						
Gentamicin				3.2	16.2	35.5	29.0	3.2	9.7	3.2				
Nalidixic acid							25.9	38.7	19.3	9.7		6.4		
Ciprofloxacin			29.0	58.1	6.5	6.4								

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

TABLE 23. *Campylobacter jejuni* isolates from patients infected outside Norway (n=119). Distribution (%) of antimicrobial susceptibility groups.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline	≤ 2	> 2	53.8	-	46.2
Erythromycin	≤ 0.5	> 4	6.7	89.9	3.4
Gentamicin	≤ 4	> 4	95.8	-	4.2
Nalidixic acid	≤ 16	> 16	37.8	-	62.2
Ciprofloxacin	≤ 1	> 2	41.2	0.0	58.8

TABLE 24. *Campylobacter jejuni* isolates from patients infected outside Norway (n=119). Distribution (%) of MICs (mg/L).

	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Tetracycline		1.7	14.3	25.2	7.5	4.2	0.8	1.6	0.8	5.0	10.1	10.1	3.4	15.1
Erythromycin				3.4	3.4	38.7	44.5	6.7	2.5					0.8
Gentamicin			0.8	3.4	21.8	36.1	31.1	2.5	2.5					1.7
Nalidixic acid						0.8	14.3	15.9	5.1	1.7	1.7	1.6		58.8
Ciprofloxacin	0.8		10.1	22.7	5.9	1.6					58.8			

*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.

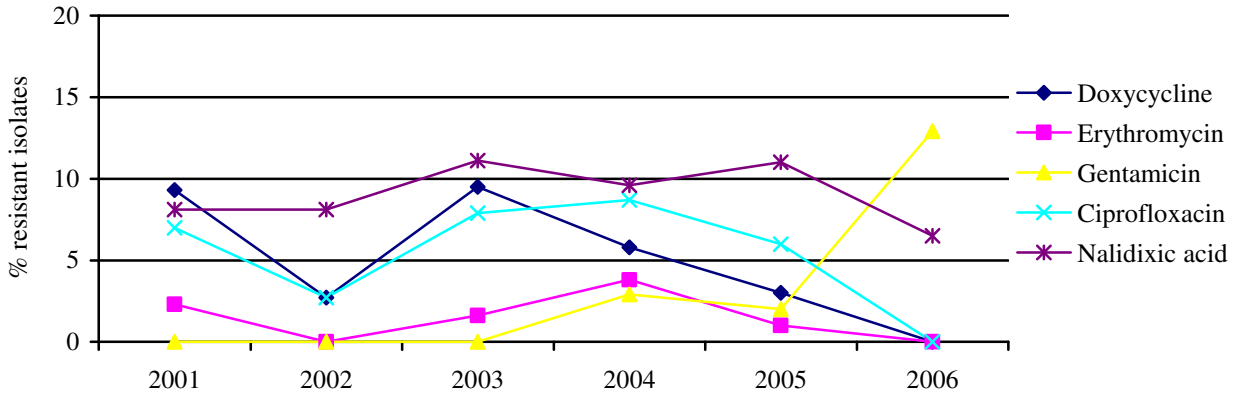

FIGURE 23. Prevalence of resistance to various antimicrobials in *Campylobacter jejuni* from humans infected in Norway 2001-2006. The breakpoints in NORM 2006 were applied.

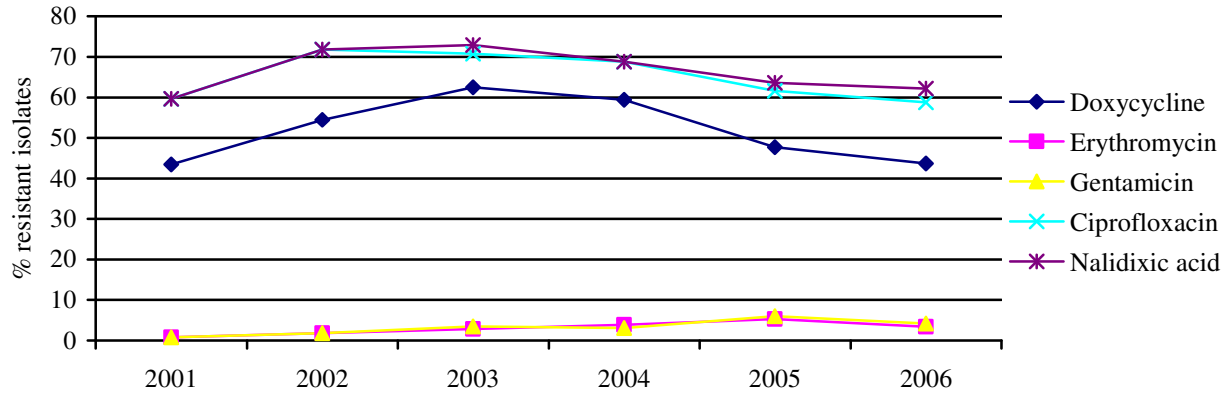

FIGURE 24. Prevalence of resistance to various antimicrobials in *Campylobacter jejuni* from humans infected outside Norway 2001-2006. The breakpoints in NORM 2006 were applied.

RESULTS AND COMMENTS

The data show that resistance was significantly more widespread among *C. jejuni* isolates recovered from patients infected abroad than in patients infected in Norway. Only 31.1% of isolates from infections acquired abroad were susceptible to all antimicrobial agents tested compared to 87.1% of the isolates from patients infected in Norway (Figure 25). The main differences between the two groups were seen for ciprofloxacin/nalidixic acid (58.8%/62.2% resistance in isolates from infections acquired abroad versus 0%/6.5% resistance in isolates from infections acquired in Norway) and tetracycline (43.7% resistance in isolates from infections acquired abroad versus 0% resistance in isolates from infections acquired in Norway), see Tables 21-25 and Figures 23-24).

The prevalence of resistance and the resistance patterns for *C. jejuni* isolated from humans infected within Norway correspond well with what was observed for *C. jejuni* isolated from Norwegian broilers, except for a higher prevalence of resistance to quinolones (nalidixic acid and ciprofloxacin) among isolates of human origin. This relationship was also observed in the NORM/NORM-VET programmes for 2001, 2002, 2003, 2004 and 2005. The prevalence of resistance to various antimicrobials in *C. jejuni* from both humans infected in Norway (Figure 23) and abroad (Figure 24) for the period 2001-2006 indicate that the occurrence of resistance is relatively stable, except for a significant ($p<0.05$) increase in gentamicin resistance among the few isolates from infections aquired in Norway. Eight *C. coli* isolates were acquired abroad, whereas the origins of the remaining three isolates were unknown. Nine of these isolates were resistant to at least one of the antimicrobial agents included, mainly quinolones or tetracycline. *C. coli* is typically associated with pigs and pork.

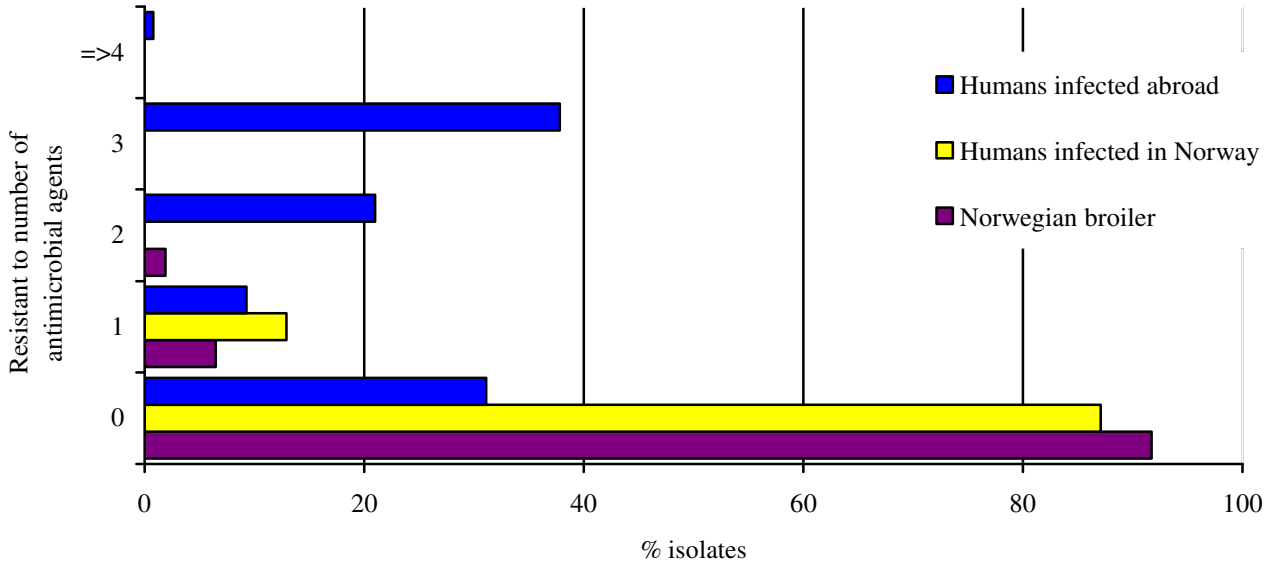

FIGURE 25. Antimicrobial resistance profiles for *Campylobacter jejuni* from Norwegian broiler (n=108), humans infected in Norway (n=31) and humans infected abroad (n=119). Proportion of isolates susceptible to all or resistant to one, two, three, or four or more antimicrobial agents are illustrated. The isolates from humans were tested for susceptibility to tetracycline, erythromycin, gentamicin, ciprofloxacin and nalidixic acid, whereas the broiler isolates in addition were tested for susceptibility to ampicillin.

Yersinia enterocolitica from human clinical specimens

Most cases of *Yersinia enterocolitica* infections in Norway are domestically acquired. A total of 165 cases of yersiniosis were reported in 2006 giving an incidence rate of 3.6 per 100,000. Nienty-nine of these cases (60%) were registered as acquired in Norway. A total of 67 *Y. enterocolitica* isolates were susceptibility tested. The results are presented in Tables 25-26.

TABLE 25. *Yersinia enterocolitica* serogroup O:3 and serogroup O:9 isolates from human clinical cases (n=67[#]). Distribution (%) of antimicrobial susceptibility groups. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 0.5	> 8	0.0	0.0	100.0
Chloramphenicol	≤ 8	> 8	91.0	-	9.0
Tetracycline	≤ 4	> 8	95.5	0.0	4.5
Nalidixic acid	≤ 16	> 16	94.0	-	6.0
Ciprofloxacin	≤ 0.5	> 1	94.0	6.0	0.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	95.5	3.0	1.5

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.
[#] Place of infection; Norway (n=40), Abroad (n=18), Unknown (n=9).

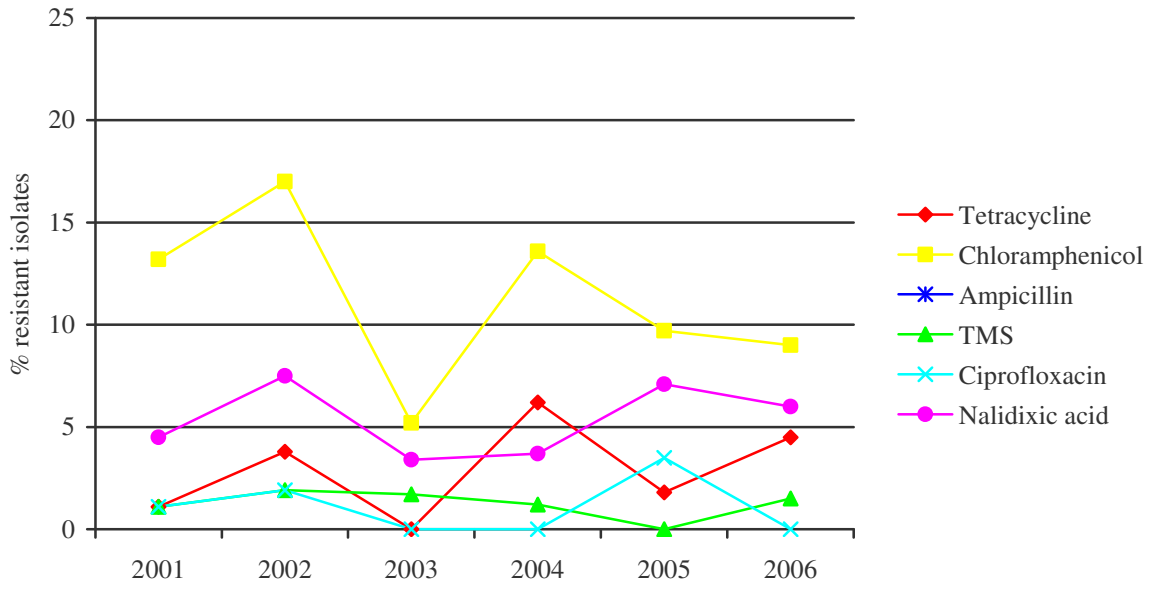

FIGURE 26. Prevalence of resistance to various antimicrobials in *Yersinia enterocolitica* from humans in Norway 2001-2006. The breakpoints in NORM 2006 were applied.

RESULTS AND COMMENTS

The infections in 2006 were mainly domestically acquired. All serogroup O:3 and O:9 isolates expressed intrinsic resistance to ampicillin. The prevalence of resistance to other antimicrobials was stable compared to earlier years (Figure 26).

In 2006, the detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefpodoxime. All isolates with reduced susceptibility to cefpodoxime were further characterized by combination Etests and/or molecular examination. A total of four isolates displayed reduced susceptibility to cefpodoxime, but none were identified as ESBL producers.

Shigella spp. from human clinical specimens

It should be emphasized that almost all reported *Shigella* infections in Norway are acquired abroad. In 2006, 20% of the 138 reported cases were classified as domestically acquired. Thus, the prevalence of resistance in this report predominantly relates to isolates originating in other countries. The species distribution of the 129 *Shigella* isolates that were susceptibility tested was as follows: *S. sonnei* 60 (46.5%), *S. flexneri* 53 (41.1%), *S. boydii* 11 (8.5%), and *S. dysenteriae* 5 (3.9%). The results for *S. sonnei* and *S. flexneri* are presented in Tables 26-27 and in the text.

TABLE 26. *Shigella sonnei* isolates from human clinical cases (n=60). Distribution (%) of antimicrobial susceptibility groups. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 0.5	> 8	0.0	86.7	13.3
Chloramphenicol	≤ 8	> 8	96.7	-	3.3
Tetracycline	≤ 4	> 8	31.7	0.0	68.3
Nalidixic acid	≤ 16	> 16	71.7	-	28.3
Ciprofloxacin	≤ 0.5	> 1	98.3	1.7	0.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	13.3	6.7	80.0

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

TABLE 27. *Shigella flexneri* isolates from human clinical cases (n=53). Distribution (%) of antimicrobial susceptibility groups. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 0.5	> 8	0.0	26.4	73.6
Chloramphenicol	≤ 8	> 8	37.7	-	62.3
Tetracycline	≤ 4	> 8	18.9	0.0	81.1
Nalidixic acid	≤ 16	> 16	94.3	-	5.7
Ciprofloxacin	≤ 0.5	> 1	92.5	1.9	5.7
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	39.6	0.0	60.4

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

RESULTS AND COMMENTS

As reported from other countries, resistance was widespread among *Shigella* isolates, regardless of the bacterial species.

The resistance frequencies observed were particularly high for tetracycline, ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole. These drugs are commonly used for various clinical purposes within human medicine in many parts of the world. For ampicillin and chloramphenicol we found differences between *Shigella* species. Resistance was most prevalent among *S. flexneri* and least prevalent among *S. sonnei* isolates. In addition, resistance to nalidixic acid was relatively common in *S. sonnei.* Clinical resistance to fluoroquinolones was rarely observed, but the detection of *Shigella* isolates intermediately susceptible to ciprofloxacin and resistant to nalidixic acid indicates that high-level fluoroquinolone resistance may be developing. The few isolates *of S. dysenteriae* (n=5) and *S. boydii* (n=11) recovered and susceptibility tested in 2006 indicate that multiresistance is also common in these species; one and five of the isolates, respectively, were resistant to two or more antimicrobial agents. Only four isolates of both *S. dysenteriae* and *S. boydii*, respectively, were susceptible to all antimicrobial agents included in the survey.

In 2006, the detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefpodoxime. All isolates with reduced susceptibility to cefpodoxime were further characterized by combination Etests and/or molecular examination. Two isolates displayed reduced susceptibility to cefpodoxime and both were verified as ESBL producers.

D. HUMAN CLINICAL ISOLATES

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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 collected 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 identity within a month from the first entry. This rule was applied irrespective of changes in the organism´s susceptibility pattern. All new 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 28, proportions are therefore estimated from all isolates and from all isolates excluding species considered to be common skin contaminants such as coagulase negative staphylococci, *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp. and *Propionibacterium* 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 prohobited in-depth analysis of these parameters.

TABLE 28. Number of blood culture isolates in 2006, and proportion of all isolates and proportion of isolates excluding possible skin contaminants (Coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp.) in 2004, 2005 and 2006.

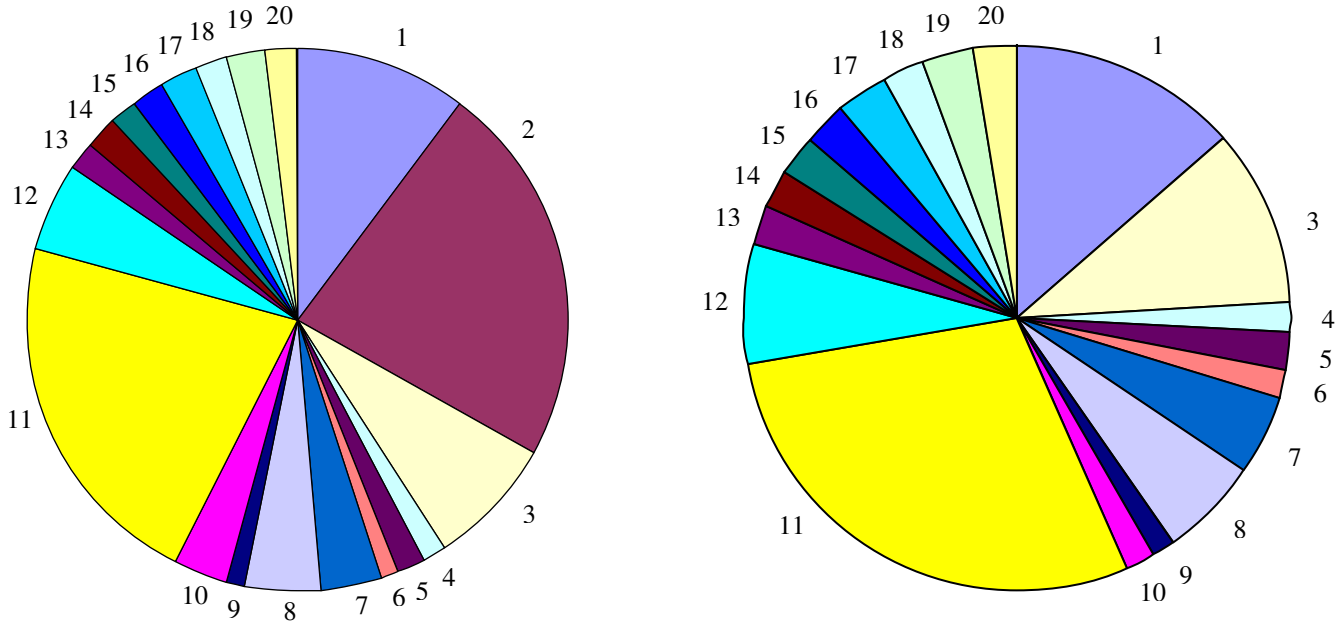
Species	No. of isolates 2006	% of all isolates			% of isolates excluding skin flora		
		2004	2005	2006	2004	2005	2006
Staphylococcus aureus	1,188	12.3	10.3	10.3	14.0	13.3	13.7
Coagulase negative staphylococci	2,630	11.3	20.3	22.7	-	-	-
Streptococcus pneumoniae	917	11.6	9.4	7.9	13.2	12.1	10.6
Streptococcus pyogenes	148	2.3	2.2	1.3	2.6	2.8	1.7
Streptococcus agalactiae	192	2.0	1.6	1.7	2.3	2.1	2.2
Betahaemolytic streptococci group C and G	134	0.7	0.8	1.2	0.9	1.1	1.5
Viridans- and non-haemolytic streptococci	430	4.6	3.8	3.7	5.3	5.0	5.0
Enterococcus faecalis	499	4.6	4.0	4.3	5.2	5.2	5.7
Enterococcus faecium	126	1.1	1.1	1.1	1.2	1.5	1.5
Other Gram positive bacteria	398	1.8	3.1	3.4	1.0	1.3	1.8
Escherichia coli	2,504	26.2	22.4	21.6	29.9	29.0	28.9
Klebsiella spp.	626	6.2	5.4	5.4	7.2	7.0	7.2
Enterobacter spp.	199	1.5	1.6	1.7	1.6	2.0	2.3
Proteus spp.	206	2.7	1.9	1.8	3.0	2.4	2.4
Other Enterobacteriaceae	220	1.8	1.8	1.9	2.0	2.3	2.5
Pseudomonas spp.	199	1.9	2.1	1.7	2.2	2.8	2.3
Other Gram negatives aerobic bacteria	269	1.7	2.2	2.3	2.0	2.8	3.1
Bacteroides spp.	215	2.1	1.8	1.9	2.4	2.4	2.5
Other anaerobic bacteria	274	1.9	2.2	2.4	2.0	2.3	2.8
Yeasts	215	1.8	2.0	1.9	2.0	2.6	2.5
Total	**11,589**	**100**	**100**	**100**	**100**	**100**	**100**

As seen in Table 28 and Figure 27, aerobic Gram positive and Gram negative bacteria represented 57.6% and 36.4% of all isolates, respectively. The predominance of Gram positives among all isolates was an increase from 2004 (52.3%) and 2005 (56.6%). The increase was mainly due to a further increase in the prevalence of coagulase negative staphylococci from 20.3% in 2005 to 22.7% in 2006. 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 43.7% Gram positives and 48.7% Gram negatives.

Among the aerobic Gram positives, the prevalences of *S. pneumoniae* declined even when skin contaminants were excluded (13.2% in 2004, 12.1% in 2005 and 10.6% in 2006). A similar trend was seen for *S. pyogenes* (group A streptococci) while a slight increase was seen for the combined group C and G streptococci (0.9% in 2004, 1.1% in 2005 and 1.5% in 2006).

Among the aerobic Gram negatives, *E. coli* (28.9%) and other Enterobacteriaceae (14.4%) accounted for the vast majority of isolates. *Pseudomonas* spp. (2.3%) decreased to the same level as in 2004 (2.2%) after a peak in 2005 (2.8%), all excluding skin flora.

Anaerobic bacteria and yeasts were less prevalent. Anaerobes accounted for 4.3% (5.3% excluding skin flora) and yeasts accounted for 1.9% (2.5% excluding skin flora). The major pathogens among anaerobes were members of the *Bacteroides fragilis* group (1.4%/1.9%) and among yeasts *Candida albicans* (1.2%/1.6%). 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 27. Distribution of all blood culture isolates (left, n=11,589) and blood culture isolates excluding common skin contaminants (right, n=8,683) such as coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp. The figure is based on data from the information systems of all Norwegian laboratories in 2006.

Escherichia coli in blood cultures

TABLE 29. *Escherichia coli* blood culture isolates (n=1,043). 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*	≤ 0.5	> 8	0.1	68.9	31.0
Piperacillin-tazobactam	≤ 8	> 16	96.5	2.2	1.2
Cefuroxime*	≤ 0.5	> 8	0.4	96.2	3.5
Cefotaxime	≤ 1	> 2	98.1	0.6	1.3
Ceftazidime	≤ 1	> 8	98.0	0.9	1.2
Cefpirome	≤ 1	> 8	97.9	1.3	0.8
Aztreonam	≤ 1	> 8	98.3	0.4	1.3
Meropenem	≤ 2	> 8	99.4	0.5	0.1
Gentamicin	≤ 2	> 4	97.7	0.5	1.8
Nalidixic acid	≤ 16	> 16	90.7	-	9.3
Ciprofloxacin	≤ 0.5	> 1	93.6	1.2	5.2
Trimethoprim-sulfamethoxazole**	≤ 2	> 8	81.0	0.5	18.5
ESBL	Negative	Positive	98.9	-	1.1

*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 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. The NWGA participates in the European breakpoint harmonization process, and the Norwegian breakpoints will therefore correspond to common EUCAST breakpoints when these have been established. Accordingly, the Entero-bacteriaceae breakpoint for resistance to cefotaxime was reduced from R > 4 mg/L to R > 2 mg/L whereas the breakpoints for meropenem were changed from S ≤ 0.5 mg/L / R > 2 mg/L to S ≤ 2 mg/L / R > 8 mg/L in 2007. The other breakpoints relevant for NORM remained unchanged from 2006 to 2007 and the resistance rates are therefore directly comparable. However, it should be noted that the transition from Etest to disk diffusion could introduce minor technical discrepancies between the results from 2005 and 2006. Aztreonam was added to the test panel in 2006.

The vast majority of isolates remained fully susceptible to broad-spectrum antimicrobials such as cefotaxime, ceftazidime, cefpirome, meropenem, gentamicin and piperacillin-tazobactam, see Table 29. The slow increase in gentamicin non-susceptibility noted in 2004 and 2005 was not observed in 2006 with 0.5% I (same as in 2005) and 1.8% R (2.0% in 2005) as seen in Figure 28. The prevalence of non-suscpetibility to fluoroquinolones continued to increase from a total of 3.3% in 2004 and 5.0% in 2005 to 6.4% in 2006. The prevalences of both intermediate susceptibility and resistance increased from 2005 to 2006 (I from 0.4% to 1.2% and R from 4.6% to 5.2%). The trend for ciprofloxacin non-susceptibility and ciprofloxacin usage is depicted in Figure 29. The prevalence of resistance to the indicator antibiotic nalidixic acid remained unchanged at 9.3%.

There were no significant changes in the SIR distributions of trimethoprim-sulfamethoxazole, ampicillin and cefuroxime from 2005 to 2006.

In 2006, the detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefotaxime, ceftazidim and cefpirome. All isolates with reduced susceptibility to ceftazidime and/or cefotaxime and/or cefpirome were further characterized by combination Etests and/or molecular examination. A total of 34 isolates displayed reduced susceptibility to one or more of the test substrates, but only 11 were verified as true ESBL producers by Etest (1.1%). This is an increase from 0.3% in 2003, 0.7% in 2004 and 0.5% in 2005. All the 11 isolates were resistant to ampicillin, cefuroxime and cefotaxime. All 11 ESBL strains were also non-susceptible to ceftazidime, but only one was fully resistant to this substance thus indicating a predominance of cefotaxime hydrolytic activity. Only eight ESBL strains were non-susceptible to cefpirome of which two were fully resistant. When looking at the specificity of individual test substrates, cefotaxime identified nine non-ESBL producers (three resistant and six intermediately susceptible), ceftazidime identified ten non-ESBL producers (two resistant and eight intermediately susceptible), and cefpirome identified 14 non-ESBL producers (two resistant and 12 intermediately susceptible). Cefotaxime was thus both a more specific indicator for ESBL production and gave a more robust signal in terms of reduced zone diameters. These findings are in accordance with previous studies documenting CTX-M enzymes as the predominant ESBL genotype in Norway.

ESBL strains were often resistant to trimethoprim-sulfamethoxazole (10/11). Six of 11 strains were classified as susceptible to ciprofloxacin (6/11), but the majority harbors quinolone resistance determinants as ten out of 11

isolates were resistant to nalidixic acid. Two isolates were fully resistant to all cephalosporines, ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole, thus leaving carbapenems as the only documented alternative for treatment. As previously noted, most ESBL strains tested susceptible to piperacillin-tazobactam (9/11) including both of the multiresistant strains.

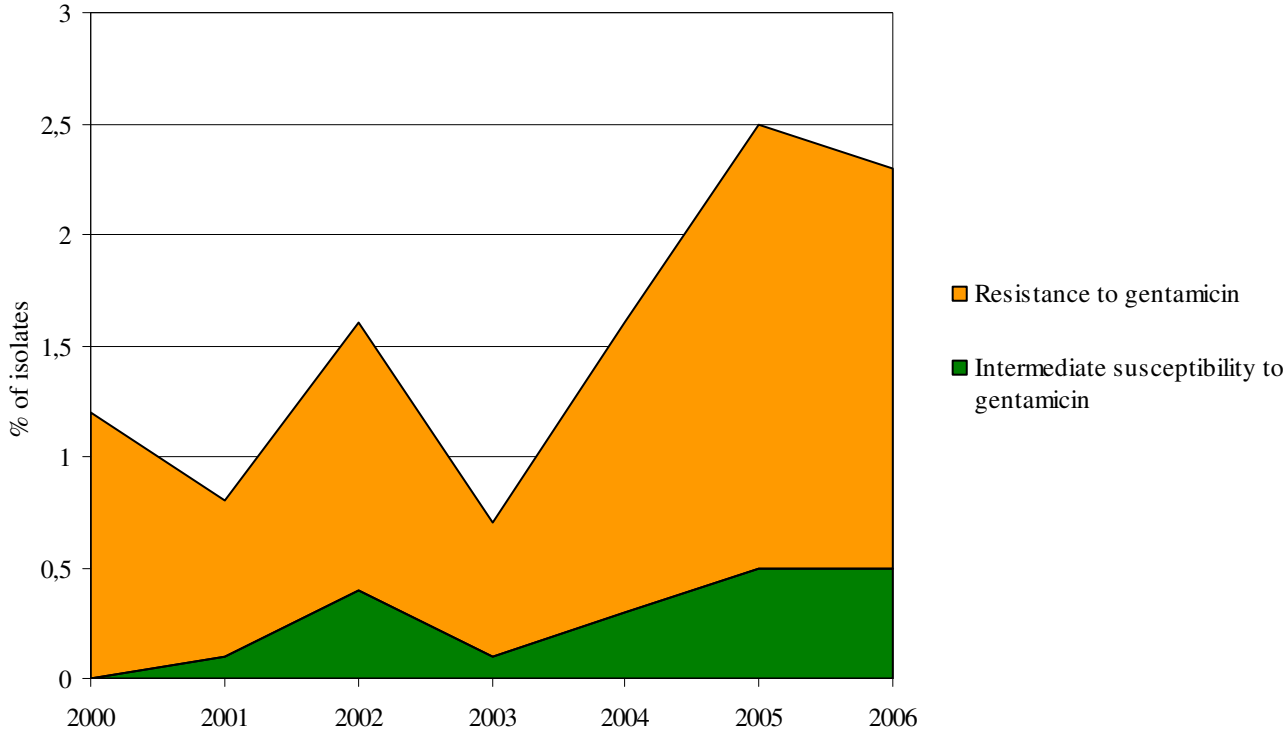


FIGURE 28. Prevalence of intermediate susceptibility and resistance to gentamicin in *Escherichia coli* blood culture isolates 2000 – 2006.

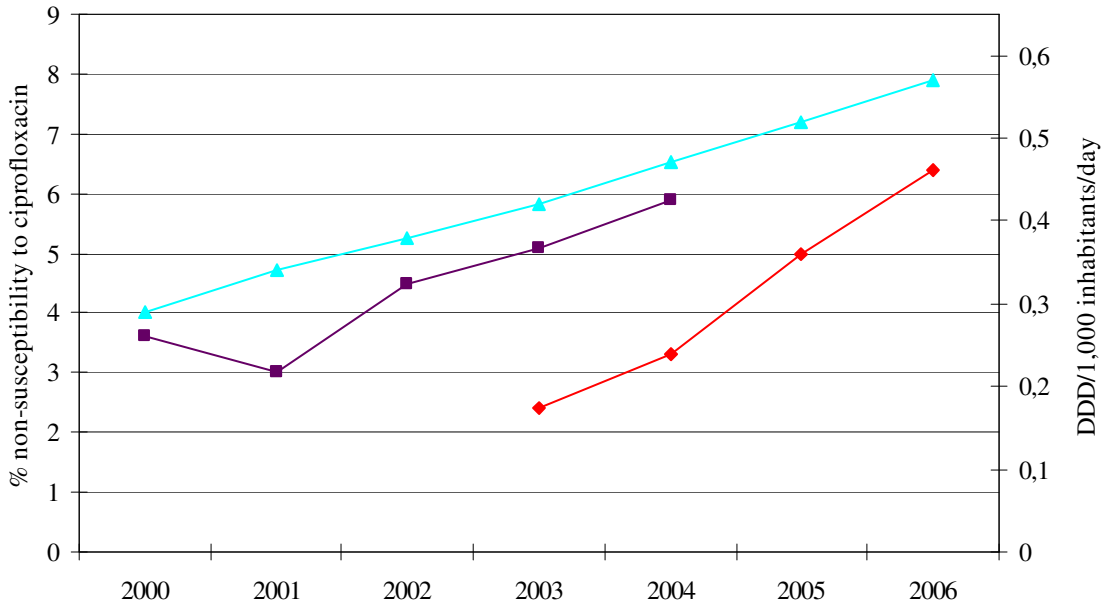


FIGURE 29. Prevalence of ciprofloxacin non-susceptibility in *Escherichia coli* blood culture isolates as defined by the former (magenta) and present (red) breakpoint protocol versus usage of ciprofloxacin (blue) 2000 – 2006.

Escherichia coli in urine

TABLE 30. *Escherichia coli* urinary tract isolates (n=1,161). 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*	≤ 0.5	> 8	0.2	68.6	31.2
Mecillinam	≤ 2	> 8	91.7	6.9	1.4
Cefuroxime	≤ 0.5	> 8	0.5	98.2	1.3
Cefotaxime	≤ 1	> 2	99.3	0.3	0.3
Ceftazidime	≤ 1	> 8	99.3	0.4	0.3
Meropenem	≤ 2	> 8	99.7	0.3	0.0
Gentamicin	≤ 2	> 4	97.7	0.8	1.6
Nalidixic acid	≤ 16	> 16	94.6	-	5.4
Ciprofloxacin	≤ 0.5	> 1	96.3	1.6	2.2
Nitrofurantoin	≤ 32	> 32	98.5	0.2	1.3
Trimethoprim	≤ 2	> 4	81.1	0.3	18.5
Trimethoprim-sulfamethoxazole**	≤ 2	> 8	82.3	0.9	16.9
ESBL	Negative	Positive	99.7	-	0.3

*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 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 prevalences of resistance for 2006 are shown in Table 30 and the results 2000 – 2006 are shown in Figure 30. Sulfonamide was omitted in 2006 whereas gentamicin, meropenem and cephalosporins for ESBL detection were added in 2005 and 2006.

The resistance rates have remained remarkably stable over the last seven years. Approximately 30 % of *E. coli* isolates are resistant to ampicillin while the remaining 70% belong to the wild type which in Norway is categorized as intermediately susceptible. Close to 20% of *E. coli* isolates are resistant to trimethoprim and trimethoprim-sulfamethoxazole. Susceptibility testing to mecillinam is technically challenging and the rates of resistance have fluctuated. The prevalence of non-susceptibility was 8.3% in 2006 which is in accordance with 6.5 – 7.9% in the years 2002 – 2004.

Ciprofloxacin is used as a second-line agent for urinary tract infections in Norway. The prevalence of non-susceptibility has remained relatively stable and was 3.8% in 2006 (1.6% intermediately susceptible and 2.2% resistant). The corresponding rates for blood culture isolates were 1.2% intermediate susceptibility and 5.2% resistance. The same difference was seen for nalidixic acid with 5.4% resistance in urinary tract isolates and 9.3% resistance in bloodstream infections. One may speculate that systemic infections are caused by selected pathogenic lineages with increased virulence and accumulation of mutations in gyrase and topoisomerase genes, whereas urinary tract isolates are more representative of the wild-type normal flora. Thirty-eight out of 63 nalidixic acid resistant isolates were non-susceptible to ciprofloxacin (25 resistant and 13 intermediately susceptible). Conversely, 38/43 (88.4%) of ciprofloxacin non-susceptible isolates were resistant to nalidixic acid. The failure to detect five (11.6%) ciprofloxacin non-susceptible isolates by the screening test indicate a potential for further quality improvement in the laboratories and/or a possible need for adjustment of zone breakpoints.

Three isolates were confirmed as ESBL producers. They were all resistant to cefotaxime, but displayed varying levels of susceptibility to ceftazidime. A fourth isolate had markedly reduced zone diameters for both substrates and was therefore included in the overall estimate of ESBL prevalence (4/1,161; 0.3%). The result was in accordance with 0.4% in 2005. Six additional isolates were non-susceptible to cefotaxime and/or ceftazidime, but zone diameters were only marginally reduced and probably represented the lower end of the wild type distribution.

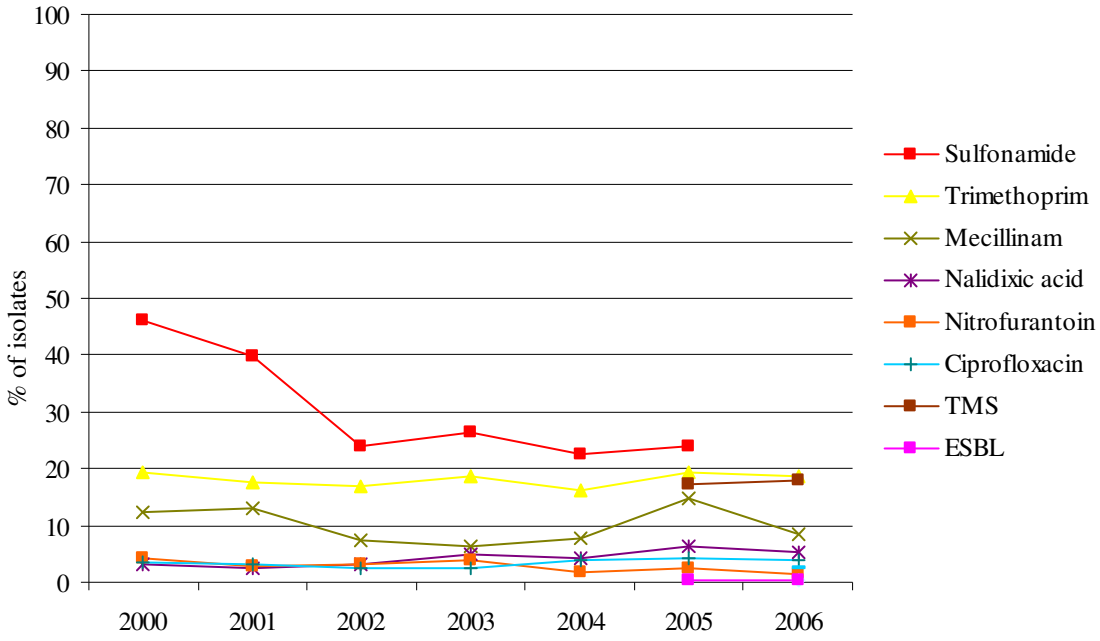


FIGURE 30. Prevalences of non-susceptibility to various antimicrobial agents in urinary tract *E. coli* isolates 2000 – 2006.

Klebsiella spp. in blood cultures

TABLE 31. *Klebsiella* spp. blood culture isolates (n=396). 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	94.2	3.0	2.8
Cefuroxime*	≤ 0.5	> 8	0.5	94.2	5.3
Cefotaxime	≤ 1	> 2	98.7	0.5	0.8
Ceftazidime	≤ 1	> 8	96.2	3.3	0.5
Cefpirome	≤ 1	> 8	96.0	3.3	0.8
Aztreonam	≤ 1	> 8	99.0	0.0	1.0
Meropenem	≤ 2	> 8	99.0	1.0	0.0
Gentamicin	≤ 2	> 4	98.7	0.5	0.8
Nalidixic acid	≤ 16	> 16	86.9	-	13.1
Ciprofloxacin	≤ 0.5	> 1	94.7	2.8	2.5
Trimethoprim-sulfamethoxazole**	≤ 2	> 8	91.9	0.3	7.8
ESBL	Negative	Positive	99.5	-	0.5

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

TABLE 32. *Klebsiella pneumoniae* blood culture isolates (n=276). 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	93.8	3.3	2.9
Cefuroxime*	≤ 0.5	> 8	0.7	93.8	5.4
Cefotaxime	≤ 1	> 2	98.6	0.7	0.7
Ceftazidime	≤ 1	> 8	94.9	4.3	0.7
Cefpirome	≤ 1	> 8	94.9	4.0	1.1
Aztreonam	≤ 1	> 8	98.9	0.0	1.1
Meropenem	≤ 2	> 8	99.3	0.7	0.0
Gentamicin	≤ 2	> 4	99.3	0.0	0.7
Nalidixic acid	≤ 16	> 16	85.5	-	14.5
Ciprofloxacin	≤ 0.5	> 1	94.2	2.2	3.6
Trimethoprim-sulfamethoxazole**	≤ 2	> 8	91.3	0.0	8.7
ESBL	Negative	Positive	99.3	-	0.7

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

TABLE 33. *Klebsiella oxytoca* blood culture isolates (n=65). 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	93.8	3.1	3.1
Cefuroxime*	≤ 0.5	> 8	0.0	95.4	4.6
Cefotaxime	≤ 1	> 2	98.5	0.0	1.5
Ceftazidime	≤ 1	> 8	98.5	1.5	0.0
Cefpirome	≤ 1	> 8	96.9	3.1	0.0
Aztreonam	≤ 1	> 8	98.5	0.0	1.5
Meropenem	≤ 2	> 8	96.9	3.1	0.0
Gentamicin	≤ 2	> 4	95.4	3.1	1.5
Nalidixic acid	≤ 16	> 16	95.4	-	4.6
Ciprofloxacin	≤ 0.5	> 1	98.5	1.5	0.0
Trimethoprim-sulfamethoxazole**	≤ 2	> 8	98.5	0.0	1.5
ESBL			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 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 276 *K. pneumoniae* (69.7%), 65 *K. oxytoca* (16.4%) and 55 (13.9%) isolates not identified to the species level, giving a total of 396 *Klebsiella* spp. isolates (Tables 31-33). The breakpoints for cefotaxime and meropenem were changed and aztreonam added to the test panel as described for *E. coli* blood culture isolates.

There were no significant changes in the overall prevalences of resistance to cephalosporins, aminoglycosides or carbapenems from 2005 to 2006. The prevalence of resistance to trimethoprim-sulfamethoxazole increased from 3.9% to 7.8%, but this may be due to the change from Etest to disk diffusion methodology.

The results for *K. pneumoniae* and *K. oxytoca* isolates identified to the species level are displayed in Tables 32 and 33. The low number of *K. oxytoca* strains precludes any firm conclusions, but the prevalences of non-susceptibility to trimethoprim-sulfamethoxazole and ciprofloxacin are still remarkably higher in *K. pneumoniae* (8.7% and 5.8%, respectively) than in *K. oxytoca* (1.5% and 1.5%, respectively).

The nalidixic acid disk was used as a screening test for detection of quinolone resistance mechanisms. A total of 10 isolates were resistant to ciprofloxacin, and 11 were intermediately susceptible to this agent. The screening test identified nine resistant strains and nine intermediately susceptible strains, thus missing one resistant and two intermediately susceptible strains. Conversely, a total of 34 ciprofloxacin susceptible strains were resistant to nalidixic acid. These 34 isolates may represent a

subpopulation with first-step mutations in the DNA gyrase responsible for quinolone resistance, but this was not further investigated.

As for *E. coli*, the detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefotaxime, ceftazidim and cefpirome disks. Isolates with reduced zone diameters were further characterized by combination Etests and/or molecular examination. Only two *K. pneumoniae* isolates were confirmed as ESBL positive, giving an overall ESBL prevalence of 0.5% among all *Klebsiella* isolates and 0.7% among speciated *K. pneumoniae* isolates. This frequency is unchanged from 2004 (0.6%) and 2005 (0.6%). One isolate was resistant to all three test substrates whereas the other isolate was resistant to cefotaxime and cefpirome but intermediately susceptible to ceftazidime.

A total of 22 *K. pneumoniae* isolates were non-susceptible to one or more of the three test substrates used for ESBL detection without being confirmed as ESBL producers. This corresponds to 8.0% of all *K. pneumoniae* isolates identified to the species level. One strain was intermediately susceptible to cefotaxime and ceftazidime, while three isolates displayed reduced susceptibility to cefpirome (one resistant and two intermediately susceptible) and ceftazidime (all three intermediately susceptible). The remaining 18 strains were only detected by a single test substrate with cefotaxime (one intermediately susceptible) being significantly more specific than ceftazidime (one resistant and seven intermediately susceptible) and cefpirome (nine intermediately susceptible). In addition, two *K. oxytoca* isolates were suggested as ESBL producers but failed in the confirmation assay. One isolate was intermediately susceptible to cefpirome and susceptible to cefotaxime and ceftazidime, whereas the other was intermediately susceptible to ceftazidime and cefpirome and resistant to cefotaxime. The overall prevalence of reduced susceptibility to any 3rd or 4th generation cephalosporin not caused by ESBL production was 3.1% among *K. oxytoca* isolates and 6.1% among all *Klebsiella* isolates.

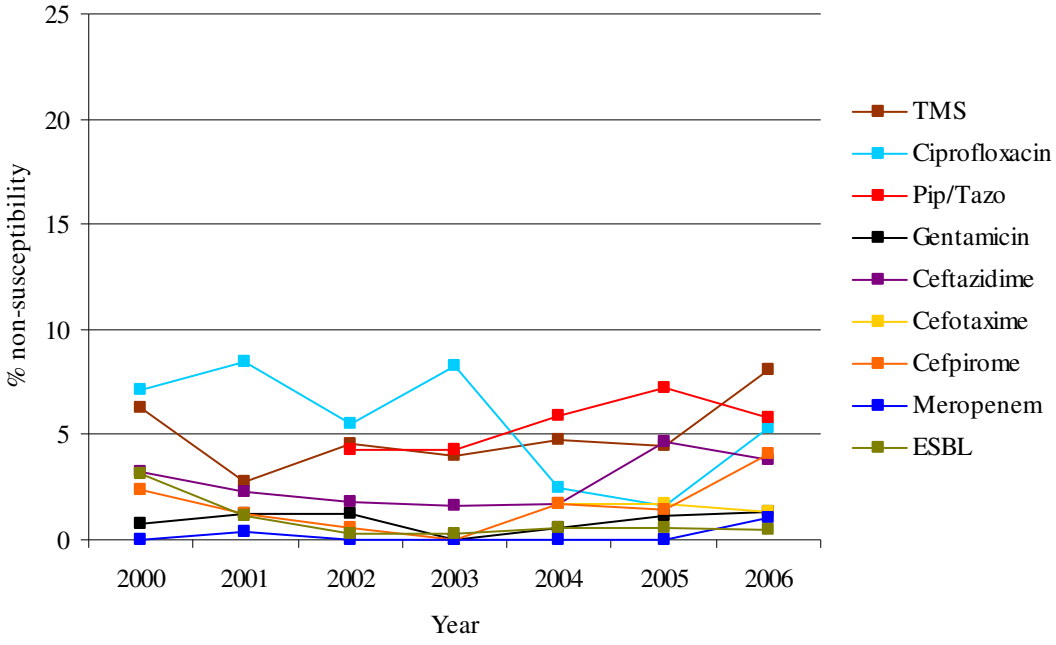


FIGURE 31. Prevalence of non-susceptibility to various antimicrobials in *Klebsiella* spp. blood culture isolates 2000-2006.

Proteus mirabilis in urine

TABLE 34. *Proteus mirabilis* urinary tract isolates (n=518). 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*	≤ 0.5	> 8	4.2	87.3	8.5
Mecillinam	≤ 2	> 8	85.9	7.3	6.8
Cefuroxime*	≤ 0.5	> 8	4.6	94.6	0.8
Cefotaxime	≤ 1	> 2	99.4	0.4	0.2
Ceftazidime	≤ 1	> 8	99.6	0.2	0.2
Meropenem	≤ 2	> 8	98.6	0.8	0.6
Gentamicin	≤ 2	> 4	97.5	1.4	1.2
Nalidixic acid	≤ 16	> 16	94.8	-	5.2
Ciprofloxacin	≤ 0.5	> 1	97.7	1.4	1.0
Nitrofurantoin	≤ 32	> 32	1.2	3.7	95.2
Trimethoprim	≤ 2	> 4	74.5	2.5	23.0
Trimethoprim-sulfamethoxazole**	≤ 2	> 8	87.3	1.5	11.2
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 in systemic infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Proteus mirabilis urinary tract isolates were included in NORM for the first time in 2006. The prevalence of high-level resistance to ampicillin (8.5%) was significantly lower than for *E. coli* (31.2%), whereas the results for mecillinam were difficult to interpret without more detailed investigations. Non-susceptibility to quinolones was present in *P. mirabilis* at a rate comparable to *E. coli*. A majority of nalidixic acid resistant isolates (16/27, 59.2%) were susceptible to ciprofloxacin, but the screening test identified 11/12 ciprofloxacin non-susceptible isolates. Trimethoprim resistance was seen in 23.0% of isolates as compared to 18.5% of *E. coli*. However, there was dissociation between resistance rates for trimethoprim (23.0%) and trimethoprim-sulfa-methoxazole (11.2%) which was not seen in *E. coli*. This may indicate a lower prevalence of resistance to the sulfonamide component in *P. mirabilis*, but this was not specifically investigated. As expected, the detected prevalence of resistance to nitrofurantoin was very high (95.2%) and should ideally be 100%. Due to structural features of the outer membrane lipopolysaccharide, nitrofurantoin does not gain access to the *P. mirabilis* cell, and the agent should therefore not be used for treatment of *P. mirabilis* infections.

ESBL production has been reported in *P. mirabilis*, but no such isolates were detected in NORM 2006. A total of five isolates (1.0%) were non-susceptible to cefotaxime or ceftazidime, but none displayed reduced susceptibility to both substances and ESBL activity was not confirmed.

The EUCAST process of breakpoint setting in antibiotic susceptibility testing

The clinical efficacy of a drug depends on several factors – some of them drug dependent while others are largely determined by host and microbe factors. Such factors include antibacterial activity (i.e. minimum inhibitory concentration, MIC), recommended dosages of a drug, as well as the drugs´ fate in the human body (bioavailability, amount of free drug, half-life, tissue distribution, site of infection and clinical efficacy).

In antibiotic susceptibility testing (AST) we determine the MIC of a given drug-bug combination and interpret the results in more simple clinically relevant terms, i.e. Susceptible, Intermediate or Resistant (S, I or R). These critical MIC-values are for short called breakpoints (BP). Given that an antibiotic is administered in appropriate dosages, S means that there is a high likelihood of therapeutic success while R indicates a high likelihood of therapeutic failure. The I category is associated with uncertain therapeutic effect. The I will in Norway often imply that a drug will have a high likelihood for therapeutic success if given in higher doses (possible for many beta-lactam antibiotics) or the drug is concentrated at the site of infection (i.e. urinary tract). The I category may also be used as a technical buffer zone to prevent unacceptable discrepancies in interpretations.

The Norwegian Working Party on Antibiotics (NWGA, in Norwegian: Arbeidsgruppen for antibiotikaspørsmål (AFA)) has for almost 30 years been responsible for setting national interpretation criteria. In collaboration with five other European national break point committees we are part of The European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST has defined 8 important steps in the process of setting and harmonizing breakpoints in Europe.

Step 1. The harmonization process means that we must ensure that we are using the antibiotic in the same way and in the same dosages. Therefore, a compilation of data on dosing, formulations, clinical indications and target organisms is reviewed and differences which might influence breakpoints are highlighted.

Step 2. To get reproducible interpretation of results when testing, it is important that the breakpoint does not divide the wild type distribution. Multiple MIC-distributions are therefore collected, the wild type MIC distribution is defined and tentative epidemiological cut-off values (wild type, WT) are determined (WT $\leq$ X mg/L). Figure 32 shows that *E. coli* organisms without resistance mechanisms to ciprofloxacin all have en MIC $\leq$ 0.064.

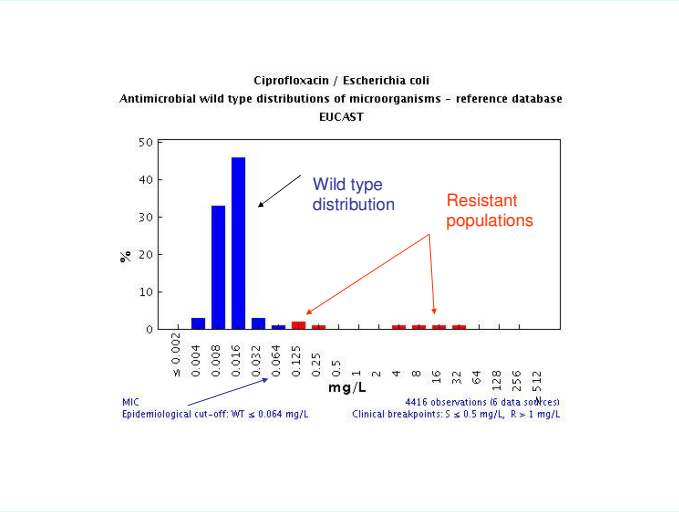


FIGURE 32. *E. coli* organisms without resistance mechanisms to ciprofloxacin all have MIC $\leq$ 0.064 mg/L.

Step 3. We tabulate the existing national breakpoints, and these breakpoints are compared and differences are discussed and the rational behind a BP explained by the national representative in EUCAST. Sometimes the dosing regimens differ so much between countries that a fully harmonized breakpoint is difficult to obtain.

Step 4. Using available Pk/Pd data, Monte Carlo simulations are performed and a Pk/Pd breakpoint calculated based on conventional dosing regimens (Figure 33). These methods give us a better understanding on how the drug is handled in a large population based on data from a smaller sample.

Step 5. Clinical data relating outcome to MIC-values, wild type distributions and resistance mechanisms are assessed in relation to the tentative breakpoint. If clinical data are sufficient, such data will tend to override BP set on other criteria.

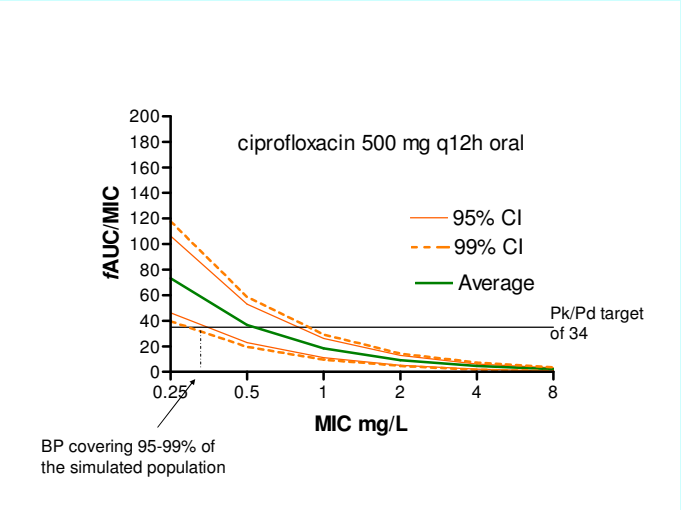


FIGURE 33. Probabilities of Target Attainment (PTA) for ciprofloxacin orally for 500 mg given two times daily. The horizontal line indicates the Pk/Pd target for *S. pneumoniae*. The pharmacokinetic parameters used are explained in detail at the EUCAST website. Results of simulations for the 400 mg IV dose not markedly influence conclusions.

Step 6. Tentative breakpoints are checked against target species wild type MIC distributions to avoid splitting the wild type to obtain tentative breakpoints. If the tentative BP splits the wild type MIC distribution, the BP is changed up or down (usually only one dilution step) to avoid splitting the wild type. This makes it possible to get reproducible categorization when reporting results of antibiotic susceptibility testing. It is important to realise that even a single susceptible strain tested many times will result in different MIC-values similar to the distribution of blue bars in Figure 32.

Step 7. Tentative breakpoints proposed by the EUCAST Steering Committee are referred to the national breakpoint committees for comments. Each national committee consist of 10-20 experts, and before the tentative breakpoints are sent back to the steering committee, probably more than 100 European experts have evaluated the suggestion.

Step 8. When the Steering Committee and national committees agree, the tentative breakpoints are subjected to the EUCAST consultation process which includes EUCAST General Committee, expert groups (e.g. *Neisseria*, anaerobes), pharmaceutical industry, susceptibility test device manufacturers and others via the EUCAST website.

Finally a rationale document is prepared and published on EUCAST website (http://www.eucast.org). The rational document contains all above mentioned steps for BP setting and concludes with a table of clinical breakpoints given.

A EUCAST technical note (ETN) is also published in Clinical Microbiology and Infection (CMI).

AFA has implemented the EUCAST breakpoints and publishes a Norwegian version at http://www.antibiotikaresistens.no (look for AFA). AFA also publishes documents on quality control, recommended panels for susceptibility testing and national guidelines on how to detect some specific resistance mechanisms.

References:

1. Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. Clin Microb Reviews 2007; 20: 391-408.

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Staphylococcus aureus in blood cultures

TABLE 35. *Staphylococcus aureus* blood culture isolates (n=750). 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	96.7	0.1	3.2
Clindamycin	≤ 1	> 2	98.7	0.4	0.9
Fusidic acid	≤ 0.5	> 0.5	94.0	-	6.0
Ciprofloxacin	≤ 1	> 1	95.7	-	4.3
Gentamicin	≤ 1	> 1	99.7	-	0.3
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 1	> 1	99.3	-	0.7
Tetracycline	≤ 1	> 2	96.4	0.0	3.6
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	98.9	0.8	0.3
Beta-lactamase	Negative	Positive	28.0	-	72.0
MRSA (*mecA*)	Negative	Positive	99.7	-	0.3
Vancomycin screen	Negative	Positive	100.0	-	0.0

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

RESULTS AND COMMENTS

Only two methicillin resistant *S. aureus* (MRSA) isolates were detected in the NORM surveillance system in 2006 (Table 35) corresponding to a prevalence of 0.27%. The two isolates were fully susceptible to all non beta-lactam antibiotics tested. The findings are in accordance with reports from the laboratory databases of the participating institutions where three out of 1,261 (0.24%) *S. aureus* blood culture isolates were MRSA. One of the sixteen *S. aureus* isolates recovered from cerebrospinal fluids was also methicillin resistant bringing the total number of systemic MRSA-isolates to 4/1,277 (0.31%). The Norwegian Surveillance System for Communicable Diseases (MSIS) reported an increase in the total number of MRSA infections in Norway from 260 in 2005 to 333 in 2006 (Table 37). However, the cases reported to MSIS are predominantly skin and soft tissue infections (271) and colonizations (270). The discrepancy between the very low prevalence of systemic MRSA infections and an increasing prevalence of non-systemic infections was thus continued in 2006. A total of 603 cases of MRSA infections and colonizations were reported to MSIS in 2006. This is a 32% increase from 2005, the only previous year with registration of MRSA colonization. Further information about MRSA cases in MSIS is presented on page 64.

Laboratory screening from MRSA using cefoxitin disks was applied for the first time in NORM in 2006. The two MRSA blood culture isolates had cefoxitin zones of 11 and 21 mm on Isosensitest agar and were thus resistant according to the breakpoint of R ≤ 21 mm. Two additional isolates had zone diameters of 21 mm but were not confirmed as true methicillin resistant isolates. A breakpoint of S ≥ 19 mm has been suggested when using cefoxitin disks on Mueller Hinton II agar and semiconfluent growth. A total of 7 isolates (2.4%) had zone diameters of 18 mm and probably represented the tail of the normal distribution (Figure 34). None of these isolates were confirmed as MRSA and no outliers with zones smaller than 18 mm were detected.

A total of 25 isolates (3.3%) were non-susceptible to erythromycin which is at the same level as in 2004 (2.7%) and slightly higher than in 2005 (1.9%). The macrolide resistance phenotype was determined by double disk diffusion (DDD) tests in 24 isolates of which 5 (21%) were constitutively MLS_B resistant, 17 (71%) were inducibly MLS_B resistant and 2 (8%) displayed efflux mediated M type resistance. The prevalences of resistance to gentamicin and fusidic acid decreased from 0.7% to 0.3% and from 7.6% to 6.0%, respectively. No isolates displayed growth on the vancomycin agar screen, and all isolates were fully susceptible to linezolid. Figure 35 shows the prevalences of non-susceptibility to various non beta-lactam antimicrobials.

72.0% of the isolates were beta-lactamase positive which is unchanged from previous years. A subgroup analysis revealed that the prevalence of tetracycline resistance was slightly higher (4.6%) among the 540 beta-lactamase positive isolates compared to the 210 beta-lactamase negative ones (1.0%). There were no significant differences between the two groups for the other non beta-lactam antimicrobials.

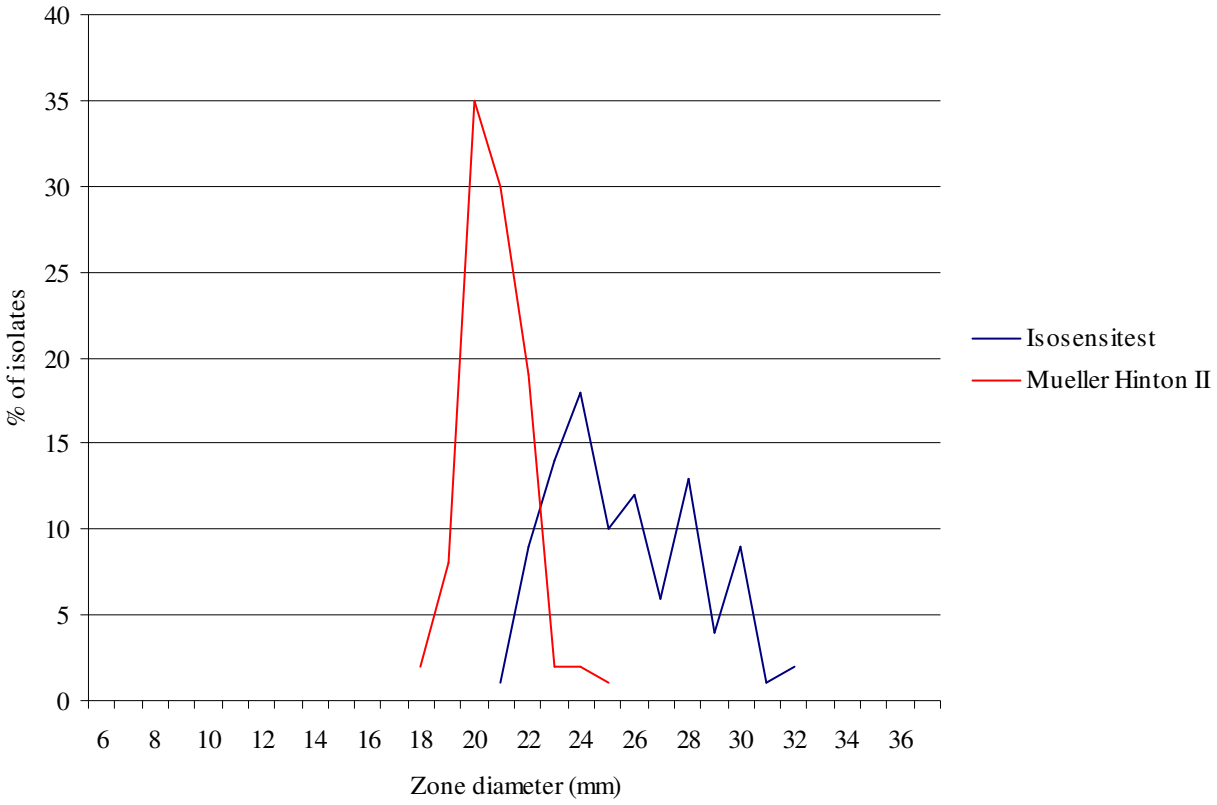

FIGURE 34. Distribution of zone diameters for blood culture *S. aureus* isolates using cefoxitin 10 µg disks on Isosensitest (blue) or Mueller Hinton II (red) agar with semiconfluent growth.

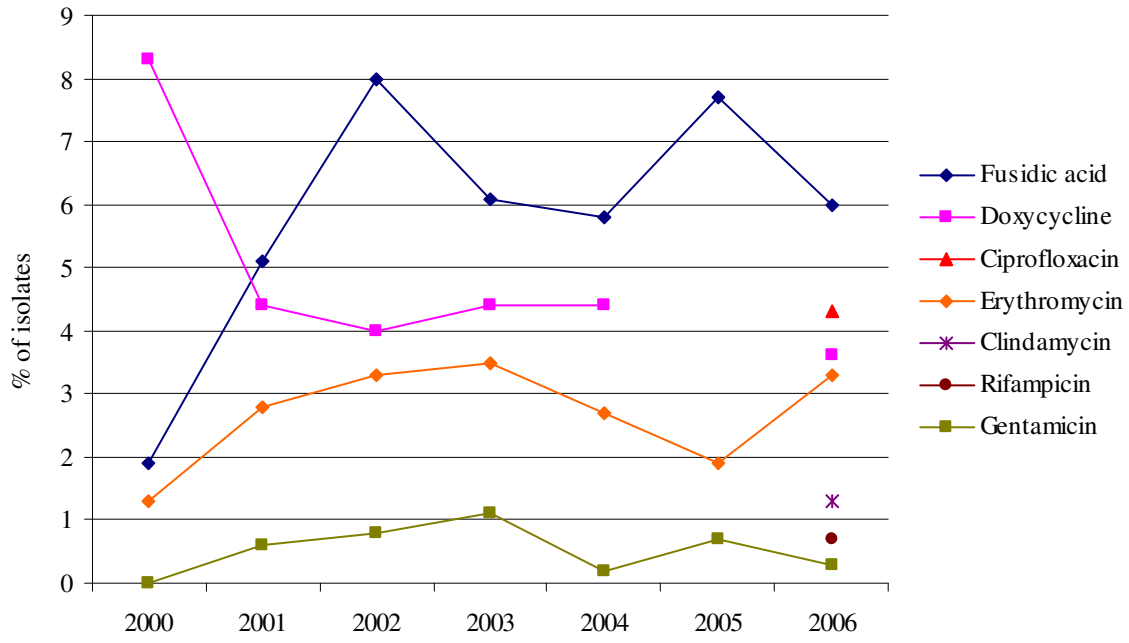

FIGURE 35. Prevalences of non-susceptibility to selected non beta-lactam antimicrobials among *Staphyloccus aureus* blood culture isolates 2000 – 2006. The breakpoint for susceptibility to gentamicin was decreased from S ≤ 2 mg/L to S ≤ 1 mg/L in 2006. Doxycycline was not included in 2005 and was then replaced by tetracycline in 2006.

Staphylococcus aureus in wound specimens

TABLE 36. *Staphylococcus aureus* isolates from wound specimens (n=1,135). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	95.1	0.4	4.6
Clindamycin	≤ 1	> 2	97.4	1.2	1.4
Fusidic acid	≤ 0.5	> 0.5	85.5	-	14.5
Ciprofloxacin	≤ 1	> 1	96.4	-	3.6
Gentamicin	≤ 1	> 1	99.5	-	0.5
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 1	> 1	99.1	-	0.9
Tetracycline	≤ 1	> 2	94.6	0.1	5.3
Trimethoprim-sulfamethoxazole*	≤ 2	> 8	98.1	1.1	0.8
Beta-lactamase	Negative	Positive	22.7	-	77.3
MRSA (*mecA*)	Negative	Positive	99.6	-	0.4
Vancomycin screen	Negative	Positive	100.0	-	0.0

*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. Three isolates were confirmed as MRSA by *mecA* PCR. One isolate was tested on Mueller Hinton II with a clearly reduced zone diameter (10 mm) whereas the other had a zone diameter (21 mm) close to the mean for all isolates tested on this agar. A total of 27/649 (4.2%) isolates tested on Isosensitest agar had zone diameters below the screening breakpoint of 22 mm, but only one isolate was verified as an MRSA (11 mm). In addition, one isolate with a zone diameter of 13 mm should be suspected of being an MRSA isolate although the confirmatory test was inconclusive. The remaining 25 isolates had zone diameters of 20 mm or more. In conclusion, four isolates were regarded as MRSA giving an overall prevalence of 0.35%. This result is in accordance with the MRSA prevalence in blood cultures of 0.3% in 2006 and the rate among isolates from wound specimens in 2003 (0.3%) and 2004 (0.5%). Two of the four isolates were resistant to erythromycin, but all four were otherwise susceptible to all antibiotics tested.

The high prevalence of resistance to fusidic acid in *S. aureus* wound isolates has been significantly reduced from 25.0% in 2004 to 14.5% in 2006. One may speculate that this may be due to herd immunity to the fusidic acid resistant clone which has caused a high incidence of bullous impetigo over the last years. The prevalence of resistance to fusidic acid is still much lower in blood culture isolates (6.0%).

For other antibiotics such as tetracyclines and macrolides there were only minor differences from the 2004 data, and the prevalences of non-susceptibility were similar for blood culture isolates and isolates from wound specimens. A total of 56 (4.9%) isolates were non-susceptible to erythromycin, and 47 of these were further examined for determination of resistance phenotype. The majority (33/47, 70.2% of macrolide resistant isolates) were inducibly resistant to clindamycin thus representing the $iMLS_B$ phenotype. Only a few isolates were either constitutively resistant to clindamycin (n=9) or low-level resistant to erythromycin (n=5) expressing efflux mediated M type resistance. The findings are in accordance with the results from blood culture isolates.

A total of 77.3% of the isolates were beta-lactamase positive which is unchanged from previous years. Resistance to fusidic acid was significantly more common among the 877 beta-lactamase positive isolates (17.1%) than among the 258 beta-lactamase-negative ones (5.8%). The prevalence of tetracycline resistance was also slightly higher (6.0%) among beta-lactamase positive isolates compared to beta-lactamase negative isolates (3.1%).

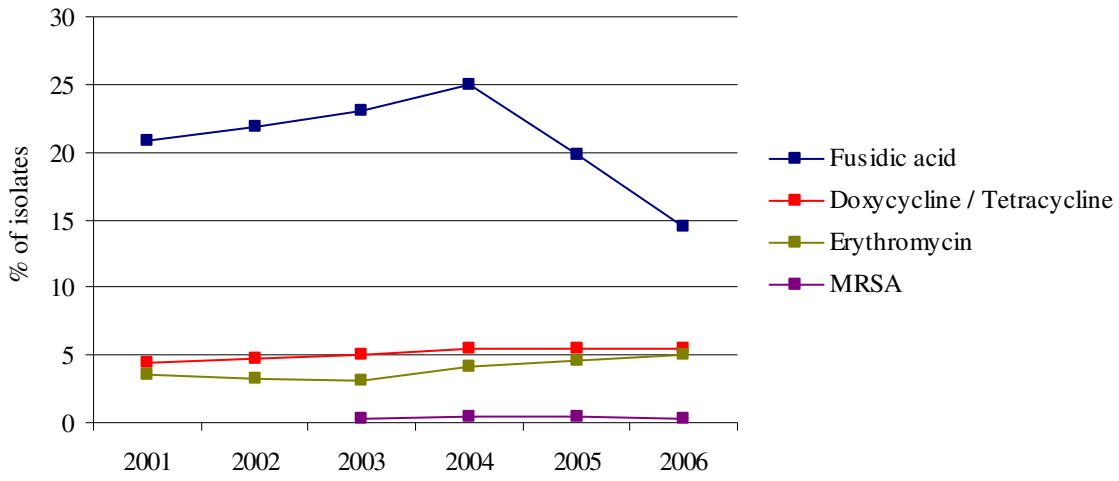

FIGURE 36. Prevalence of methicillin resistance (MRSA) and non-susceptibility to fusidic acid, tetracyclines and erythromycin among *S. aureus* isolates from wound specimens 2001 – 2006. Doxycycline was replaced by tetracycline in 2006.

MRSA infections in Norway 2005

Infections with methicillin resistant *Staphylococcus aureus* (MRSA) have been notifiable in Norway since 1995, and asymptomatic colonization was made notifiable in 2005. Consistent discrimination between infection and colonization can be difficult. The total number of reported cases of MRSA increased from 457 in 2005 to 603 cases in 2006. Three hundred and thirty three cases in 2006 were notified as MRSA-infections while 270 (45 %) were reported as MRSA colonization, see Figure 37. One hundred and twenty four (21 %) cases were diagnosed in hospitals while 434 (72 %) were diagnosed outside hospitals of which 108 were residents of long term care institutions.

The number of reported MRSA infections has increased steadily over the past twelve years. The overwhelming majority consists of wound infections and abscesses. The number of severe infections is still very low. MRSA was isolated from blood cultures in three patients in 2006 compared to four patients in 2005 and ten in 2004. For all twelve years reported, MRSA have been isolated from blood cultures in only 50 patients (Table 37). National guidelines for prevention of MRSA infection and colonization were published in 2004. Revised guidelines including measures in long term care institutions and primary health care will be published in 2007.

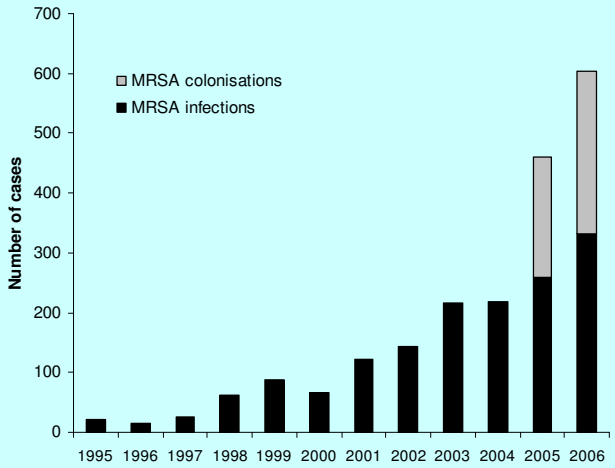

FIGURE 37. Reported cases of MRSA infections 1995-2006 and reported cases of MRSA colonizations 2005-2006 in Norway.

TABLE 37. Clinical diagnosis of reported MRSA cases in Norway 1995-2006 (numbers adjusted from previous reports).

Clinical diagnosis	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total
Septicaemia	1	1	3	4	4	2	6	6	6	10	4	3	50
Meningitis				1								2	3
Osteomyelitis	2	1		2			2	1	2	2	1	2	15
Arthritis			1	1				1				2	5
RTI*, incl. otitis media	1		1	4	8	6	9	11	7	9	12	18	86
Urinary tract infection		1		4	3	4	2	9	12	13	16	19	83
Wound infection, abscess	10	9	15	31	63	49	95	110	169	156	214	271	1192
Other, unknown	8	4	5	15	10	6	7	5	18	29	13	16	136
Total, infections	22	16	25	62	88	67	121	143	214	219	260	333	1570
Colonization											197	270	467
Total, all cases	22	16	25	62	88	67	121	143	214	219	457	603	2037

* RTI = Respiratory tract infection

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Enterococcus spp. in blood cultures

TABLE 38. *Enterococcus* spp. blood culture isolates (n=366). 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	≤ 2	> 8	84.2	0.8	15.0
Gentamicin	≤ 128	> 128	69.1	-	30.9
Linezolid	≤ 4	> 4	100.0	-	0.0
Vankomycin	≤ 4	> 8	98.9	1.1	0.0

TABLE 39. *Enterococcus faecalis* blood culture isolates (n=265). 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	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 128	> 128	72.1	-	27.9
Linezolid	≤ 4	> 4	100.0	-	0.0
Vankomycin	≤ 4	> 8	100.0	0.0	0.0

TABLE 40. *Enterococcus faecium* blood culture isolates (n=58). 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	≤ 2	> 8	17.2	1.7	81.0
Gentamicin	≤ 128	> 128	53.4	-	46.6
Linezolid	≤ 4	> 4	100.0	-	0.0
Vankomycin	≤ 4	> 8	100.0	0.0	0.0

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 2006 included 265 (72.4%) *E. faecalis* isolates, 58 (15.8%) *E. faecium* isolates and 43 (11.7%) unspeciated enterococcal isolates.

The panel of antibiotics examined and the breakpoints used were not changed from 2005 to 2006. However, the results for penicillin G, streptomycin and quinupristin-dalfopristin have been removed from the printed tables as one of the disk diffusion systems does not provide breakpoints for these substances. Distributions of zone diameters for both systems are available at www.antibiotikaresistens.no except for quinupristin-dalfopristin in *E. faecalis* which is not relevant due to inherent resistance.

E. faecalis was universally susceptible to ampicillin. The prevalence of non-susceptibility to ampicillin in *E. faecium* remained relatively stable at 82.7% compared to 85.9% in 2005 (Table 40 and Figure 38), but the prevalence of resistance increased from 77.1% to 81.0%. This change is probably due to the transition from Etest to disk diffusion methodology.

The prevalence of high-level gentamicin resistance (HLGR) continued to increase in *E. faecium* from 35.8% in 2005 to 46.6% in 2006 (Figure 39). Virtually all (26/27, 96.3%) HLGR *E. faecium* isolates were concomitantly resistant to ampicillin. Conversely, 26 out of 48 (54.2%) ampicillin non-susceptible *E. faecium* also displayed HLGR.

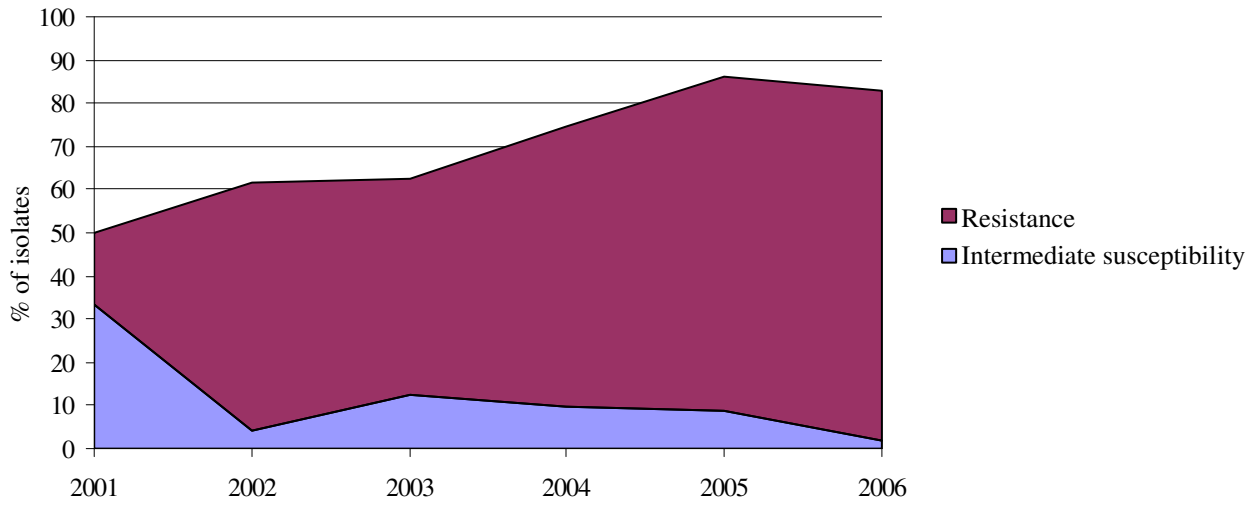

FIGURE 38. Prevalence of intermediate susceptibility and resistance to ampicillin in *E. facium* blood culture isolates. The breakpoints applied were S ≤ 1 mg/L and R > 16 mg/L in 2001-2002, and S ≤ 2 mg/L and R > 8 mg/L in 2003-2006.

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 prevalence of HLGR in *E. faecalis* increased from 24.3% in 2005 to 27.9% in 2006. 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 established in clinical enterococcal isolates in Norway. Four isolates were reported as vancomycin resistant (1.1%), but three of these were identified as *E. gallinarum* (n=2) or *E. casseliflavus* (n=1) which are inherently low-level resistant to vancomycin due to expression of the VanC ligase. All the three isolates had MIC values of 8 mg/L. The fourth VRE was not fully investigated, but as it was reported as *Enterococcus* sp. and not as either *E. faecalis* or *E. faecium* one may suspect that this was also an inherently resistant species. All isolates were fully susceptible to linezolid.

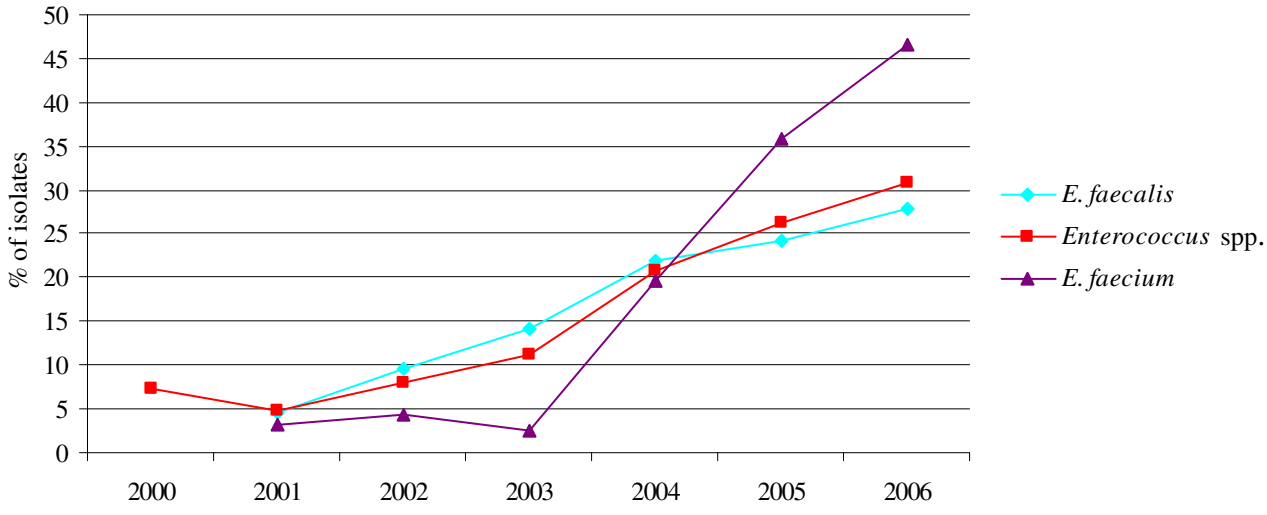

FIGURE 39. Prevalence of high-level resistance to gentamicin in blood culture isolates of *E. faecalis*, *E. faecium* and all enterococci combined during 2000-2006. The breakpoint for high-level resistance was decreased from R ≥ 1,024 mg/L to R > 128 mg/L in 2004.

Streptococcus pneumoniae in blood cultures

TABLE 41. *Streptococcus pneumoniae* blood culture isolates (n=688). 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	> 1	98.1	1.3	0.6
Cefuroxime	≤ 0.5	> 1	99.3	0.3	0.4
Cefotaxime	≤ 0.5	> 2	99.7	0.3	0.0
Erythromycin	≤ 0.5	> 0.5	87.6	-	12.4
Clindamycin	≤ 0.25	> 2	98.4	0.6	1.0
Tetracycline	≤ 2	> 2	97.8	-	2.2
Trimethoprim-sulfamethoxazole*	≤ 0.5	> 2	90.1	6.1	3.8
Oxacillin screen (mm)	≥ 20	< 20	96.1	-	3.9

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

TABLE 42. *Streptococcus pneumoniae* blood culture isolates (n=688). 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		7.0	53.9	35.2	2.0	0.3	0.3	0.4	0.3	0.1	0.4					
Cefuroxime			23.1	63.2	10.2	1.5	0.9	0.4	0.3	0.3		0.1				
Cefotaxime	0.3	5.4	53.6	35.2	3.3	0.7	0.9	0.3	0.3							
Erythromycin				2.2	16.4	43.3	25.7			0.4	0.4	3.8	4.4	2.0	0.6	0.7
Clindamycin			1.9	7.3	22.1	52.2	15.0	0.4	0.1		0.1					0.9
Tetracycline				1.0	27.5	57.8	9.9	0.7	0.3	0.6	0.1	0.7	0.9	0.4	0.1	
TMS**					0.1	4.5	55.4	30.1	3.6	2.5	0.9	0.4	0.3	2.2		
Norfloxacin			0.1	0.1					0.6	5.2	38.1	42.9	11.6	1.2		0.1

	< 20	20	21	22	23	24	25	26	27	28	29	30	31	32	33	≥ 34
Oxacillin disk	3.9	1.1	1.6	2.2	5.5	9.3	13.2	12.9	9.6	11.3	6.4	9.2	4.1	3.9	2.2	3.5

*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 summarised in Tables 41-42 and Figures 40-42. A total of 13 *S. pneumoniae* isolates were non-susceptible to penicillin G comprising 1.9% of the total. This is comparable to 2.0% non-susceptibility in 2004 and 2.1% in 2005. Nine isolates (1.3%) were defined as intermediately susceptible (MIC 0.125-1 mg/L) whereas four isolates (0.6%) were resistant (MIC 2-4 mg/L). Two of the penicillin G non-susceptible isolates were also resistant to cefuroxime (MIC 2 and 8 mg/L, respectively) and intermediately susceptible to cefotaxime (MIC 1 mg/L). These were the only strains non-susceptible to cefotaxime. Three additional isolates were non-susceptible to both penicillin G and cefuroxime (one resistant and two intermediately susceptible). The remaining eight penicillin non-susceptible isolates were fully susceptible to cephalosporins.

Five of the 13 penicillin non-susceptible isolates were resistant to macrolides (38.5%). The oxacillin disk screen for penicillin non-susceptibility identified 11 of the 13 strains, see Figure 40. The two strains not identified by the oxacillin screen were both fully penicillin resistant with MICs of 4 mg/L. Conversely, 11/27 oxacillin resistant isolates (40.7%) were verified as penicillin non-susceptible. The oxacillin screening test identified all cephalosporin non-susceptible isolates.

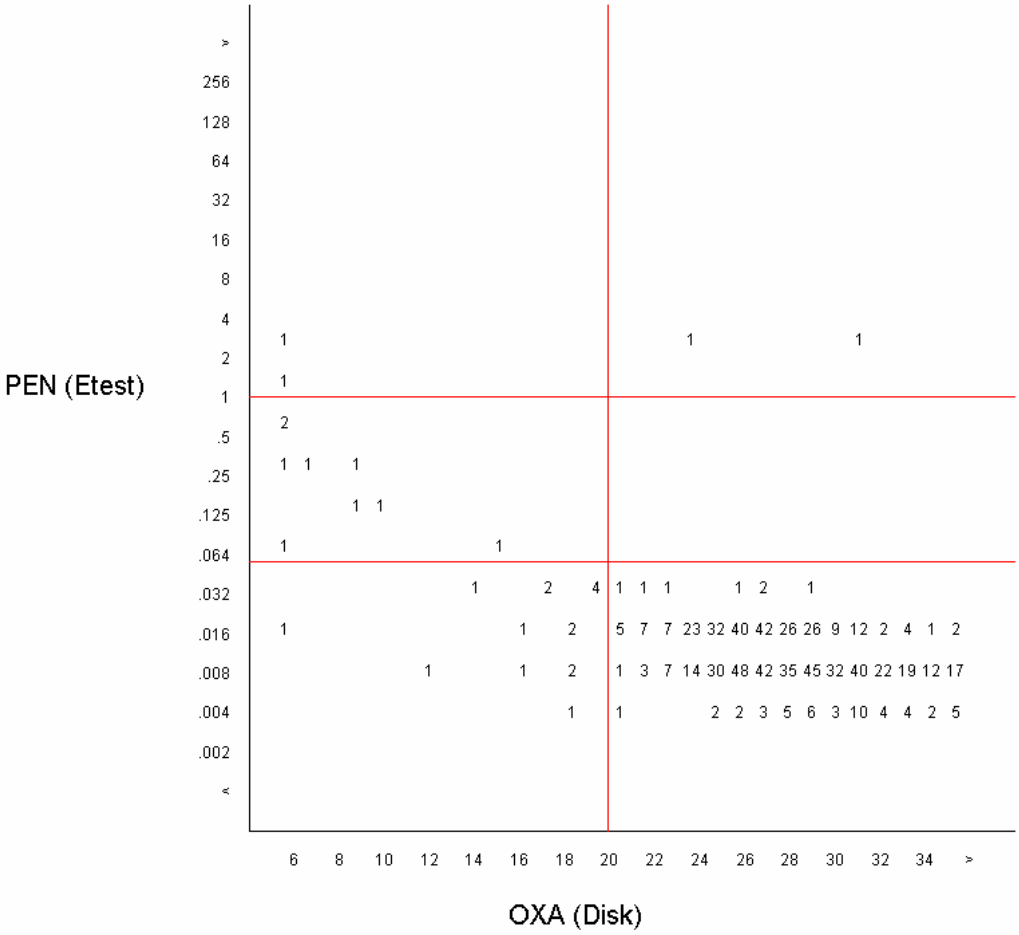

FIGURE 40. Correlation between oxacillin disk zone diameters and MICs of penicillin G among 688 *S. pneumoniae* blood culture isolates in 2006. The figures indicate number of strains. Red lines represent breakpoints for the oxacillin screening disk (S ≥ 20 mm, R < 20 mm) and penicillin G (S ≤ 0.064 mg/L, R > 1 mg/L).

The prevalence of macrolide resistance continued to increase from 9.7% in 2004 and 10.8% in 2005 to 12.4% in 2006, see Figure 41. Eighty out of 85 erythromycin non-susceptible islolates were further subjected to double disk diffusion (DDD) tests for characterization of MLS phenotypes. A majority of isolates (85.0% of erythromycin non-susceptible isolates, 10.5% of all isolates) displayed a phenotype compatible with efflux-based M-type resistance to erythromycin only. However, 15.0% of erythromycin non-susceptible isolates (1.9% of all isolates) were either inducibly (n=2) or constitutively (n=10) resistant to clindamycin, thus indicating the presence of *erm*-encoded MLS resistance to both macrolides and lincosamides. The increase in macrolide resistance from 2001 to 2006 was predominantly caused by dissemination of *mef*-encoding M-type strains, but the prevalence of *erm*-encoded MLS_B resistance has also gradually increased.

In a subgroup analysis, a distinction was made between isolates from adults born before 1991 (90.0%) and children born in 1991 or thereafter (10.0%). The prevalence of macrolide resistance was 19.8% among children and 11.1% among adults. There was also a marked difference in the resistance phenotypes as all MLS-typed macrolide resistant isolates from children (n=18) displayed M-type resistance, while isolates from adults also included 19.7% MLS_B resistance (10 constitutive and 2 inducible strains among the 61 typed isolates). The finding of age-related differences in macrolide resistance rates probably reflects the clonal distribution of serotypes in different age-groups, but no information was available regarding serotype distribution in the sample. It is also unknown whether a similar effect is present in *S. pneumoniae* from other clinical samples such as respiratory tract specimens. There were no age-related differences in resistance rates for antibiotics other than macrolides.

There was a marked increase in the prevalence of both intermediate susceptibility and resistance to trimethoprim-sulfamethoxazole in 2006. Susceptibility testing to this agent is technically demanding and may be prone to errors. The results for 2007 will reveal whether this is an emerging trend or a temporary phenomenon. There were no significant changes for tetracyclines (Figure 42). The low prevalence of high-level norfloxacin resistance (Table 42) is in accordance with the absence of levofloxacin and other “respiratory fluoroquinolones” from the Norwegian market.

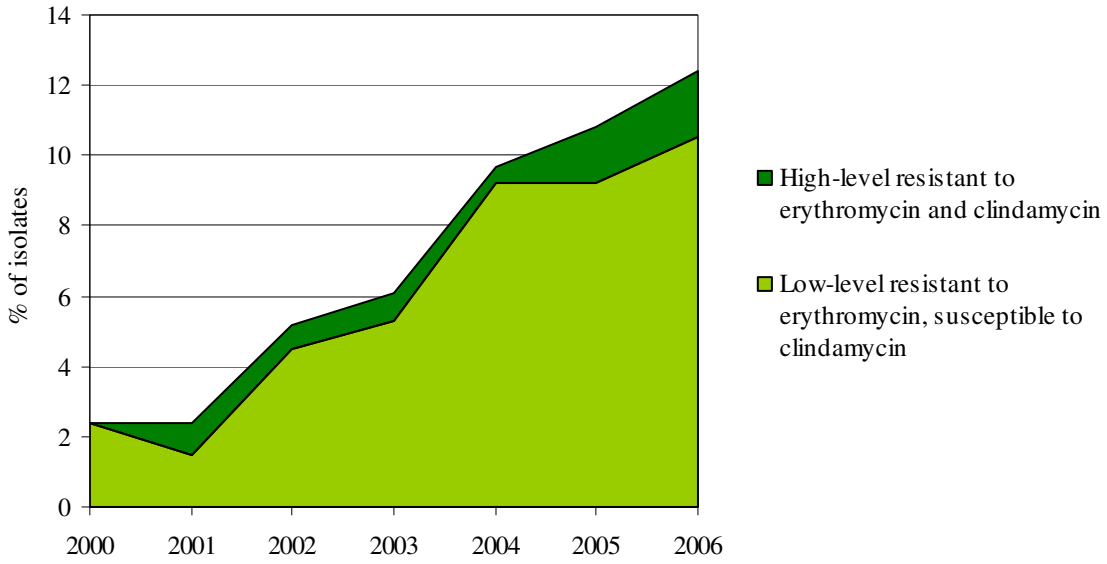

FIGURE 41. Prevalence (%) of macrolide resistant *Streptococcus pneumoniae* blood culture isolates with consitutive or inducible MLS_B phenotype (high-level resistance to erythromycin and clindamycin) and M phenotype resistance (low-level resistance to erythromycin, susceptibility to clindamycin) 2000-2006.

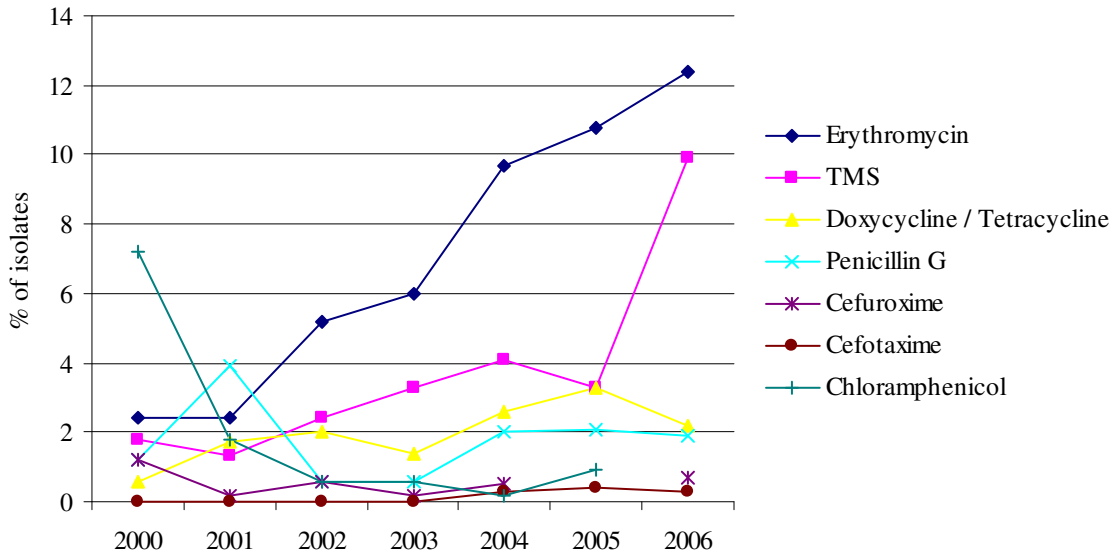

FIGURE 42. Prevalences (%) of non-susceptibility to various antimicrobials in *Streptococcus pneumoniae* blood culture isolates during 2000 – 2006. The breakpoints have not been adjusted since 2002. Doxycycline was substituted by tetracycline in 2005, and chloramphenicol was omitted from the surveillance programme in 2006.

Streptococcus pyogenes in blood cultures, respiratory tract specimens and wound specimens

TABLE 43. *Streptococcus pyogenes* blood culture isolates (n=302). 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	≤ 0.125	-	100.0	-	-
Erythromycin	≤ 0.5	> 0.5	99.3	-	0.7
Clindamycin	≤ 0.25	> 2	99.0	0.3	0.7
Tetracycline	≤ 2	> 2	91.4	-	8.6
Trimethoprim-sulfamethoxazole*	≤ 0.5	> 2	88.4	7.0	4.6

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

TABLE 44. *Streptococcus pyogenes* blood culture isolates (n=302). 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
Penicillin G	0.7	7.3	77.8	13.9		0.3										
Erythromycin				4.3	16.9	62.3	15.6	0.3				0.7				
Clindamycin			1.0	8.6	47.7	40.4	1.3	0.3								0.7
Tetracycline				2.3	39.7	43.4	5.0	1.0				0.7	4.6	3	0.3	
TMS**					3.0	15.2	43.0	27.2	6.6	0.3	0.3			4.3		

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

TABLE 45. *Streptococcus pyogenes* from respiratory tract specimens (n=530). 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	≤ 0.125	-	100.0	-	-
Erythromycin	≤ 0.5	> 0.5	96.8	-	3.2
Clindamycin	≤ 0.25	> 2	99.2	0.2	0.6
Tetracycline	≤ 2	> 2	95.7	-	4.3
Trimethoprim-sulfamethoxazole*	≤ 0.5	> 2	82.6	13.6	3.8

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

TABLE 46. *Streptococcus pyogenes* from respiratory tract specimens (n=530). 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
Penicillin G	0.4	11.3	83.4	4.2	0.4	0.2										
Erythromycin				6.2	28.5	44.7	17.2	0.2		0.4	0.4	0.2	0.8	0.4		1.1
Clindamycin			1.9	10.4	37.4	44.2	5.5			0.2						0.6
Tetracycline				1.5	35.1	45.5	10.0	3.2	0.2	0.2	0.6	0.9	1.5	0.9	0.4	
TMS**				0.2	6.6	25.7	29.2	20.9	10.0	3.6	0.9	0.8		2.1		

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

TABLE 47. *Streptococcus pyogenes* from wound specimens (n=508). 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	≤ 0.125	-	100.0	-	-
Erythromycin	≤ 0.5	> 0.5	98.4	-	1.6
Clindamycin	≤ 0.25	> 2	99.4	0.2	0.4
Tetracycline	≤ 2	> 2	87.0	-	13.0
Trimethoprim-sulfamethoxazole*	≤ 0.5	> 2	84.3	14.2	1.6

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

TABLE 48. *Streptococcus pyogenes* from wound specimens (n=508). 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
Penicillin G	0.2	11.0	78.9	9.6	0.2											
Erythromycin			0.2	9.3	27.8	43.9	17.1	0.2			0.2	0.2	0.4	0.2		0.6
Clindamycin			3.0	12.4	39.4	39.0	5.7	0.2					0.2			0.2
Tetracycline				1.8	31.3	43.7	7.5	2.8			0.6	1.8	4.5	5.3	0.2	0.6
TMS**			0.2	0.8	8.7	27.0	27.6	20.1	9.6	4.5	0.6	0.2	0.2	0.6		

*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. pyogenes (beta-haemolytic streptococci group A - GAS) isolates from respiratory tract specimens and wound swabs have previously been surveyed in NORM in 2002 and 2004. Group A streptococcal blood culture isolates have never been surveyed in NORM before.

As expected, all isolates were fully susceptible to penicillin G (Tables 43-48). Penicillin non-susceptibility has never been detected in group A streptococci, and the highest MIC values recorded in this survey were 0.125 mg/L which is equivalent to the breakpoint. Most isolates displayed MICs of 0.016 mg/L.

The prevalence of non-susceptibiliy to tetracyclines has decreased since 2004 (Figure 43). Doxycycline was replaced by tetracycline in 2006, but it is still noteworthy that the overall prevalence of resistance to this group declined from 15.6% in 2002 to 11.7% in 2004 and further to 8.6% in 2006. The same trend was seen when subgroups of isolates from specific clinical samples were analysed. Isolates from wound samples were much more likely to be tetracycline resistant (13.0%) than isolates from respiratory tract samples (4.3%), but for both sample locations there was a substantial decrease from 2004 (15.1% in wound samples and 8.0% in respiratory tract samples). Blood cultures isolates had a prevalence of 8.6%.

Macrolide resistant group A streptococci has been a problem in many countries including Finland and Italy. In NORM, the prevalence of erythromycin resistance has remained stable (range 0.7 – 3.2%) and is unchanged from 2004. There were no significant differences between isolates from the different clinical samples. Twenty-six out of 27 erythromycin resistant isolates were further investigated using the double disk diffusion (DDD) assay for determination of resistance phenotype. The largest group of 15/26 isolates (57.7% of macrolide resistant isolates) corresponding to 1.2% of the total sample displayed *erm*-encoded MLS_B resistance either inducibly (n=9) or constitutively (n=6). The remaining group of 11/26 isolates (42.3% of macrolide resistant isolates) corresponding to 0.8% of the total sample displayed low-level resistance to erythromycin seen in the *mef*-encoded M-phenotype. It is remarkable that macrolide resistance has remained so rare in Norwegian group A streptococci when both *mef* and *erm* resistance determinants have disseminated widely in *Streptococcus pneumoniae* in the same time period. This observation indicates that herd immunity and other selective forces are acting in addition to the effect of antibiotic exposure.

In Norway, trimethoprim-sulfamethoxazole (TMS) is rarely used for treatment of infections caused by group A streptococci. There was a significant increase in the overall prevalence of non-susceptibility to TMS from 6.4% in 2004 to 15.4% in 2006. The trend was similar for wound samples (6.2% to 15.8%) and respiratory tract specimens (6.3% to 17.4%), whereas the prevalence of non-susceptibility was somewhat lower in blood culture isolates (11.6%). Most isolates were intermediately susceptible (12.3%) as opposed to fully resistant (3.1%). It is uncertain whether the increased prevalences represent epidemiological changes or inconsistent technical performance of the antibiotic susceptibity testing.

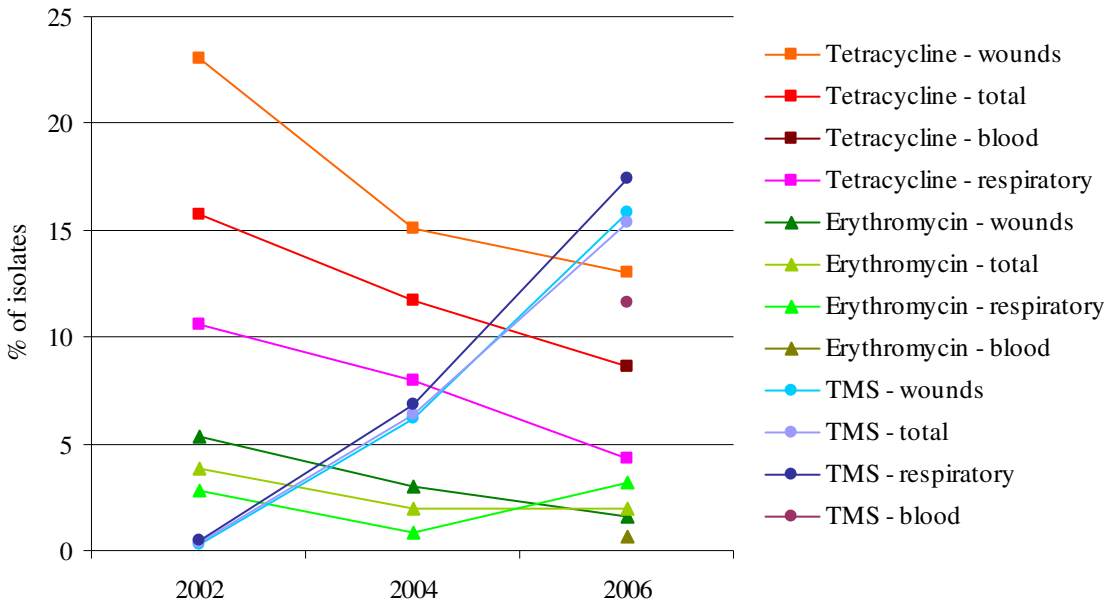

FIGURE 43. Prevalences of non-susceptibility to various antimicrobials in *Streptococcus pyogenes* from the respiratory tract, wound specimens and blood cultures in 2002, 2004 and 2006. Blood cultures were only included in 2006. Doxycycline was used in 2002 and 2006 but replaced by tetracycline in 2006.

Strepcococcus agalactiae from systemic infections

TABLE 49. *Streptococcus agalactiae* from systemic infections (n=50). 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	≤ 0.125	-	100.0	-	-
Cefotaxime	≤ 0.5	> 0.5	100.0	-	-
Erythromycin	≤ 0.5	> 0.5	80.0	-	20.0
Clindamycin	≤ 0.25	> 2	80.0	14.0	6.0
Vancomycin	≤ 4	> 4	100.0	-	0.0

TABLE 50. *Streptococcus agalactiae* from systemic infections (n=50). Distribution (%) of MICs (mg/L).*

	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 256
Penicillin G	14.0	74.0	12.0												
Cefotaxime	14.0	74.0	12.0												
Erythromycin				10.0	72.0	2.0			2.0	6.0	2.0				8.0
Clindamycin		2.0	20.0	58.0	12.0		2.0	2.0							4.0
Gentamicin										2	16	40	26	10	6
Vancomycin					74	26									

*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.

Streptococcus agalactiae (beta-haemolytic group B streptococci) was included in NORM for the first time in 2006. All systemic isolates in Norway are referred to the national reference laboratory at St. Olavs Hospital in Trondheim, and confirmatory identification and susceptibility testing was performed there. A collection of 50 consecutive strains were included in the survey. Seventeen isolates originated from neonates and small children (< 1 year), 13 from eldrely patients (> 70 years) and the remaining 20 from other adults. Fourty-four isolates were recovered from blood cultures while six were collected from other clinical materials (cerebrospinal fluid = 2, placenta = 1, nasal secretion = 1, unknown = 2).

As seen in Tables 49 and 50 there were no isolates with reduced susceptibility to penicillin G, cefotaxime or vancomycin. A total of 13 isolates (26%) displayed intermediate susceptibility to erythromycin (n=10, 20%) and/or clindamycin (n=10, 20%). Four isolates were fully resistant to both agents, and two of these displayed high level resistance with MICs ≥ 256 mg/L. Nine of the 17 isolates from neonates and small children (53%) were non-susceptible to erythromycin and/or clindamycin as compared to four of the 33 (12.1%) from the other patients. The possible clonal spread of macrolide resistant *S. agalactiae* among neonates and small children is presently being investigated and will be published as a separate study.

Aminoglycosides are used in combination treatment of systemic *S. agalactiae* infections. The Norwegian Reference Group for Antimicrobial Susceptibility Testing (AFA) does not issue breakpoints for gentamicin in streptococci, but the MIC distribution (Table 50) indicates that the prevalence of high-level resistance ≥ 256 mg/L is relatively low.

Mycobacterium tuberculosis

A total of 294 cases of tuberculosis were reported to the Norwegian Surveillance System for Communicable Diseases (MSIS) in 2006. Of these, 276 individuals had not previously been treated with antituberculosis drugs. *Mycobacterium tuberculosis* was isolated from 217 cases, and 216 isolates were susceptibility tested. The strain from the remaining case was contaminated before susceptibility testing was performed. The results are presented in Table 51.

TABLE 51. Antimicrobial susceptibility of 216 isolates of *M. tuberculosis* complex isolated in 2006 from patients not previously treated for tuberculosis. One isolate was lost before susceptibility testing.

Geographical origin of patient	No. of isolates	Resistance to antimicrobial agents (No. of isolates)					
		Isoniazid	Rifampicin	Ethambutol	Streptomycin	Pyrazinamid	MDR TB*
Norway	38				1		
Europe outside Norway	15	3	1		5	1	1
Asia	59	7		2	5	3	
Africa	104	14		1	21	1	
America	1						
Total	217	24	1	3	32	5	1
Proportion of resistant isolates (%)		11.1	0.5	1.4	14.8	2.3	0.5

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

RESULTS AND COMMENTS

Susceptibility tests were also performed on *M. tuberculosis* isolates from 9 patients who had previously received antituberculosis drug treatment. One of these isolates, from an African patient, was monoresistant to isoniazid. Two isolates from patients of European origin (excl. Norway) were multidrug resistant. One of these showed resistance to all first-line drugs except pyrazinamid and in addition resistance to clarithromycin, rifabutin, PAS, ethionamid, thiacetazone and kanamycin. The other isolate was resistant to all first-line drugs in addition to amikacin, ofloxacin, rifabutin, PAS, ethionamid, capreomycin, thiacetazone, kanamycin and amoxicillin and therefore met the current criteria for extensive drug resistant tuberculosis (XDR-TB).

Candida spp. in blood cultures

TABLE 52. Antimicrobial susceptibility of *Candida albicans* blood culture isolates (n=112). 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*	≤ 4	> 32	100.0	0.0	0.0
Voriconazole**	≤ 1	> 2	100.0	0.0	0.0
Caspofungin***	≤ 1	> 1	100.0	-	0.0

*Breakpoints from the Norwegian Reference Group on Antibiotic Susceptibility Testing – AFA.
** Proposed breakpoints (Pfaller, M. A., et al. J Clin.Microbiol 2006:44:819-826).
***There are no recommended breakpoints. Strains with MIC ≤ 1mg/L are presumably susceptible.

TABLE 53. *Candida albicans* blood culture isolates (n=112). 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				1.8	3.6	9.8	50.9	29.5	4.5								
Fluconazole							11.6	42.9	42.0	3.6							
Voriconazole	8.9	51.8	38.4		0.9												
Caspofungin			0.9	6.3	23.2	39.3	28.6	1.8									

*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.

TABLE 54. Antimicrobial susceptibility of *Candida glabrata* blood culture isolates (n=34). 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*	≤ 4	> 32	55.9	35.3	8.8
Voriconazole**	≤ 1	> 2	94.1	5.9	0.0
Caspofungin***	≤ 1	> 1	100.0	-	0.0

*Breakpoints from the Norwegian Reference Group on Antibiotic Susceptibility Testing – AFA.
** Proposed breakpoints (Pfaller, M. A., et al. J Clin.Microbiol 2006:44:819-826).
***There are no recommended breakpoints. Strains with MIC ≤ 1mg/L are presumably susceptible.

TABLE 55. *Candida glabrata* blood culture isolates (n=34). 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							35.3	64.7									
Fluconazole										14.7	41.2	23.5	5.9	5.9	5.9		2.9
Voriconazole				2.9	20.6	38.2	14.7	17.6		5.9							
Caspofungin					2.9	17.6	79.4										

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

TABLE 56. Antimicrobial susceptibility of *Candida tropicalis* blood culture isolates (n=15). 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*	≤ 4	> 32	100.0	0.0	0.0
Voriconazole**	≤ 1	> 2	100.0	0.0	0.0
Caspofungin***	≤ 1	> 1	100.0	-	0.0

*Breakpoints from the Norwegian Reference Group on Antibiotic Susceptibility Testing – AFA.
** Proposed breakpoints (Pfaller, M. A., et al. J Clin.Microbiol 2006:44:819-826).
***There are no recommended breakpoints. Strains with MIC ≤ 1mg/L are presumably susceptible.

TABLE 57. *Candida tropicalis* blood culture isolates (n=15). 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							6.7	40.0	53.3								
Fluconazole							13.3	46.7	33.3		6.7						
Voriconazole		6.7	20.0	20.0	40.0	6.7	6.7										
Caspofungin				6.7	6.7	33.3	53.3										

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

RESULTS AND COMMENTS

In 2006, 183 strains of 11 different yeast species isolated from patients with bloodstream infections were received at the national mycology reference laboratory. In 2005, 171 strains of 13 different species were received. All isolates were tested for susceptibility to amphotericin B, fluconazole, voriconazole and caspofungin. The results for the three most common species *Candida albicans* (n=112, 61.2%), *Candida glabrata* (n=34, 18.6%) and *Candida tropicalis* (n=15, 8.2%) are shown in the Tables 52-57.

The *C. albicans* and *C. tropicalis* strains were all susceptible to fluconazole. About half of the *C. glabrata* isolates had decreased susceptibility to fluconazole (8.8% were resistant and 35.3% intermediately susceptible). Of the 34 *C. glabrata* strains, two stains with high fluconazole MIC also had decreased voriconazole susceptibility (MIC ≥ 2 mg/L). Patients with serious infections caused by *C. glabrata* should probably be treated with other antifungals than azoles. All the *C. albicans* and *C. tropicalis* isolates were susceptible to voriconazole.

All isolates were susceptible to amphotericin B and caspofungin. Compared to earlier studies on susceptibility of Norwegian blood stream yeast isolates (NORM/NORM-VET 2005, Sandven P. et al. J Clin Microbiol 2006:44; 977-81) there has been no increase in resistance.

The breakpoints used in yeast susceptibility testing are under discussion. In this report the breakpoints recommended by the Norwegian Reference Group on Antibiotic Susceptibility Testing (AFA) are used for amphotericin B and fluconazole. AFA has no recommendations for voriconazole and caspofungin. Their recommendation is common breakpoints for all yeast species. In a preliminary document the European Committee on Antimicrobial Susceptibility Testing - Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) recommends epidemiological breakpoints for all *Candida* spp except for *C. glabrata* and *C. krusei*. For *C. albicans* and *C. tropicalis* EUCAST-AFST propose MIC ≤ 2 mg/L sensitive for fluconazole and MIC ≤ 0.25 mg/L sensitive for voriconazole.

HIV resistance among persons diagnosed with HIV-infection in Norway

Highly active antiretroviral therapy has during the last ten years greatly improved the life expectancy for persons living with HIV infection. However, treatment response is threatened by the development of drug resistance. The consequences may be even more severe if resistance occurs as a result of treatment in an individual (acquired resistance). Further harm can be inflicted if others are infected with resistant virus (primary resistance), usually as a result of prior inadequate treatment of person who is the source of infection.

Internationally there is concern related to the development of HIV resistance and transmission of drug resistant virus. A previous European study showed increased prevalence of resistance among recently infected individuals (infected within the last 12 months of diagnosis) and in persons with HIV subtype B (1). This has been explained by recently infected individuals being more likely to have been infected with a resistant virus, but also by resistant virus reverting to wild type virus after some time. Subtype B is the most common subtype among Europeans, who generally have had more exposure to antiretroviral treatment than persons with subtypes more common in other parts of the world.

In Norway surveillance of HIV drug resistance among persons newly diagnosed with HIV infection was started in January 2006. The main purpose of this programme is to study resistance at the population level over time among untreated individuals. Knowledge about the occurrence of HIV resistance among persons with recently diagnosed HIV infection is important when developing guidelines for empiric therapy, e.g. for post-exposure prophylaxis. Surveillance data can also be used to identify deficiencies in infection control programmes, and to implement counter-measures, among population groups with known, treated HIV infection.

To exclude error in sample handling, all patients having a positive HIV-test have a second blood sample analysed for HIV antibodies. At the same time a plasma sample is sent to one of the three laboratories performing HIV resistance testing in Norway (Haukeland University Hospital, Ullevål University Hospital and Rikshospitalet). After DNA sequencing of relevant areas of the genome coding for HIV resistance, the electronic sequence is sent anonymously to the Norwegian Institute of Public Health. The sequences are analysed using the HIV Drug Resistance Database at Stanford University. By use of a common identity number, results of resistance testing can be linked to other information about the cases in the anonymous HIV surveillance database at the Norwegian Institute of Public Health.

In this first year of HIV resistance surveillance, DNA sequences were received from 118 of 275 persons (42.9 %) newly diagnosed with HIV infection in 2006. Preliminary analysis shows that 14 cases (11.9 %) had virus with one or more mutations coding for resistance. One person had a mutation coding for resistance against protease inhibitors, two for nucleoside reverse transcriptase inhibitors, and eleven for non-nucleoside reverse transcriptase inhibitors. The prevalence of resistance among persons with newly diagnosed HIV infection in 2006 was at the same level as reported in a previous European study (1).

1. Wensing AM, van de Vijver DA, Angarano G et al. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. J Infect Dis 2005; 192: 958-66.

HIV resistance – clinical implications and current laboratory methods for clinical use

Antiviral therapy in HIV infection is aimed at restoring immune function, and thus preventing the occurrence of HIV related disease. All current antiretroviral drugs act by hampering viral replication in infected CD4 positive cells. The blocking of viral replication restores the levels and also the function of affected immunocompetent cells. Thus, the patients with restored immune function will stay well, and are at low risk of developing HIV related diseases. However, a proportion of patients do not achieve adequate treatment response, defined as full viral suppression (HIV RNA lower than the level of detection) (1,2). The most important causes for not reaching this treatment goal are (3,4).

- Lack of adherence
- Side effects
- Development of resistance
- Subtherapeutic drug concentrations.

Of course, these factors also interact, because side effects lead to lack of adherence, which in turn leads to subtherapeutic drug levels, which then may lead to drug resistance.

The HIV virus is highly prone to developing mutations in its genome during replication (5,6). Viruses containing mutations with a survival benefit in a milieu where antiviral drugs are present will by evolutionary mechanisms overgrow wild type virus. In a situation of drug levels too low to suppress the replication of mutated virus, but high enough to suppress wild type virus, the selection of mutated, resistant viruses are likely to occur. Hence, patients taking drugs without full viral suppression will subsequently develop resistance directed at the drugs the patients are taking. In this situation, the level of HIV will rise, and a state of immunodeficiency may develop in spite of antiretroviral therapy. In this situation the patient's current treatment regimen needs to be changed, in order to obtain better viral suppression. The development of drug resistance in patients receiving antiretroviral therapy is clearly documented (7,8). Prospective, comparative intervention studies, in which one group of patients with treatment failure has changed their treatment based upon results from resistance testing, and the other group has changed their treatment based upon clinical judgement, have shown divergent results. However, most studies show better outcome for the patients who have undergone resistance testing (9-13). Thus, most treatment guidelines recommend resistance testing in routine clinical practice (14,15). On the other hand, it is shown that resistant viruses often are hampered in their replicative capacity, and have reduced fitness (16-18). Thus, having resistant virus would not necessarily lead to higher risk of HIV related disease, compared to patients with wild type virus. The presence of high level drug resistance has been shown to be associated with increased risk of morbidity (AIDS) and mortality in cohort studies (19,20).
The prevalence of primary drug resistance in recently infected persons has been reported to be in the range of 8-15 % in studies from different parts of the world, and studies have shown reduced clinical response to antiviral therapy in patients with primary drug resistance (21-24).

When analysing drug resistance in HIV infected individuals, most laboratories perform genotypic resistance testing. The reverse transcriptase (RT) and the protease (PR) genes are sequenced and matched with a consensus HIV strain. In this way, mutations in the RT and PR genes are identified. Some of the mutations ultimately will alter the composition of the RT and PR enzymes, and thus the affinity to the drugs will decrease. Thus, mutations in the viral genome known to be associated with resistance to a certain drug will be reported by the laboratory. The results subsequently need to be interpreted, according to the patient's current and previous medical regimens. This interpretation requires a great extent of co-operation between the laboratory doctor and the physician, and there is lacking knowledge about the clinical significance of many resistance mutations. Different algorithms and scoring systems for interpreting genotypic HIV resistance results have been published (25-27). However, the ability of these interpretation systems to predict outcome related to achieving HIV related disease prospectively is not well documented.

A more expensive, and therefore less frequently performed method is phenotypic resistance testing. The patient's virus´ ability to grow in cell culture in the presence of different levels of drugs is investigated, hereby aiming at finding the inhibitory concentration for each drug. This method is more parallel to methods applied in resistance testing in bacteria; providing a more direct interpretation of drug resistance present in that particular patient's virus. However, the genotypic method provides information of the presence of mutations not necessarily giving higher inhibitory concentrations, but known to be associated with subsequent evolution of resistance mutations, and drug resistance in the long run. Thus, the genotypic and phenotypic test results give complementary information. The phenotypic method is not routinely performed in Norway.

Some laboratories perform a "virtual" phenotypic drug resistance test. This method is based on samples from patients on antiviral therapy, where both genotypic and phenotypic resistance tests have been performed. Thus, association between certain mutations and inhibitory concentrations can be established, and kept in a database. The patient sample tested will be sequenced. Mutations found are by advanced statistical methods correlated to the samples in the database, and "virtual" phenotypic inhibitory concentrations are calculated.

In conclusion, drug resistance in antiviral therapy in HIV infected individuals is an issue of major concern. The clinical impact of antiviral drug resistance may be severe, and efforts should be made towards keeping resistance levels at a minimum.

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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. From 2003, 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 antimicrobials 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 only supplied by drug wholesalers. An exemption from the pharmacy/ wholesaler monopoly has been granted for medicated feeds (i.e., feeds into which drugs for therapeutic use are mixed prior to sale). Medicated feeds have to be prescribed by veterinarians and produced by feed mills authorized by the Norwegian Medicines Agency. In Norway, medicated feeds produced and supplied by feed mills are only used for farmed fish. Medicated feeds for livestock are not produced in feed mills due to the small size of livestock herds compared to most other European countries. However, herd/flock treatment of livestock with antimicrobial agents is possible, again subject to veterinary prescription, through the drinking water or as a top dressing on the feed. The sales figures for veterinary antimicrobials from wholesalers and feed mills are thought to roughly equal the use of these drugs. Sales figures for veterinary antimicrobials are therefore used as a synonym of veterinary antimicrobial use. 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. The number of items sold in 2006 for the various veterinary drug formulations, strengths and package sizes were obtained from the Norwegian Institute of Public Health and calculated to express kilogram active substance.
Veterinarians have since 1989 been obliged by regulation to submit copies of all prescriptions to farmed fish to the Norwegian Directorate of Fisheries (NDF), and since 2004 to the Norwegian Food Safety Authority (NFSA). NFSA (and formerly NDF) compiles all relevant information such as the drug substance and the amounts prescribed, fish species to be treated and the date of prescribing into a prescription database. Data on annual usage of antimicrobials per fish species was obtained from this prescription database. These data have since 1996 been regularly validated against overall national sales statistics of drugs sold for use in farmed fish, and this validation shows that the data from these two sources are highly correlated.

Drug classification system
The Anatomical Therapeutic Chemical vet (ATCvet) classification system was used to categorize 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 approved veterinary antimicrobial (AM) specialities belonging to the following ATCvet groups: QA07AA (gastrointestinal infections), QG01AA+AX (uterine infections) and, QJ [AM agents for systemic use that includes intramammary dose applicators (QJ51)]. Additionally, a few AMs preparations sold on special exemption from market authorization have been included following a case by case assessment (se footnotes for the various tables and figures). Sales of AMs 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 AMs premix approved for farmed fish only (trimethoprim+sulfadiazine 1:5) but sold solely for use in terrestrial animals since 1995 (unpublished data). In the present report, the sales of this premix have for the first time been included in Table 5 that presents detailed sales figures for AMs for terrestrial animals for the latest year; while for Fig. 1 and 2 this premix has been included for the whole period. Consequently, the sales of the AM drug usage in terrestrial animals reported for the previous years (1995-2005) were underestimated, although only slightly. The updated usage figures are highly positively correlated (r=0.998) with the data reported previously (1995-2005) confirming the formerly reported reduction in the usage of AMs 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.

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

Data sources
In Norway, antibacterials are prescription only medicines (POM), and only sold by pharmacies. These data cover total sales of antibacterials for humans in Norway and are based on sales of antimicrobials from drug wholesalers to pharmacies and hospitals in Norway. The figures presented should be regarded as maximum figures based on the assumption that all drugs sold are actually consumed. The actual drug consumption will probably be somewhat lower.
Data on drug use have been collected by The Norwegian Institute of Public Health since the beginning of the seventies.
Data on antibacterial use in hospitals are retrieved from *Sykehusapotekenes Legemiddelstatistikk* (drug statistics in hospital pharmacies): a cooperation of the Norwegian pharmacies delivering drugs to hospitals and *LIS* (Drug Purchasing Cooperation). *Sykehusapotekenes Legemiddel-statistikk* collects sales data from each hospital pharmacy. Data are collected as sales to wards/hospitals from the pharmacy.
Data on the use in ambulatory care are estimated from the use in hospitals and total use of antibacterials for humans. The use in ambulatory care includes the use of antibacterials in nursing homes. From 1 January 2004, a national prescription database, NorPD, has been established. These data gives exact population prevalence of antibacterial prescription in ambulatory care.

Drug Classification
The data are categorized according to the ATC classification system. Defined Daily Doses (DDDs) are employed as units of measurement. The ATC/DDD index of 2007 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 inter-nationally. 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 antiinfectives are as a main rule based on the use in infections of moderate severity. Some antiinfectives 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 J01 antibacterials for systemic use. Oral vancomycin (A07AA09) and oral and rectal metronidazole (P01AB01) are also included. Of the antimycobacterials, only rifampicin is included and 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 report.

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

Sampling strategy

The isolates of indicator bacteria (*E. coli* and *Enterococcus* spp.) included in the NORM-VET monitoring programme in 2006 were collected from healthy broilers (faecal and meat samples). The sampling period was from January to November. The faecal samples from broilers were obtained at four of the Regional Laboratories at the National Veterinary Institute from samples collected according to the Norwegian Salmonella control programme for live animals. The first sample to be processed on a specific weekday during the whole sampling period was collected at each laboratory. The number of samples from each laboratory was proportionate to the total number of samples from broilers obtained for each laboratory in the previous year. Samples of broiler meat were collected weekly at retail level by personnel at the National Veterinary Institute.

Isolation and identification of bacteria

Escherichia coli

The *E. coli* strains included in NORM-VET 2006 were isolated and identified at the National Veterinary Institute. Meat: Five grams of material from each specimen were incubated in 45 ml of MacConkey broth (Oxoid). After incubation at 44°C for 24 h, a small amount (approx. 10 µl) of broth was plated onto the surface of lactose-saccarose-bromthymol blue agar. Faeces: Intestinal content was swabed and plated directly onto the surface of lactose-saccarose-bromthymol blue agar without broth enrichment.

After incubation of the agar plates at 37°C for 24 h, a typical colony was plated onto blood agar (Heart infusion agar (Difco) containing 5% bovine blood). Colonies were identified as *E. coli* by typical appearance, lactose and/or saccarose fermentation and a positive indole reaction.

***Enterococcus* spp.**

The enterococcal strains included in NORM-VET 2006 were isolated and identified at the National Veterinary Institute. Meat: Five grams of material from each specimen were incubated in 45 ml of Azide dextrose broth (Oxoid). After incubation at 44°C for 24 h, a small amount (approx. 10µl) of broth was plated onto the surface of Slanetz & Bartley agar (Oxoid). Faeces: Intestinal content was swabed and plated directly onto the surface of Slanetz & Bartley agar (Oxoid) without broth enrichment.

After incubation of the agar plates at 44°C for 48h, typical colonies were plated onto blood agar (Heart infusion agar (Difco) with 5% bovine blood). Typical colonies were tested by catalase reaction and *E. faecium* and *E. faecalis* were identified by *ddl*-PCR (Dutka-Malen et al., 1995).

For the selective isolation of vancomycin resistant *Enterococcus* spp. (VRE), the samples were treated as described above, and plated out on additional Slanetz and Bartley's agar plates containing 32 mg/L vancomycin. Colonies from each positive sample were selected, and the isolates confirmed as *Enterococcus* spp. by phenotypic characterization. The isolates were further identified to species level and tested for the presence of the *vanA* gene using PCR (Dutka-Malen et al, 1995, Simonsen et al, 2000).

Susceptibility testing

Only one isolate per herd or product were tested for antimicrobial susceptibility. Bacterial isolates were tested for antimicrobial susceptibility at the National Veterinary Institute. A broth microdilution method; VetMIC™ (Dept. of Antibiotics, National Veterinary Institute, Sweden) was used for susceptibility testing of all isolates. For interpretation of results for indicator bacteria (*E. coli* and *Enterococcus* spp.) epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used (http://www.escmid.org). When no cut-off value were available, or the range of concentrations tested was inappropriate for the recommended value, a cut-off value was defined on the basis of the actual MIC distributions obtained in the NORM-VET programme. The same approach was used when recommended cut-off values would have divided distributions of MIC in a manner not in agreement with the concept of wild type distributions, causing an erroneously high frequency of resistance. This applies to streptomycin and ciprofloxacin in *E. coli* and to ampicillin in *Enterococcus* spp.

Quality assurance systems

The following susceptible bacteria were included as quality controls on a weekly basis, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212. The following resistant bacteria were tested on a regular basis: *E. faecium* CCUG 33829, CCUG 36804. The results were approved according to reference values given by CLSI when available. Additional control strains were included when necessary.

The participating laboratories at the National Veterinary Institute are accredited according to the requirements of NS-EN ISO/IEC 17025 and participate in external quality assurance programmes for veterinary pathogens (VLQAS Veterinary Laboratories Agency Quality Assurance Unit, Loughborough, England).

Data processing

Susceptibility data were recorded and processed in WHONET 5.3, a program developed by the World Health Organization (WHO) for analysis of antimicrobial resistance data (http://www.who.int/drugresistance/whonetsoftware/en/index.html). The susceptibility data were stored as continuous values (MIC).

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 samples were obtained from animals during clinical examinations or necropsies at the National Veterinary Institute. One isolate of each serovar per incident was included for susceptibility testing.

Investigation of resistance in a sample collection of historical *Salmonella* spp. from reptiles submitted to the the National Veterinary Institute during 1997-2006 was performed. Only one isolate from each serovar from each localisation (mainly zoo's) and year were included.

Campylobacter jejuni

As part of the Norwegian action plan against *Campylobacter* in broilers (www.zoonose.no), caecal samples were collected at slaughter plants. One isolate per positive farm was included for susceptibility testing.

Sampling strategy - humans

Salmonella, Yersinia enterocolitica* and *Shigella

All human isolates were obtained from clinical specimens. One isolate per patient or one isolate per outbreak was included for susceptibility testing.

Campylobacter

A total of 250 human 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* from animals was carried out by the National Veterinary Institute according to the Nordic Committee on Food Analyses (NMKL) method number 71. Isolation of *Campylobacter* spp. from broiler was carried out by local laboratories according to the Nordic Committee on Food Analyses (NMKL) method number 119, with minor modifications. Identification of *C. jejuni* was carried out by the Norwegian Institute of Public Health.

Isolation and identification of bacteria from humans was 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). The identification of all isolates from animals and humans was verified at the National Reference Laboratory for Enteropathogenic Bacteria at the Norwegian Institute of Public Health.

Susceptibility testing

Isolates from animals were tested for antimicrobial susceptibility at the National Veterinary Institute. MIC values were obtained using the VetMIC™ microdilution method (Dept. of Antibiotics, National Veterinary Institute, Sweden).

Salmonella, *Yersinia* and *Shigella* isolates from humans were susceptibility tested at the Norwegian Institute of Public Health by an agar disk diffusion test using BD Sensi-Disc and Mueller-Hinton II-medium. The *Campylobacter* isolates from humans were tested for antimicrobial susceptibility at the Norwegian Institute of Public Health using Etest (AB Biodisk).

For animal isolates, epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used (http://www.escmid.org). When no cut-off value were available, or the range of concentrations tested was inappropriate for the recommended value, a cut-off value was defined on the basis of the actual MIC distributions obtained in the NORM-VET programme. The same approach was used when recommended cut-off values would have divided MIC distributions in a manner not in agreement with the concept of wild type distributions and thereby causing an erroneously high frequency of resistance in (a) single year(s). This applies to gentamicin and *C. jejuni*.

For human isolates, MIC breakpoints defined by AFA (Norwegian Reference Group on Antibiotic Susceptibility Testing) were applied when available and appropriate. For disk diffusion results, population based breakpoints were used. Breakpoints for *Campylobacter* are preliminary and based on MIC distributions.

Quality assurance systems

The National Veterinary Institute and the Reference Laboratory for Enteropathogenic Bacteria at the Norwegian Institute of Public Health have a quality assurance system according to the requirements of NS-EN ISO/IEC 17025.

Campylobacter jejuni subsp. *jejuni* CCUG 33057 and CCUG 11284 were used as quality control strains at the National Veterinary Institute on a weekly basis. The National Veterinary Institute participates in an external quality assurance programme for veterinary pathogens organized by the VLQA (Veterinary Laboratories Agency Quality Assurance Unit, Loughborough, England) and also in the external quality assurance programmes organized by ARBAO-II http://www.dfvf.dk/. The Norwegian Institute of Public Health participates in the external quality assessment programme for *Salmonella* organized by Enter-Net.

Data processing

Susceptibility data were recorded and processed in WHONET 5.3, a program developed by the World Health Organization (WHO) for analysis of resistance data (http://www.who.int/drugresistance/whonetsoftware/). The susceptibility data were stored as discrete values (MIC).

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

General considerations
NORM is based upon periodic sampling and testing in each participating laboratory of microbial isolates from defined clinical conditions such as respiratory tract infections, wound infections, urinary tract infections, or septicaemiae. For enteric infections see Appendix 4. 2006 was the seventh year of surveillance, and all 23 laboratories in Norway participated in the surveillance system in addition to the Norwegian Institute of Public Health. All laboratories followed the same sampling strategy and use identical criteria for the inclusion of microbial strains. Only one isolate per patient and infectious episode was included. 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 up to a defined maximum of isolates for each surveillance category. The surveillance categories in 2006 and the maximum number of isolates from each laboratory were as follows: *E. coli* (n=50), *Klebsiella* spp. (n=25), *Staphylococcus aureus* (n=50), *Streptococcus pneumoniae* (n=50), *Streptococcus pyogenes* (all), *Enterococcus* spp. (n=20), *S. agalactiae* (50 in total) and *Candida* spp. (all) from blood cultures; *S. aureus* (n=50) and *S. pyogenes* (n=25) from wound samples, *S. pyogenes* (n=25) from respiratory tract infections, and *E. coli* (n=50) and *Proteus mirabilis* (n=25) from urinary tract infections. The report further includes all isolates of *Mycobacterium tuberculosis* and viral strains from all patients with newly diagnosed HIV infections.

Susceptibility testing
E. coli, *Klebsiella* spp., *P. mirabilis*, *Enterococcus* spp. and *S. aureus* isolates were examined by disk duffusion using either Oxoid disks on Isosensitest agar, or Beckton Dickinson disks on Mueller Hinton II agar. Suitable antibiotics were selected for each bacterial species, and the results were interpreted according to the respective manufacturers´ recommendations using the breakpoints of the Norwegian Reference Group on Antibiotic Susceptibility Testing (AFA). The AFA breakpoints are identical to EUCAST breakpoints where such have been established. 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 *S. aureus* and *Enterococcus* spp. isolates were screened for glycopeptide resistance using the vancomycin 6 mg/L BHI agar. *S. pneumoniae* and *S. pyogenes* isolates were susceptibility tested using Etest on MH II agar supplemented with 5% lysed horse blood as specified by AB Biodisk (Solna, Sweden). All resistance values were recorded either as mm inhibition zone sizes or MIC values in order to monitor trends in the occurrence of resistance.

Confirmation of resistance phenotypes
E. coli and *Klebsiella* spp. with reduced susceptibility to one or more 3rd or 4th generation cephalosporins were examined for ESBL production using the ESBL Etest according to the instructions of the manufacturer. *S. aureus* isolates with reduced susceptibility to cefoxitin were examined by *mecA* PCR for confirmation of methicillin resistance (MRSA). *Enterococcus* spp. isolates displaying growth on the vancomycin screening agar were examined by *van* gene PCRs for confirmation of VRE. Erythromycin resistant *S. pneumoniae*, *S. pyogenes* and *S. aureus* 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, *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 (heterogeneous MRSA), *S. aureus* CCUG 35600 (homogeneous MRSA).

Data processing
The specially designed eNORM computer program was used for the registration of patient data, sample data and resistance data. The results were further analysed by WHONET5 with the aid of the NORMlink program, both developed by 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 1 month after the initial finding were considered duplicates and omitted from the survey. No attempt was made to evaluate the clinical significance of each finding.

Mycobacterium tuberculosis
Susceptibility testing (DST) was performed at the Norwegian Institute of Public Health, Ullevål University Hospital and Rikshospitalet. All isolates were tested using the BACTEC 460 or BACTEC MGIT 960 systems. All three laboratories participate in the WHO external DST quality control program. The same three laboratories and Haukeland University Hospital also perform tests for mutations in *rpoB* gene to detect resistance to rifampicin.

Yeasts
All systemic yeast isolates in Norway are submitted to Rikshospitalet where susceptibility testing by Etest was performed.

HIV
Genotypic susceptibility tesing of HIV isolates from primary infections was performed at Haukeland University Hospital, Ullevål University Hospital and Rikshospitalet. Relevant areas of the reverse transcriptase (RT) and the protease (PR) genes were DNA sequenced and submitted to the Norwegian Institute of Public Health. The results were subsequently analysed online in the HIV Drug Resistance Database at Stanford University.

Appendix 6: Breakpoints NORM-VET

For interpretation of results for zoonotic bacteria (*Salmonella* and *Campylobacter*) and indicator bacteria (*E. coli* and *Enterococcus* spp.), epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used (http://www.escmid.org). When no cut-off values were available, or the range of concentrations tested was inappropriate for the recommended value, a cut-off value was defined on the basis of the actual MIC distributions obtained in the NORM-VET programme. The same approach was used when recommended cut-off values would have cut through distributions of MICs in a manner not in agreement with the concept of wild type distributions, causing an erroneously high frequency of resistance in (a) single year(s). This applies to gentamicin in *C. jejuni*, streptomycin and ciprofloxacin in *E. coli* and ampicillin in *Enterococci* spp.

Bacitracin values in this report are given in units/mL. In an attempt to convert unit/mL to mg/L we discovered that there is some confusion on this issue. The bacitracin compound used in NORM-VET is obtained from Sigma and meets the standards set by the United States Pharmacopoeia (USP), stating that one unit is equivalent to 26 µg of the US standard. However, according to the International Standard Preparations, one international unit is equivalent to 13.51 µg. On the other hand, if the bacitracin is of a very high degree of purity, though unstable, it corresponds to 66 (-70) units/mg, that is, one unit is equivalent to approximately 15 µg. Feedingstuff grade of bacitracin correspond to 42-50 units/mg (one unit=20-24 µg) (Otten et al., 1975).

Antimicrobial	Resistant (MIC values, mg/L)	*Campylobacter*	*E. coli* / *Salmonella*	*Enterococcus*
Tetracycline	> 2	■		■
	> 8		■	
Chloramphenicol	> 16		■	
	>32			■
Florfenicol	> 16		■	
Ampicillin	>4		■[a]	
	> 8	■	■[a]	■
Ceftiofur	> 1		■	
Cefotaxime	>0.25		■[b]	
	>0.5		■[b]	
Trimethoprim	> 2		■	
Sulfonamides	> 256		■	
Erythromycin	> 4	■		■
Streptomycin	> 16		■[c]	
	> 32		■[c]	
	> 512			■
Gentamicin	> 2	■	■	
	> 32			■
Kanamycin	>16		■	
	> 1024			■
Enrofloxacin	> 0.5	■		
Ciprofloxacin	>0.06		■	
Nalidixic acid	> 16	■	■	
Vancomycin	> 4			■
Bacitracin**	> 32			■
Linezolid	> 4			■
Virginiamycin*	> 4			■
Narasin	> 2			■

[a] > 8 for *E. coli*, > 4 for *Salmonella* spp.
[b] > 0.25 for *E. coli*, > 0.5 for *Salmonella* spp.
[c] > 16 for *E. coli*, > 32 for *Salmonella* spp.
* applies only for *E. faecium*
** units

Appendix 7: Breakpoints NORM

NORM data are categorized according to the breakpoints of the Norwegian Reference Group on Antibiotic Susceptibility Testing – AFA (isolates from humans) which are harmonized with EUCAST breakpoints when available. Breakpoints for *Campylobacter* are preliminary and based on MIC-distributions. For details regarding bacteria and antimicrobial panels, see tables in text. AFA breakpoints are available at www.antibiotikaresistens.no.

	MIC values mg/L														
Antimicrobials	S	R	*E. coli*	*Klebsiella* spp.	*Proteus mirabilis*	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp.	*S. aureus*	*Enterococcus* spp.	*S. pneumoniae*	*S. pyogenes*	*S. agalactiae*	Yeasts
Amphotericin B															■
Ampicillin	≤ 0.5	> 8	■		■	■	■	■							
	≤ 2	> 8									■				
Aztreonam	≤ 1	> 8	■	■											
Caspofungin															■
Cefotaxime	≤ 0.5	> 0.5												■	
	≤ 0.5	> 2										■			
	≤ 1	> 2	■	■	■										
Cefpirome	≤ 1	> 8	■	■											
Ceftazidime	≤ 1	> 8	■	■	■										
Cefuroxime	≤ 0.5	≥ 1										■			
	≤ 0.5	≥ 8	■	■	■										
Chloramphenicol	≤ 8	> 8				■	■	■							
Ciprofloxacin	≤ 0.5	> 1	■	■	■	■	■	■							
	≤ 1	> 1								■					
	≤ 1	> 2							■						
Clindamycin	≤ 0.25	> 2										■	■	■	
	≤ 1	> 2								■					
Erythromycin	≤ 0.5	> 0.5										■	■	■	
	≤ 0.5	> 4							■						
	≤ 1	> 2								■					
Fluconazole															■
Fusidic acid	≤ 0.5	> 0.5								■					

Antimicrobials	MIC values mg/L S	R	*E. coli*	*Klebsiella* spp.	*Proteus mirabilis*	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp.	*S. aureus*	*Enterococcus* spp.	*S. pneumoniae*	*S. pyogenes*	*S. agalactiae*	Yeasts
Gentamicin	≤ 1	> 1								■					
	≤ 2	> 4	■	■	■										
	≤ 4	> 4							■						
	≤ 128	> 128									■				
Linezolid	≤ 4	> 4								■	■				
Mecillinam	≤ 2	> 8	■		■										
Meropenem	≤ 2	> 8	■	■	■										
Nalidixic acid	≤ 16	> 16	■	■	■	■	■	■	■						
Nitrofurantoin	≤ 32	> 32	■		■										
Penicillin G	≤ 0.064	> 1										■			
	≤ 0.125												■	■	
Pip./Tazo.	≤ 8	> 16	■	■											
Rifampicin	≤ 1	> 1								■					
Tetracycline	≤ 1	> 2								■					
	≤ 2	> 2							■			■	■		
	≤ 4	> 8				■	■	■							
Trimethoprim	≤ 2	> 4	■		■										
TMS*	≤ 0.5	> 2										■	■		
	≤ 2	> 8	■	■	■	■	■	■		■					
Vancomycin	≤ 4	> 4												■	
Voriconazole															■

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