2× and 4× the MIC of DW-224a. The regrowth of test organisms was prevented completely by DW-224a at concentrations of 1×, 2× and 4× MIC by 24 h, although regrowth occurred in the presence of ciprofloxacin at 1× MIC concentration. DW-224a also showed a dose-dependent bactericidal activity against all test organisms up to 4× the MIC. The MBCs of DW-224a in Mueller–Hinton broth (MHB) were identical to 1× MIC or at most twice the MIC for all strains tested. Because the bactericidal activities of DW-224a increased with increasing concentrations (up to a concentration of 2 mg/L) for all test strains, and because the slope of the lines (rate of activity) in the time–kill curve varied with concentration, we concluded that DW-224a had concentration-dependent bactericidal activities regardless of the tested strains. Mutations within QRDR of the genes encoding DNA gyrase in *S. pneumoniae* strains did not affect the bactericidal activities of DW-224a, ciprofloxacin and gemifloxacin.

PAE of DW-224a was determined using a broth technique in MHB.[6] The PAE was calculated by the following equation: PAE = $T - C$, where T is the time to achieve 1 $\log_{10}$ cfu/mL growth for the antibiotic-exposed sample and C is the time to achieve 1 $\log_{10}$ cfu/mL growth for the untreated control sample (Table 1).

In summary, DW-224a showed very potent and dose-dependent bactericidal effect against all strains tested. The clinical usefulness of DW-224a should be established by further studies.

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Transparency declarations

None to declare.

References

1. Kwak JH, Seol MJ, Kim HJ *et al. In vitro* and *in vivo* antibacterial activities of the new fluoroquinolone, DW-224a. In: *Programs and Abstracts of the Forty-third Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2003*. Abstract F-415, p. 222. American Society for Microbiology, Washington, DC, USA.

2. Hooper DC. Mechanisms of action of antimicrobials: focus on fluoroquinolones. *Clin Infect Dis* 2001; **32** Suppl 1: S9–15.

3. Weigel LM, Anderson GJ, Facklam RR *et al.* Genetic analyses of mutations contributing to fluoroquinolone resistance in clinical isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2001; **45**: 3517–23.

4. Kwak JH, Seol MJ, So MK *et al. In vitro* development of resistant mutants to DW-286a, a new quinolone antibiotic, in *Streptococcus pneumoniae*. In: *Programs and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA*, 2002. Abstract F-578, p. 191. American Society for Microbiology, Washington, DC, USA.

5. National Committee for Clinical Laboratory Standards. *Methods for Determining Bactericidal Activity of Antimicrobial Agents; Document M26-A*. NCCLS, Wayne, PA, USA, 1999.

6. Craig WA, Gudmundsson S. The postantibiotic effect. In: Lorian V, ed. *Antibiotics in Laboratory Medicine*. Baltimore: Williams & Wilkins, 1996; 296–329.

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First description of *bla*$_{\text{CTX-M-1}}$-carrying *Escherichia coli* isolates in Danish primary food production

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Sir,

Escherichia coli is a common commensal of the intestinal tract of animals and humans, but can also be an important pathogen. *E. coli* isolates resistant to oxyiminocephalosporins due to the production of extended-spectrum β-lactamases (ESBLs) have emerged worldwide and a number of different ESBL genes such as the *bla*$_{\text{SHV}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{CTX}}$ and *bla*$_{\text{CMY}}$ genes have been identified in *E. coli*.[1–3] The same genes have been shown to encode resistance in different countries, which could indicate a global spread of these genes. The first ESBL-producing bacterium from food animals in Denmark was found in August 2003. This was a *bla*$_{\text{CMY-2}}$-containing *Salmonella* Heidelberg isolate obtained from the intestine of a boar imported from Canada.[4] In the same year in October the Danish Institute for Food and Veterinary Research (DFVF) received three *Salmonella* Virchow isolates found in quails imported from France, which were found to contain *bla*$_{\text{CTX-M-9}}$.[5]

In Denmark ESBL-producing isolates of *E. coli* have not previously been isolated from animals in primary food production. However, in July 2005 two *E. coli* isolates from infections in pigs showing resistance to cephalosporins were identified during the routine diagnostic testing at the Laboratory for Pig Diseases in Kjellerup, Denmark. The isolates were from two different farms, and one was from a case of diarrhoea (isolate A) and the other one from septicaemia (isolate B).

Isolate A was haemolytic and serogrouped as O149, while isolate B was non-haemolytic and serogrouped as O20. Both isolates were examined for antimicrobial susceptibility by MIC determinations using a commercially dehydrated panel Sensititre (Trek Diagnostic Systems, UK). The following antimicrobials were assayed: ampicillin, apramycin, cefalotin, ceftiofur, cefpodoxime, chloramphenicol, ciprofloxacin, co-amoxiclav, florfenicol, gentamicin, nalidixic acid, neomycin, spectinomycin, streptomycin, sulfamethoxazole, tetracycline and trimethoprim.

In addition, the presence of ESBL genes was determined as described previously.[6] Both isolates were resistant to ampicillin, cefalotin, ceftiofur and cefpodoxime, and had reduced susceptibility to co-amoxiclav; they both contained $bla_{CTX\text{-}M\text{-}1}$ and the ESBL resistance was transferable to nalidixic acid-resistant *E. coli* MT102 recipients. Isolate B was also resistant to apramycin, gentamicin, neomycin and tetracycline, but resistance to these antimicrobials was not co-transferred with the ESBL resistance. Plasmid purification and hybridization to undigested and *Pst*I-digested plasmids from the donors and transconjugants revealed that the $bla_{CTX\text{-}M\text{-}1}$ gene was located on a plasmid with a size of >70 kb and on a band of ~4.5 kb in both isolates and their transconjugants. This could indicate that the plasmids from the two *E. coli* isolates are related.

The origin of the ESBL genes in the two farms is not known. Contacts to the veterinarians visiting the two farms did not reveal any indication of connections between the farms or trading contacts with other countries. Data from the continuous monitoring of drug usage in Denmark showed that a fourth-generation cephalosporin, cefquinome, had been used for three individual treatments in farm A in 2004, while a third-generation cephalosporin, ceftiofur, had been widely used in farm B in 2004 and 2005.[7]

E. coli with $bla_{CTX\text{-}M\text{-}1}$ have also been reported from both animals and humans in other countries.[1,2,8,9] Thus, there are several international reservoirs from where the resistance could have spread to these two Danish farms.

The present observation showed that ESBL-producing isolates have also emerged in the primary food production in Denmark. Thus, it must be expected that these genes will spread further among food-producing animals and thereby constitute a reservoir of resistant strains and resistance genes that can transfer to and cause treatment problems for humans. The global spread of ESBL-mediated resistance in *E. coli* and other Enterobacteriaceae is one of the emerging problems we are currently facing. Thus, studies determining the way in which the spread occurs are urgently needed. This is to our knowledge the first report of $bla_{CTX\text{-}M\text{-}1}$ from pigs.

Transparency declarations

None to declare.

References

1. Brinas L, Moreno MA, Teshager T *et al.* Monitoring and characterization of extended-spectrum β-lactamases in *Escherichia coli* strains from healthy and sick animals in Spain in 2003. *Antimicrob Agents Chemother* 2005; **49**: 1262–4.

2. Carattoli A, Lovari S, Franco A *et al.* Extended-spectrum β-lactamases in *Escherichia coli* isolated from dogs and cats in Rome, Italy, from 2001 to 2003. *Antimicrob Agents Chemother* 2005; **49**: 833–5.

3. Jeong SH, Bae IK, Lee JH *et al.* Molecular characterization of extended-spectrum β-lactamases produced by clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* from a Korean nationwide survey. *J Clin Microbiol* 2004; **42**: 2902–6.

4. Aarestrup FM, Hasman H, Olsen I *et al.* International spread of $bla_{CMY\text{-}2}$-mediated cephalosporin resistance in a multiresistant *Salmonella enterica* serovar Heidelberg isolate stemming from the importation of a boar by Denmark from Canada. *Antimicrob Agents Chemother* 2004; **48**: 1916–7.

5. Aarestrup FM, Hasman H, Jensen LB. Resistant *Salmonella* Virchow in quail products. *Emerg Infect Dis* 2005; **11**: 1984–5.

6. Hasman H, Mevius D, Veldman K *et al.* β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. *J Antimicrob Chemother* 2005; **56**: 115–21.

7. Stege H, Bager F, Jacobsen E *et al.* VETSTAT-the Danish system for surveillance of the veterinary use of drugs for production animals. *Prev Vet Med* 2003; **57**: 105–15.

8. Brigante G, Luzzaro F, Perilli M et al. Evolution of CTX-M-type β-lactamases in isolates of *Escherichia coli* infecting hospital and community patients. *Int J Antimicrob Agents* 2005; **25**: 157–62.

9. Pitout JD, Hossain A, Hanson ND. Phenotypic and molecular detection of CTX-M-β-lactamases produced by *Escherichia coli* and *Klebsiella* spp. *J Clin Microbiol* 2004; **42**: 5715–21.

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Klebsiella pneumoniae strains carrying the chromosomal SHV-11 β-lactamase gene produce the plasmid-mediated SHV-12 extended-spectrum β-lactamase more frequently than those carrying the chromosomal SHV-1 β-lactamase gene

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Sir,

Klebsiella pneumoniae produces species-specific class A chromosomal β-lactamases that confer resistance to ampicillin, amoxicillin, carbenicillin and ticarcillin. Three families of chromosomal β-lactamases, including SHV, LEN and OKP, have been identified in clinical *K. pneumoniae* isolates.[1] Plasmid-mediated SHV-type extended-spectrum β-lactamases (ESBLs) are widespread in *K. pneumoniae* isolates, and there has been a predominance of SHV-12 in south-east Asia.[2] In this study, we investigated the diversity of chromosomal β-lactamase genes in clinical *K. pneumoniae* isolates from Korean hospitals and the incidence of SHV-12 according to genotypes of chromosomal β-lactamases.