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Imported food and feed as contributors to the introduction of plasmid-mediated colistin-resistant Enterobacteriaceae to a 'low prevalence' country

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Sir,

In the last year, it has become evident that the plasmid-located colistin resistance gene *mcr-1* has a worldwide distribution.[1] Colistin is widely used in veterinary medicine, particularly in pigs and poultry, and the selection pressure in livestock production is a likely driver of the persistence and dissemination of *mcr-1*.[2] It is not surprising that a large proportion of *mcr-1*-positive isolates described thus far originate from food-producing animal reservoirs.[1] However, plasmid-mediated colistin resistance is also reported from diseased and healthy humans. This is concerning, as colistin is particularly important as one of the last therapeutic options for human infections caused by multiresistant bacteria. Furthermore, plasmid-mediated colistin resistance has been documented for other animal categories such as companion animals, reptiles and birds as well as in environmental samples of sewage, water and vegetables.[1]

In Europe, sales of veterinary medicinal products, including the polymyxin colistin, are documented through the European Surveillance of Veterinary Antimicrobial Consumption reports, and wide variations are observed between countries.[3] In Norway, the sale of polymyxin veterinary medicinal products was zero during the years 2010–15. Historical data also show that there has been no sale of polymyxins for use in animals during the years 1993–2009 in Norway (http://www.vetinst.no/overvaking/antibiotikaresistens-norm-vet). Retrospective molecular screening of Enterobacteriaceae exhibiting colistin MICs above the breakpoint recommended by EUCAST (>2 mg/L), has demonstrated absence of *mcr-1*-positive isolates in samples from animals, food and feed originating from Norway for the years 2010–15.[4] However, in 2016 the monitoring programme NORM-VET included samples of imported seafood and imported raw dog food. From two samples, one of each category, *mcr-1*-positive *Escherichia coli* were detected. The seafood sample was imported scampi from Bangladesh. The frozen dog food sample originated from the UK and contained turkey meat, fruit and vegetables. These samples were screened selectively for quinolone-resistant *E. coli* (QREC). Presumptive QREC were susceptibility tested using broth microdilution (Sensititre®; TREK Diagnostic Systems Ltd, Thermo Scientific, Waltham, MA, USA). The two isolates had colistin MICs of 4 mg/L and were subjected to further investigations. WGS was performed using a MiSeq platform. Data were analysed using online tools available from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org) or manually by search via CLC Main Workbench (CLC bio; QIAGEN, Aarhus, Denmark). WGS data documented *mcr-1* in both isolates and the multilocus STs 3014 and 48. In the scampi isolate, a 132 kb contig contained both *mcr-1* and the IncHI2 replicon marker, indicating that *mcr-1* was located on this plasmid. Both isolates contained genes encoding resistance to several classes of antimicrobial agents (Table 1). Furthermore, a gene responsible for quaternary ammonium compound resistance (*qacH*) and tellurite resistance operons were present in both isolates. The dog food isolate also contained genes encoding mercury resistance. Conjugation experiments were performed in triplicate, but no transconjugants containing *mcr-1* were obtained.

Only one finding of plasmid-mediated colistin resistance has been reported in humans in Norway. This was an *E. coli* with *mcr-1* from a traveller returning from India with enteritis.[4] Human travellers have probably contributed to the worldwide spread of antimicrobial resistance. However, international trade in live animals, animal products, feed and food may represent an important route of dissemination, enabling the global spread of important resistance types and their introduction into geographic areas where they may be rare or absent. Humans may acquire antimicrobial-resistant bacteria through handling or consumption of contaminated food. Likewise, companion animals may acquire resistant bacteria via their feed. Sharing of common Enterobacteriaceae clones among family members and their dogs has been described,[5] including *mcr-1*-positive *E. coli*,[6] emphasizing the possibility of zoonotic transmission.

Interestingly, we found *mcr-1*-positive isolates via screening for QREC. European studies have described that turkeys and broilers are associated with the highest prevalence of *mcr-1*.[7,8] According to European surveillance data these animal species are also associated with high occurrences of QREC.[9] So far, most detections of *mcr-1* have been a result of retrospective screening of historical strain collections or WGS databases, whereas fewer reports have dealt with detection in 'real time'. Our findings show the importance of risk-based screening of relevant samples to uncover possible sources of bacteria resistant to last-resort antimicrobials. Food products imported from areas with a high environmental load of resistant bacteria and with higher usage of antimicrobials should receive special attention. This is exemplified by the findings in the present study, but also by findings from Switzerland where

Table 1. Characteristics of *mcr-1*-positive *E. coli* isolates

Isolate	2016-22-851 (scampi)	2016-22-902 (dog food)
Month/year of isolation	September 2016	October 2016
Sequence type	ST48	ST3014
Phylogenetic group	A	A
Serotype (genotype)	O39:H45	O9:H4
Plasmid replicon type(s)	IncHI2 (pMLST: ST4), IncN, IncX3	IncHI2 (pMLST: ST4), IncR, IncI1, IncQ1
Mutations		
gyr	*gyrA*: Ser83Leu	*gyrA*: Ser83Leu, Asp87Asn
par	–	*parC*: Ser80Ile
Additional resistance genes	bla_{TEM-1B}, *dfrA12, dfrA15, mph(A), sul1, sul3, tet(A), cmlA1, strA, strB, aadA2, aadA1, qnrS1, qepA*	bla_{TEM-1C}, *dfrA1, dfrA12, sul1, sul2, sul3, tet(A), catA1, cmlA1, aadA2, aac(3)-IIa, aadA1, strA, strB, aph(3′)-Ic*
MICs (mg/L)	SMX (1024), TMP (>32), CIP (>8), TET (>64), MEM (<0.03), AZM (16), NAL (>128), CHL (32), TGC (≤0.25), CST (4), CTX (≤0.25), CAZ (≤0.5), AMP (>64), GEN (≤0.5)	SMX (>1024), TMP (>32), CIP (>8), TET (>64), MEM (<0.03), AZM (8), NAL (>128), CHL (64), TGC (≤0.25), CST (4), CTX (≤0.25), CAZ (≤0.5), AMP (>64), GEN (>32)

SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; TET, tetracycline; MEM, meropenem; AZM, azithromycin; NAL, nalidixic acid; CHL, chloramphenicol; TGC, tigecycline; CST, colistin; CTX, cefotaxime; CAZ, ceftazidime; AMP, ampicillin; GEN, gentamicin.

mcr-1-positive *E. coli* in vegetables imported from Thailand and Vietnam have been reported.[10] Furthermore, a study performed in Canada found carbapenem-resistant bacteria in samples of imported seafood from Asia.[11]

Our data represent single findings from two categories of imported food and feedstuff. More comprehensive monitoring to follow up these reservoirs is required. Furthermore, there is a need to extend our knowledge regarding how to perform a future risk-based sampling for antimicrobial resistance in 'low prevalence' settings to achieve maximum sensitivity for the detection of rare, but highly important mechanisms of resistance.

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Transparency declarations

None to declare.

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