Methicillin-resistant *Staphylococcus aureus* ST9 from a case of bovine mastitis carries the genes *cfr* and *erm*(A) on a small plasmid

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Sir,

Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause a wide variety of infections in animals with bovine mastitis being the predominant staphylococcal infection in dairy cattle. MRSA isolates—mainly of multilocus sequence type (ST) 398—have recently been shown to be also involved in bovine mastitis. Although a variety of antimicrobial resistance genes have been identified in MRSA isolates from cases of bovine mastitis, the multidrug resistance gene *cfr* has not yet been identified in bovine CC398 isolates, although it has been found in a single porcine MRSA ST398 isolate and a single porcine methicillin-susceptible *S. aureus* ST9 isolate. Although pilot studies have indicated that MRSA ST9 isolates are commonly found among pigs in China, cfr-positive MRSA isolates of animal origin have not yet been reported in China.

In 2010, during a routine surveillance study on antimicrobial resistance on dairy farms in China, an MRSA strain (designated SA16) was isolated from fresh raw milk of a cow suffering from mastitis. The MRSA strain was further characterized by multilocus sequence typing (MLST; http://saureus.mlst.net/), *spa* typing (http://spaserver.ridom.de) and SCCmec typing, as previously described. This strain belonged to ST9 (allelic profile 3-3-1-1-1-1-10), belonged to spa type t899 (allelic profile 07-16-23-02-34), and harboured an SCCmec element of type III. The strain displayed resistance to oxacillin (MIC = 128 mg/L), erythromycin (MIC ≥ 64 mg/L) and clindamycin (MIC ≥ 64 mg/L), had a linezolid MIC of 4 mg/L and showed high florfenicol and tiamulin MICs of ≥128 mg/L and 256 mg/L, respectively. The genes *mecA* (oxacillin resistance), *cfr* and *fexA* (phenicol resistance) were detected by PCR and confirmed by sequencing of the respective amplicons.

Plasmid preparation using the DNA midi kit (Qiagen, Hilden, Germany) identified an ~7 kb plasmid, designated pMSA16. This plasmid was transformed into the recipient strain *S. aureus* RN4220 by electrotatransformation using 0.2 cm cuvettes with a Gene Pulser apparatus at 2.5 kV (Bio-Rad, Munich, Germany). Transformants were selected on BHI agar containing erythromycin (15 mg/L) or florfenicol (10 mg/L). Southern blot analysis using the *cfr* and *fexA* amplicons as probes confirmed that the *cfr* gene was located on pMSA16 in the original strain and its transformants, while the *fexA* gene was most likely located on the chromosomal DNA. The pMSA16-harbouring transformants showed ≥4-fold increases in the MICs of chloramphenicol, florfenicol, clindamycin, linezolid and tiamulin, which are indicative for the *cfr*-associated resistance phenotype and demonstrated that the pMSA16-associated *cfr* gene is functionally active. In addition, the pMSA16-harbouring transformants showed an elevated MIC of erythromycin of ≥64 mg/L, which suggested that a macrolide resistance gene is also located on plasmid pMSA16.

To gain insight into the structure and organization of plasmid pMSA16, the sequence of this 7054 bp plasmid was determined by primer walking. Using the open reading frame (ORF) finder software (http://www.ncbi.nlm.nih.gov/gorf/f), five reading frames for proteins of ≥100 amino acids (aa) were identified in pMSA16 (Figure 1a). The rep gene codes for a 327 aa plasmid replication protein, which shares 100% and 98.5% aa identity with the same-sized RepU protein of a plasmid from the porcine *S. aureus* strain 7612628-4 (GenBank accession number JF968539) and the 326 aa RepU protein of the *Staphylococcus saprophyticus* plasmid pSES22, respectively. A macrolide-lincosamide–streptogramin B resistance gene coding for a 243 aa rRNA methylase was detected, the deduced aa sequence of which was indistinguishable from the same-sized Erm(A) protein of *Tn554*.7 Analysis of the *erm*(A)-flanking regions identified a potential recombination site that included the translational start codon of the *erm*(A) gene and might have contributed to the replacement of the pSES22-associated *erm*(C) gene by a *Tn554*-borne *erm*(A) gene (Figure 1b). At the other end, homology to *Tn554* ended in the translational attenuator 135 bp upstream of the *erm*(A) translational start codon (Figure 1a and b). The *erm*(C) and *erm*(A) translational attenuators are composed of several pairs of inverted repeats, and areas characterized by inverted repeats are considered as preferential areas for illegitimate recombination events. Therefore, recombination in the *erm*(A)
upstream part has most likely occurred between the \textit{erm}(A) translational attenuator of Tn554 and the \textit{erm}(C) translational attenuator of pSES22. Further upstream, another stretch of 525 bp proved to be identical to the corresponding sequence of pSES22 (Figure 1a). It should be noted that the co-existence of the genes \textit{cfr} and \textit{erm}(33) or \textit{cfr} and \textit{erm}(B) on the same plasmid, pSCFS1\textsuperscript{8} or pBS-01\textsuperscript{5} has been reported previously. Analysis of the region upstream of the \textit{cfr} gene revealed the presence of a truncated \textit{pre/mob} gene whose deduced 272 aa Pre/Mob protein showed 99.6% identity to the C-terminal 272 aa of the 420 aa Pre/Mob protein of plasmid pSES22.\textsuperscript{6} Another \textit{pre/mob} gene, encoding a 376 aa Pre/Mob protein,
which shared 95.2% identity with the 392 aa Pre/Mob protein from plasmid pSCFS1, was found downstream of the cfr gene in pMSA16. The 2848 bp fragment containing the cfr gene and its downstream pre/mob gene showed 100% nucleotide sequence identity to the corresponding parts in plasmids pBS-01 and pBS-02.  

In conclusion, this is the first report describing the presence of the cfr gene in a bovine MRSA ST9 strain. Moreover, this is also the first report of an erm(A) gene on a small plasmid. The findings of this study suggested that plasmid pMSA16 consists of a pSES22 backbone from which the erm(C) gene was exchanged for an erm(A) gene and into which a pBS-01/pBS-02-related cfr-carrying segment was integrated. These observations underline that inter-plasmid recombination, but also recombination between a plasmid and a transposon, plays a role in the dissemination of cfr and erm(A).

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**Transparency declarations**

None to declare.

**References**


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**Emergence of CTX-M-2-producing *Escherichia coli* in diseased horses: evidence of genetic exchanges of bla<sub>CTX-M-2</sub> linked to ISCR1**

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Sir,

The impact of CTX-M-producing bacteria on animal health remains unclear.  The CTX-M-2 enzyme was first identified in a *Salmonella* Mbandaka strain isolated from faeces of a 4-month-old child.  Later on, CTX-M-2-producing Enterobacteriaceae were also reported in faeces from healthy poultry and horses.  In this study, we describe the molecular characterization of different *bla<sub>CTX-M-2</sub>*-carrying plasmids in multidrug-resistant *Escherichia coli* isolates recovered from cases of diseased horses. From November 2008 through June 2010, five cephalosporin-resistant *E. coli* isolates were obtained from diseased horses hospitalized at the Faculty of Veterinary Medicine, Ghent University, Belgium. The isolates were recovered from horses with an abdominal fistula (*E. coli* 2657), abdominal wound infection (*E. coli* 3744), peritonitis (*E. coli* 1443), furunculosis (*E. coli* 1454) and arthritis (*E. coli* 4410) (Table S1, available as Supplementary data at JAC Online). Most horses received a prolonged empirical therapy with several antimicrobial agents, such as β-lactams, aminoglycosides and fluoroquinolones. Antimicrobial susceptibility testing of the *E. coli* isolates was assessed by the disc diffusion method and interpreted according to the guidelines of the Antibigram Committee of the French Society for Microbiology (Table S1).  PCRs to screen for extended-spectrum β-lactamase (ESBL) genes were performed.  All isolates harboured the plasmid-encoded *bla<sub>CTX-M-2</sub>* gene.  *E. coli* 4410 and 3744 also carried a narrow-spectrum *bla<sub>TEM-1</sub>* gene.

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