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Retrospective analysis of genome sequences revealed the wide dissemination of *optrA* in Gram-positive bacteria

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Sir,

Oxazolidinones, including linezolid and tedizolid, are highly effective against infections of enterococci, staphylococci and streptococci, which are clinically important Gram-positive pathogens.[1] *cfr* is the first gene that is transferable and causes resistance to oxazolidinones; *cfr* also mediates resistance to phenicols, lincosamides, pleuromutilins and streptogramin A.[2] Recently, Wang *et al.*[3] first reported a novel transferable resistance gene, *optrA*, which confers resistance to oxazolidinones and phenicols in enterococcal isolates in China. Shortly after, the *optrA* gene was detected in clinical enterococcal isolates in Italy[4] and in *Staphylococcus sciuri* in China.[5]

WGS is now routine and thousands of bacterial genomes are available in public databases. To investigate the prevalence of *optrA* in the sequenced genomes, we retrieved the *optrA* sequence and traced this gene in the GenBank database (Table S1, available as Supplementary data at *JAC* Online). We found that *optrA* presented in two enterococcal isolates from human blood in the USA, two environmental samples from swine manure metagenome in China and six *Streptococcus suis* isolates from healthy pigs in China. These results are the first reported presence of *optrA* in clinical enterococci in the USA, and in environmental microorganisms and streptococci in China.

optrA genes in those isolates showed 99% nucleotide identity with the original *optrA* from *Enterococcus faecalis* pE349. Comparison of the OptrA amino acid sequences revealed seven variants with alterations at nine positions (Table S1). Further analysis showed that the genetic context of *optrA* in two US isolates, especially that from *E. faecalis* 599, was similar to that in the chromosome of *E. faecalis* E016 though seemingly lacking Tn*558* and the Δ*erm*(A)-like segment,[6] and distinct from that in the plasmid of *E. faecalis* pE349 and *S. sciuri* pWo28-3 (Figure 1). The genetic contexts of *optrA* in two swine manure samples, IN-7 and IN-8 (Figure 1), were similar to those in plasmid pFX13,[6] i.e. flanked by IS*1216E*, with the Δ*erm*(A)-like gene located upstream instead of downstream of *optrA*. We also found that *optrA* coexisted with *tet*(O/W/32/O) and *tet*(L) in sample IN-7 and with *lsa*(A) and *tet*(O/W/32/O) in sample IN-8 (Figure 1). The genetic composition of the mosaic segments is unique and no similar structure was found in known mobile genetic elements.

We found that six *S. suis* isolates from healthy pigs in 2011 also harbour *optrA* (Table S1). This is surprising as oxazolidinones, often last-resort drugs in clinical treatment, have not been proved to be used in livestock and *optrA* has not been reported in streptococci. All *optrA*-carrying segments except strain YS39 were flanked downstream by IS*1216E* (Figure 1). As the genetic contexts of YS35 and YS39 were not intact due to the over-truncated contigs, we only discuss the other four isolates. In isolate YS57, the ∼8.1 kb *optrA* contig was comprised of *optrA*, an upstream *araC* and downstream an Δ*erm*(A)-like gene, an *S*-adenosylmethionine (SAM)-dependent methyltransferase (*met*) gene and two hypothetical genes. The *optrA* gene block was flanked by IS*1216E* at both ends in the same orientation, which was found only in plasmids before.[6] The *optrA*-carrying IS*1216E* fragment was inserted between *SNF2* and *agg*, two conserved genes of the 89K pathogenicity island (PAI), which presented in a strain of human *S. suis* outbreaks in China in 2005.[7] However, the other conserved genes of the 89K PAI were split into other contigs due to incomplete genome assembly. Significantly, the 89K PAI belongs to the ICE*Sa*2603 family of integrative conjugative elements, which is widely distributed in and demonstrates horizontal transfer between streptococci and enterococci.[8] Figure S1 shows the schematic genetic diagram of the *optrA*-carrying contig of YS57 with *Streptococcus agalactiae* ICE*Sa*2603 and *S. suis* 89K. On contigs of YS21, YS49 and YS50, the ∼7.4 kb *optrA* fragment contains *optrA*, an upstream truncated *araC*, a nickase gene and three hypothetical genes. Upstream of *optrA*, the fragment was flanked by a truncated IS*1272*-like element not fully sequenced (Figure 1). Downstream of *optrA*, the fragment was flanked by an IS*3L*-like element and IS*1216E*. The *optrA* fragment was integrated into a larger prophage by replacing the Mega-like element of *Streptococcus pyogenes* Φm46.1 and the *cad*A/C-*tet*(W) fragment of *S. suis* ΦSsUD.1 (Figure S1).[9] The Φm46.1 prophage family, which was originally found integrated in the 3′-terminal part of *rum* loci in *S. pyogenes* and thereafter in *S. agalactiae* and *S. suis*, is transferable to other streptococci.[10] These results suggest the important role of IS*1216E* in chromosomal

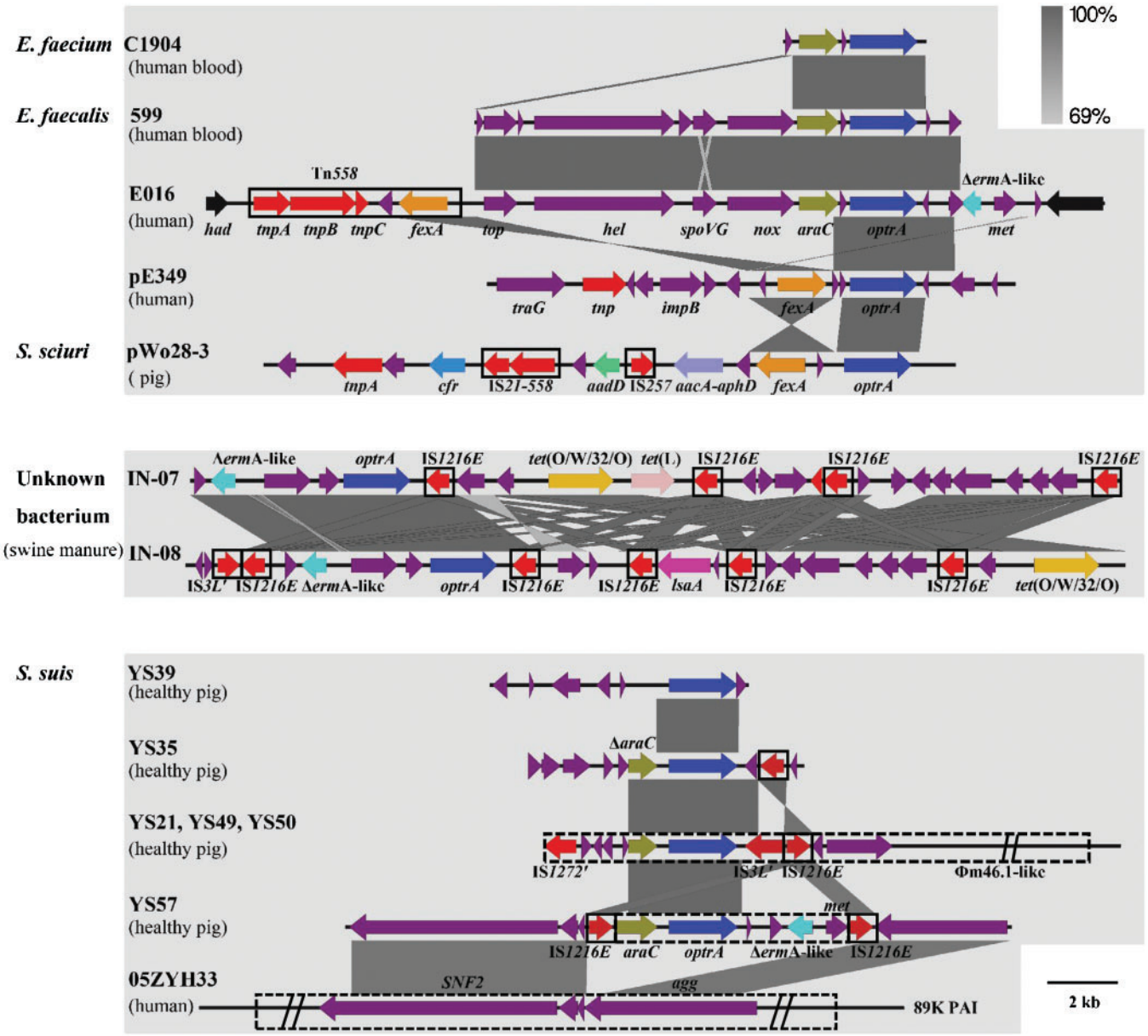

Figure 1. Comparison of genomic sequences harbouring the *optrA* gene. The Basic Local Alignment Search Tool (BLAST) was used to compare the sequence similarity of *optrA* using NCBI GenBank nr/nt and WGS databases. Any isolates previously reported were excluded. Isolate information can be seen in Table S1, available as Supplementary data at *JAC* Online. *E. faecalis* E016 and pE349 served as chromosome- and plasmid-borne *optrA* references, respectively. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

integration of the *optrA* gene and highlight a potential dissemination between streptococci by mobile genetic elements of ICE*Sa*2603 and the Φm46.1 family.

The analysis suggests that the *optrA* gene might have been spreading in Gram-positive bacteria especially in *S. suis*. We screened the *optrA* gene in 154 *S. suis* isolates from routine surveillance for swine streptococcosis in south-east China. *optrA* was detected in three *S. suis* isolates (AH0906, SH0918 and NJ1112). Compared with *optrA*-negative strains, the strains exhibited 4- to 8-fold elevated MICs (2–4 mg/L) of linezolid. Our screen of *S. suis* and other streptococcal isolates is still in progress and the overall results of the survey will be described later.

Our results showed that *optrA*-carrying segments can be inserted into the chromosome via IS*1216E* elements and integrated into ICE*Sa*2603 and Φm46.1—a process that plays a role in the chromosomal dissemination of *optrA* among Gram-positive bacteria. Based on the occurrence of *optrA* sampled from humans, animals and the environment in China, Italy and the USA, the worldwide distribution of *optrA* might be underestimated. Therefore, routine surveillance for the presence of *optrA* in Gram-positive bacteria is warranted.

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Transparency declarations

None to declare.

Supplementary data

Table S1 and Figure S1 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Flamm RK, Mendes RE, Hogan PA *et al*. Linezolid surveillance results for the United States (LEADER Surveillance Program 2014). *Antimicrob Agents Chemother* 2016; **60**: 2273–80.

2 Shen J, Wang Y, Schwarz S. Presence and dissemination of the multiresistance gene *cfr* in Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother* 2013; **68**: 1697–706.

3 Wang Y, Lv Y, Cai J *et al*. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. *J Antimicrob Chemother* 2015; **70**: 2182–90.

4 Brenciani A, Morroni G, Vincenzi C *et al*. Detection in Italy of two clinical *Enterococcus faecium* isolates carrying both the oxazolidinone and phenicol resistance gene *optrA* and a silent multiresistance gene *cfr*. *J Antimicrob Chemother* 2016; **71**: 1118–9.

5 Li D, Wang Y, Schwarz S *et al*. Co-location of the oxazolidinone resistance genes *optrA* and *cfr* on a multiresistance plasmid from *Staphylococcus sciuri*. *J Antimicrob Chemother* 2016; **71**: 1474–8.

6 He T, Shen Y, Schwarz S *et al*. Genetic environment of the transferable oxazolidinone/phenicol resistance gene *optrA* in *Enterococcus faecalis* isolates of human and animal origin. *J Antimicrob Chemother* 2016; **71**: 1466–73.

7 Tang J, Wang C, Feng Y *et al*. Streptococcal toxic shock syndrome caused by *Streptococcus suis* serotype 2. *PLoS Med* 2006; **3**: e151.

8 Huang J, Liang Y, Guo D *et al*. Comparative genomic analysis of the ICE*Sa*2603 family ICEs and spread of *erm*(B)- and *tet*(O)-carrying transferable 89K-subtype ICEs in swine and bovine isolates in China. *Front Microbiol* 2016; **7**: 55.

9 Brenciani A, Bacciaglia A, Vignaroli C *et al*. Φm46.1, the main *Streptococcus pyogenes* element carrying *mef*(A) and *tet*(O) genes. *Antimicrob Agents Chemother* 2010; **54**: 221–9.

10 Giovanetti E, Brenciani A, Morroni G *et al*. Transduction of the *Streptococcus pyogenes* bacteriophage Φm46.1, carrying resistance genes *mef*(A) and *tet*(O), to other *Streptococcus* species. *Front Microbiol* 2014; **5**: 746.

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Increasing resistance to fusidic acid among clinical isolates of MRSA

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Sir,

It has been pointed out that oral anti-MRSA antibiotics can be used as step-down therapy for healthcare-associated infections or as initial therapy for community-associated infections.[1] Fusidic acid (FA) is one of the oral antibiotics with activity against MRSA and has been widely used in different parts of the world including Taiwan.[2–4] The prevalence of FA resistance among MRSA varies in different countries and regions.[2,3,5–7] The most common mechanisms of FA resistance are mutation of the *fusA* gene and carriage of transferable determinants *fusB* or *fusC*.[2,3,5,8]

Taiwan is an area with high prevalence of MRSA infection and FA has been used clinically for decades.[4] However, longitudinal and multicentre surveillance of FA resistance among MRSA isolates has not been conducted in Taiwan. The present study investigated the prevalence and mechanisms of FA resistance among clinical MRSA isolates collected from 28 hospitals located in different regions of Taiwan by the Taiwan Surveillance on Antimicrobial Resistance (TSAR) programme.[9] The genetic characteristics of the FA-resistant (FA-R) isolates were also studied.

A total of 1417 non-duplicate MRSA isolates, including 466, 475 and 476 from 2004, 2008 and 2012, respectively, were studied. A significant increase in FA resistance occurred: from 3.2% in 2004 to 10.7% in 2008 and 18.1% in 2012 (P for trend <0.001). Compared with FA-susceptible isolates ($n = 1265$, FA MIC range ≤ 0.125 to 1 mg/L, $MIC_{50/90} \leq 0.125$ mg/L), the FA-R isolates ($n = 152$, FA MIC range 2 to >128 mg/L, $MIC_{50/90}$ 64/>128 mg/L) were significantly more resistant to ciprofloxacin (48.7% versus 94.7%), gentamicin (58.3% versus 88.8%), trimethoprim/sulfamethoxazole (31.8% versus 69.7%) and tetracycline (57.9% versus 81.6%) (all $P < 0.001$).

Among the 152 FA-R isolates, 84 had *fusA* mutations, another 64 were *fusC* positive and only 1 isolate was *fusB* positive. Three isolates had no *fusA* mutation and were negative for *fusB* and *fusC*. The prevalence of MRSA isolates with *fusA* mutations increased significantly from 2004 (3.2%) to 2008 (7.4%) ($P = 0.005$), but then stabilized in 2012 (7.6%). A total of 20 *fusA* mutation profiles were found, with the L461K single mutation (40 isolates) being most common, followed by the H457Q/L461F double mutation (19 isolates). Details of *fusA* mutation profiles can be seen in Figure S1 (available