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Identification of the Novel *dfrK*-Carrying Transposon Tn*559* in a Porcine Methicillin-Susceptible *Staphylococcus aureus* ST398 Strain[▽]

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The trimethoprim resistance gene *dfrK* was found to be part of the novel Tn*554*-related transposon Tn*559* integrated in the chromosomal *radC* gene of a porcine methicillin-susceptible *Staphylococcus aureus* ST398 strain. While Tn*559* and Tn*554* had similar arrangements of the transposase genes *tnpA*, *tnpB*, and *tnpC*, the Tn*554*-associated resistance genes *erm*(A) and *spc* were replaced by *dfrK* in Tn*559*. Circular forms of Tn*559* were detected and suggest the functional activity of this transposon.

The trimethoprim resistance gene *dfrK* was recently detected on plasmid pKKS2187 in a porcine methicillin-resistant *Staphylococcus aureus* (MRSA) strain of the multilocus sequence type 398 (ST398) (6). In this plasmid, the gene *dfrK* was physically linked to the tetracycline resistance gene *tet*(L). Further analysis of plasmids from porcine MRSA ST398 strains identified the *tet*(L)-*dfrK* genes in close proximity to the kanamycin/neomycin resistance gene *aadD* on plasmid pKKS825 (7) or to the macrolide-lincosamide-streptogramin B (MLS_B) resistance gene *erm*(T) on plasmid pKKS25 (8). While *dfrK* was usually plasmid borne and linked to *tet*(L), a recent survey on the presence of *dfrK* among German coagulase-positive staphylococci from animals (3, 5, 19) identified the porcine *S. aureus* strain 2171 in which *dfrK* was neither located on a plasmid nor linked to *tet*(L).

This strain was obtained from a sow suffering from a genital tract infection (19). Susceptibility testing by broth microdilution (2) revealed susceptibility to oxacillin (MIC, 0.5 μg/ml) but resistance to penicillins (MICs, 8 μg/ml [penicillin] and 4 μg/ml [ampicillin]), macrolides and lincosamides (MICs, ≥64 μg/ml [erythromycin] and ≥128 μg/ml [clindamycin]), tetracycline (MIC, 32 μg/ml), enrofloxacin (MIC, 8 μg/ml), and trimethoprim (MIC, ≥128 μg/ml). Specific PCR assays (3, 13, 18) (Table 1) revealed the presence of the β-lactamase gene *blaZ*, the MLS_B resistance gene *erm*(B), the tetracycline resistance gene *tet*(M), and the trimethoprim resistance gene *dfrK*. *S. aureus* 2171 proved to be negative for *mecA* (11). Multilocus sequence typing and *spa* typing (9) revealed the sequence type 398 and *spa* type t011. Plasmid analysis showed the presence of three plasmids of sizes below 10 kb, none of which conferred trimethoprim resistance in repeated protoplast and electrotransformation experiments nor showed the presence of *dfrK* in Southern blot hybridization experiments. The *dfrK* probe consisted of the internal PCR-generated 214-bp fragment of the *dfrK* gene (Table 1). A single band of ca. 3.7 kb hybridizing with the *dfrK* probe was seen in whole-cell DNA digested with EcoRI. This observation suggested that a single copy of *dfrK* was most likely present in the chromosomal DNA of *S. aureus* 2171.

To gain information about the *dfrK* flanking regions, the EcoRI fragments were religated and subjected to an inverse PCR using the primers dfrKinv1 and dfrKinv2 (Table 1). These primers are located 29 bp apart from each other within the *dfrK* gene. The resulting amplicon was cloned into pCR-Blunt II-Topo (Invitrogen, Karlsruhe, Germany) with subsequent transformation into chemically competent *Escherichia coli* TOP10 and sequenced using the M13 universal and reverse primers as well as with the dfrKinv1 and dfrKinv2 primers. Completion of the sequence was done by primer walking using primers derived from the sequence obtained with the abovementioned primers. Sequence analysis revealed similarities to part of *tnpB* and *tnpC* of transposon Tn*554* in the *dfrK* upstream region and to the 3′ end of the chromosomal *radC* gene in the *dfrK* downstream region. Database searches identified a Tn*554* element integrated into the *radC* reading frame in the whole-genome sequence of *S. aureus* N315 (12). Assuming that there is a similar situation in the genome of *S. aureus* 2171, we designed a PCR assay using one primer located in the 5′-terminal part of *radC* and the other from the *S. aureus* 2171-specific *tnpB* sequence (Table 1). An amplicon of 2,305 bp was obtained and completely sequenced by primer walking starting with the radC-fw and tnpB-rev primers.

Analysis of the complete sequence revealed the presence of a transposon-like element of 4,289 bp, designated Tn*559* (Fig. 1). Tn*559* has three partly overlapping transposase genes, *tnpA*, *tnpB*, and *tnpC*. The 361-amino-acid (aa) TnpA protein of Tn*559* showed 97.2% identity, the 630-aa TnpB protein showed 99.4% identity, and the 125-aa TnpC protein showed 99.2% identity to the same-sized proteins of Tn*554* (1). Downstream of the *tnpC* gene, the reading frame for a 163-aa dihydrofolate reductase was detected. The gene revealed 97.4% nucleotide sequence identity to the *dfrK* genes, and the deduced protein showed 95.7% amino acid identity to the DfrK proteins known from staphylococcal plasmids pKKS2187, pKKS825, and pKKS25 (6–8). The sequences up- and downstream of the *dfrK* gene in Tn*559* revealed two areas of ho-

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TABLE 1. PCR primers used in this study

Primer designation	Primer sequence (5′→3′)	Annealing temp (°C)	Amplicon size (in bp)	Reference
blaZ_fw	TGCTTCAACTTCAAAAGCGATA	55	563	This study
blaZ_rv	GTTCAGATTGGCCCTTAGGA			
dfrK_fw	GCTGCGATGGATAAGAACAG	50	214	3
dfrK_rv	GGACGATTTCACAACCATTAAAGC			
dfrK_inv1	CGGAAGAGCATTACCTGGAA	55	3,642	This study
dfrK_inv2	AATTGGATATCCCTTTGTAGTATTTTT			
Tn559_circ-fw	TCCATGAACTCGTACAGCAA	55	778	This study
Tn559_circ-rv	TGGTTGTGAAATTGTCCATTC			
radC_fw	GGAAAGGATGGGGAGAAGAG	55	2,305	This study
tnpB_rv	TGCTTCAATTTCCACTCTCG			

mology which might have served for recombination of a plasmid-borne *dfrK* gene region with a Tn*554* transposon (Fig. 2). Such a recombination resulted in the replacement of the former resistance gene region, consisting of the rRNA methylase gene *erm*(A), the spectinomycin resistance gene *spc*, and an open reading frame (ORF) of unknown function, by the *dfrK* gene.

As previously reported for Tn*554* (14–17) and other members of this transposon family, such as Tn*5406* (4) and Tn*558* (10), Tn*559* also neither contains inverted repeats at its ends nor generates a duplication of the target sequence at the integration site. Tn*559* exhibited the hexanucleotide sequence 5′-GATGTA-3′ at the left-end junction and the sequence 5′-CAAGTT-3′ at the right-end junction. Studies of serial transposition of Tn*554* into novel target sites revealed that the sequences at the junctions of Tn*554* varied with respect to the target sites: with each new transposition event, the sequence originally present in the target site is found at the left end of Tn*554*, whereas the former left-end junction is now found at the right end and the former right-end junction is lost (14, 15, 17). Since transposition of Tn*554* and its relatives includes the formation of circular forms which precede the integration of the transposon into a new target sequence (4, 14), inverse PCR assays using the primers Tn559_circ-fw and Tn559_circ-rv (Table 1) were conducted to detect such circular Tn*559* intermediates. Amplicons of the expected size were obtained in repeated experiments from *S. aureus* 2171 and sequenced completely. In agreement with the transposition model (14, 15), this amplicon consisted of 322 bp of *tnpA* and its upstream region including the 6-bp core sequence 5′-GATGTA-3′ at the left end of Tn*559*, while the remaining 456 bp represented part of the *dfrK* gene and the right end of Tn*559* up to—but not including—the sequence 5′-CAAGTT-3′. Evi-

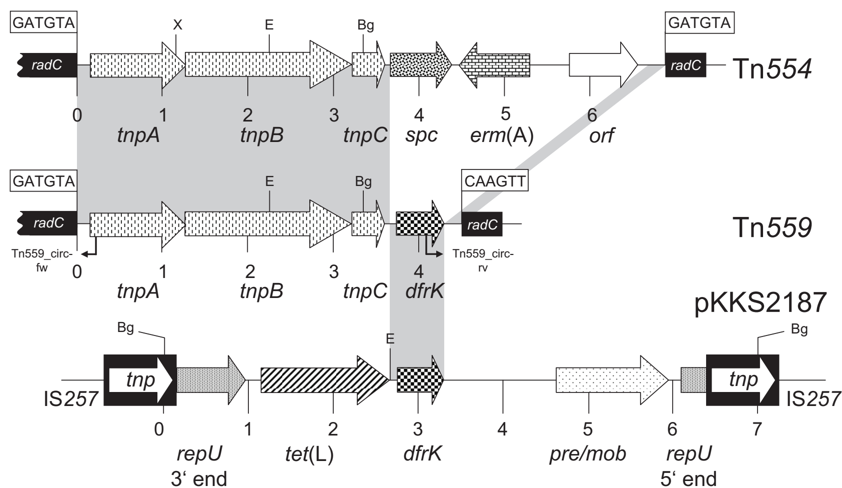

FIG. 1. Comparison of transposon Tn*559* (FN677369) with transposon Tn*554* (X03216) and the resistance gene region of plasmid pKKS2187 (FM207105). A distance scale in kb is given below each map. The position and orientation of the genes coding for transposition functions (*tnpA*, *tnpB*, and *tnpC*), antimicrobial resistance [*erm*(A), resistance to macrolides, lincosamides, and streptogramin B antibiotics; *spc*, spectinomycin resistance; *dfrK*, resistance to trimethoprim], plasmid replication (*repU*), plasmid recombination/mobilization (*pre*/*mob*), or unknown functions (*orf*) are indicated by arrows with the direction of transcription being shown by the arrowhead. The IS*257* elements are shown as black boxes with the white arrow indicating the transposase gene *tnp*. Restriction endonuclease cleavage sites are abbreviated as follows: Bg (BglII), E (EcoRI), and X (XhoI). The positions of primers used for the detection of circular Tn*559* forms are labeled Tn559_circ-fw and Tn559_circ-rv and indicated by arrows. The hexanucleotide sequences at the transposon junctions of the two transposons are shown in boxes. The regions of ≥97% nucleotide sequence identity between Tn*554* and Tn*559* as well as between Tn*559* and pKKS2187 are marked by gray shading. The *radC* gene sequences up- and downstream of Tn*554* and Tn*559* are shown as black boxes. It should be noted that the *radC* sequence determined in *S. aureus* 2171 corresponds exactly to the primary target sequence of Tn*554* known as *att554* (14, 15).

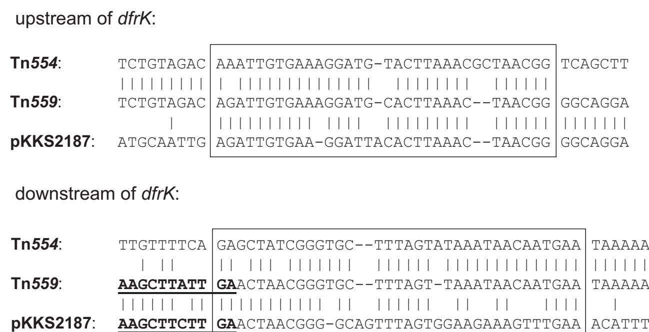

FIG. 2. Potential sites used for the development of Tn*559* via recombination between a *dfrK*-carrying plasmid and a Tn*554*-like transposon. Vertical bars indicate identical bases compared to the Tn*559* sequence. The recombination sites, where crossover is believed to have occurred, are boxed. The sequence displayed in bold underlined type in Tn*559* and pKKS2187 represents the 3′ end of the *dfrK* gene.

dence of the presence of circular Tn*559* forms suggested the functional activity of this transposon in *S. aureus* 2171.

Nucleotide sequence accession number. The sequence of Tn*559* and its flanking regions has been deposited in the EMBL database under accession number FN677369.

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REFERENCES

1. **Bastos, M. C., and E. Murphy.** 1988. Transposon Tn*554* encodes three products required for transposition. EMBO J. **7:**2935–2941.
2. **CLSI.** 2008. Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals; approved standard, 3rd ed. CLSI document M31-A3. CLSI, Wayne, PA.
3. **Fessler, A., C. Scott, K. Kadlec, R. Ehricht, S. Monecke, and S. Schwarz.** 2010. Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. J. Antimicrob. Chemother. **65:**619–625.
4. **Haroche, J., J. Allignet, and N. El Solh.** 2002. Tn*5406*, a new staphylococcal transposon conferring resistance to streptogramin A and related compounds including dalfopristin. Antimicrob. Agents Chemother. **46:**2337–2343.
5. **Kadlec, K., R. Ehricht, S. Monecke, U. Steinacker, H. Kaspar, J. Mankertz, and S. Schwarz.** 2009. Diversity of antimicrobial resistance pheno- and genotypes of methicillin-resistant *Staphylococcus aureus* ST398 from diseased swine. J. Antimicrob. Chemother. **64:**1156–1164.
6. **Kadlec, K., and S. Schwarz.** 2009. Identification of a novel trimethoprim resistance gene, *dfrK*, in a methicillin-resistant *Staphylococcus aureus* ST398 strain and its physical linkage to the tetracycline resistance gene *tet*(L). Antimicrob. Agents Chemother. **53:**776–778.
7. **Kadlec, K., and S. Schwarz.** 2009. Identification of a novel ABC transporter gene, *vga*(C), located on a multiresistance plasmid from a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. Antimicrob. Agents Chemother. **53:**3589–3591.
8. **Kadlec, K., and S. Schwarz.** 2010. Identification of a plasmid-borne resistance gene cluster comprising the resistance genes *erm*(T), *dfrK*, and *tet*(L) in a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. Antimicrob. Agents Chemother. **54:**915–918.
9. **Kehrenberg, C., C. Cuny, B. Strommenger, S. Schwarz, and W. Witte.** 2009. Methicillin-resistant and -susceptible *Staphylococcus aureus* strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene *cfr*. Antimicrob. Agents Chemother. **53:**779–781.
10. **Kehrenberg, C., and S. Schwarz.** 2005. Florfenicol-chloramphenicol exporter gene *fexA* is part of the novel transposon Tn*558*. Antimicrob. Agents Chemother. **49:**813–815.
11. **Kondo, Y., T. Ito, X. X. Ma, S. Watanabe, B. N. Kreiswirth, J. Etienne, and K. Hiramatsu.** 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob. Agents Chemother. **51:**264–274.
12. **Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu.** 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet **357:**1225–1240.
13. **Lüthje, P., and S. Schwarz.** 2006. Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide-lincosamide resistance phenotypes and genotypes. J. Antimicrob. Chemother. **57:**966–969.
14. **Murphy, E.** 1990. Properties of the site-specific transposable element Tn*554*, p. 123–135. *In* R. P. Novick (ed.), Molecular biology of the staphylococci. VCH Publishers, New York, NY.
15. **Murphy, E.** 1989. Transposable elements in gram-positive bacteria, p. 269–288. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, DC.
16. **Murphy, E., L. Huwyler, and M. C. F. Bastos.** 1985. Transposon Tn*554*: complete nucleotide sequence and isolation of transposition-defective and antibiotic-sensitive mutants. EMBO J. **4:**3357–3365.
17. **Murphy, E., and S. Löfdahl.** 1984. Transposition of Tn*554* does not generate a target duplication. Nature **307:**292–294.
18. **Roberts, M. C., Y. Pang, D. E. Riley, S. L. Hillier, R. C. Berger, and J. N. Krieger.** 1993. Detection of Tet M and Tet O tetracycline resistance genes by polymerase chain reaction. Mol. Cell. Probes **7:**387–393.
19. **Schwarz, S., E. Alešík, C. Werckenthin, M. Grobbel, A. Lübke-Becker, L. H. Wieler, and J. Wallmann.** 2007. Antimicrobial susceptibility of coagulase-positive and coagulase-variable staphylococci from various indications of swine, dogs and cats as determined in the BfT-GermVet monitoring program 2004–2006. Berl. Münch. Tierärztl. Wochenschr. **120:**372–379.