

First report of class 2 integron in clinical *Enterococcus faecalis* and class 1 integron in *Enterococcus faecium* in South China

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Abstract

During 2003 to 2004, class 1 integron was detected in 8 out of 10 tested enterococci isolates, with 2 of them positive for class 2 integron. This is the first report of class 2 integron in *Enterococcus faecalis* and class 1 integron in *Enterococcus faecium*.

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Enterococci are members of the normal intestinal flora and emerged as a major cause of nosocomial infections, which exhibit intrinsic resistance to several antimicrobial agents and the propensity to acquire antibiotic resistance rapidly. Integrons have been newly regarded as substantial contributors to the spread of antibiotic resistance genes (Mazel, 2006). Class 1 integron has been identified as a primary source of resistance genes within Gram-negative and Gram-positive bacteria (Shi et al., 2006; Xu et al., 2007, 2008a, 2008b). Conversely, class 2 integron has only been observed in a few Gram-negative organisms (Van Essen-Zandbergen et al., 2007; Xu et al., 2009).

The first evidence of the integron-related gene, *aadA*, was found in *Enterococcus faecalis* strain W4470 (Clark et al., 1999). Our preliminary study conducted at the First Affiliated Hospital of Jinan University (FAHJU) demonstrated an unexpected spread of class 1 integrons to Gram-

positive organism, among which were 5 *E. faecalis* strains. In this study, we report class 1 and 2 integron-positive enterococci strains sampled from FAHJU during 2003 to 2004.

Enterococci strains were identified as *E. faecalis* and *Enterococcus faecium* by API Strep strip (API systems SA, La Balme Les Grottes, France) and Vitek 2 automated system (bioMérieux, Marcy, l'Etoile, France). Antimicrobial susceptibility testing was performed by standard disk diffusion method (Clinical and Laboratory Standards Institute, 2005). Detection and characterization of class 1 and 2 integrons were performed as described previously (Su et al., 2006; Xu et al., 2009). Briefly, polymerase chain reaction products of the variable region were characterized by restriction fragment length polymorphism and further confirmed by sequencing.

Ten tested enterococci strains were positive for class 1 integrase and 3'-conserved region of *qacEΔI-sulI*. Three different types of gene cassette arrays were found in 8 strains, with no amplicon of the variable region obtained in 2 isolates (Table 1). When the *aadA* gene was compared

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Table 1
Clinical data and phenotypic characteristics of 15 enterococci isolates during 2001 to 2004

| Strain no. | Species | Age and sex | Department ^a | Isolated date | Source | Class 1 integron | | Class 2 integron | | Resistance profile ^b |
|---------------------|--------------------|-------------|-------------------------|---------------|----------|------------------|--|------------------|-------------------------|---------------------------------|
| | | | | | | <i>Int11</i> | Cassettes | <i>Int12</i> | Cassettes | |
| 011042 ^c | <i>E. faecalis</i> | 65, F | IM | 2001 | Urine | + | <i>dfrA12-orfF-aadA2</i> | - | - | CfChGeLvSmSpTc |
| 011056 ^c | <i>E. faecalis</i> | 74, M | IM | 2001 | Urine | + | <i>dfrA12-orfF-aadA2</i> | - | - | CfChClEmGeLvPcSmSpStTc |
| 011059 ^c | <i>E. faecalis</i> | 74, M | IM | 2001 | Urine | + | <i>dfrA12-orfF-aadA2</i> | - | - | CfEmGeLvPcSmSt |
| 011021 ^c | <i>E. faecalis</i> | 38, M | Sur | 2001 | Blood | + | <i>dfrA12-orfF-aadA2</i> | - | - | ClEmLvSpSt |
| 021225 ^c | <i>E. faecalis</i> | 74, F | IM | 2002 | Sputum | + | <i>dfrA12-orfF-aadA2</i> | - | - | CfChClEmLvPcSpStTc |
| 032111 | <i>E. faecalis</i> | 61, F | Sur | 2003 | Urine | + | | - | - | EmPcTc |
| 032251 | <i>E. faecalis</i> | 63, M | Sur | 2003 | Blood | + | <i>dfrA12-orfF-aadA2</i> | - | - | CfChClEmGeLvPcSmSpStTc |
| 032438 | <i>E. faecalis</i> | 78, F | Gen | 2003 | Pus | + | | - | - | EmPcTc |
| 042474 | <i>E. faecalis</i> | 59, F | Sur | 2004 | Perineum | + | <i>dfrA12-orfF-aadA2</i> | - | - | CfEmGeLvPcSmSpStTc |
| 042833 | <i>E. faecalis</i> | 69, M | Gen | 2004 | Pus | + | <i>dfrA12-orfF-aadA2</i> | - | - | CfEmGeLvPcSmSpTc |
| 042925 | <i>E. faecalis</i> | 58, M | IM | 2004 | Blood | + | <i>dfrA17-aadA5</i> | + | <i>dfrA1-sat1-aadA1</i> | CfChClEmGeLvPcSmSpStTc |
| 042926 | <i>E. faecalis</i> | 53, M | IM | 2004 | Blood | + | <i>dfrA17-aadA5</i> | + | <i>dfrA1-sat1-aadA1</i> | CfChClEmGeLvPcSmSpStTc |
| 042952 | <i>E. faecalis</i> | 3, M | Ped | 2004 | Urine | + | <i>dfrA12-orfF-aadA2</i> ; <i>aadA2</i> | - | - | CfClEmGeLvPcSmSpStTc |
| 042668 | <i>E. faecium</i> | 51, M | IM | 2004 | Pus | + | <i>dfrA12-orfF-aadA2</i> | - | - | AmCfEmGeLvPcSmSpTc |
| 042910 | <i>E. faecium</i> | 59, M | Sur | 2004 | Pus | + | <i>dfrA12-orfF-aadA2</i> | - | - | AmCfEmGeLvPcSmSpSt |

^a IM = internal medicine; Sur = surgery; Ped = pediatrics; Gen = general ward.

^b Antibiotics used included ampicillin (Am), ciprofloxacin (Cf), chloramphenicol (Ch), clindamycin (Cl), erythromycin (Em), gentamicin (Ge), levofloxacin (Lv), penicillin (Pc), streptomycin (Sm), spectinomycin (Sp), teicoplanin, tetracycline (Tc), trimethoprim-sulfamethoxazole (St) and vancomycin.

^c Included in the preliminary study (Shi et al., 2006).

to that from *E. faecalis* strain W4770 (Clark et al., 1999), homology of nucleotides and amino acids approached 88.1% and 83.8%, respectively. Class 2 integrons with *dfrA1-sat1-aadA1* were discovered in 2 *E. faecalis* strains, the sequence of which was 99.8% and 99.7% homologous

to that from *Escherichia coli* and *Pseudomonas aeruginosa* in preliminary investigations (Su et al., 2006; Xu et al., 2009). High homology was observed when the entire enterococcal sequences were compared with integron sequences from isolates sampled in the same hospital

Table 2
Comparison of integron sequences from enterococci and other species in the same hospital setting

| Enterococci | Other organisms | Homology | GenBank no. | Reference |
|--------------------------|------------------------------------|--|---------------|--------------------------|
| Class 1 integron | | | | |
| <i>dfrA12-orfF-aadA2</i> | <i>Staphylococcus epidermidis</i> | 99.8% homology with 398 G-A, 458 G-A, 984 G-T, and 1603 A-G | AB297447 | Xu et al. (2008a, 2008b) |
| | <i>Staphylococcus hominis</i> | 99.8% homology with 398 G-A, 458 G-A, 984 G-T, and 1603 A-G | AB297448 | Xu et al. (2008a, 2008b) |
| | <i>Staphylococcus haemolyticus</i> | 99.7% homology with 398 G-A, 458 G-A, 860 C-T, 984 G-T, and 1603 A-G | AB297449 | Xu et al. (2008a, 2008b) |
| | <i>Staphylococcus warneri</i> | 99.8% homology with 398 G-A, 458 G-A, 984 G-T, and 1603 A-G | AB297450 | Xu et al. (2008a, 2008b) |
| | <i>S. aureus</i> | 99.8% homology with 398 G-A, 458 G-A, 984 G-T, and 1603 A-G | AB191048 | Shi et al. (2006) |
| | <i>S. aureus</i> | 99.8% homology with 398 G-A, 458 G-A, 984 G-T, and 1603 A-G | AB481129 | None |
| <i>dfrA17-aadA5</i> | <i>S. aureus</i> | 99.9% homology with 1289 C-T | AB481128 | None |
| | <i>S. epidermidis</i> | 100% homology | AB291061 | Xu et al. (2008a, 2008b) |
| | <i>S. hominis</i> | 99.9% homology with 1289 C-T | AB291062 | Xu et al. (2008a, 2008b) |
| <i>aadA2</i> | <i>E. coli</i> | 100% homology | AB189264 | Su et al. (2006) |
| | <i>S. aureus</i> | 99.5% homology with 217 T-C, 322 A-C, 586 A-G, 717 T-G, and 857 G-A | AB481131 | None |
| | <i>S. epidermidis</i> | 99.5% homology with 217 T-C, 322 A-C, 586 A-G, 717 T-G, and 857 G-A | AB291063 | Xu et al. (2008a, 2008b) |
| Class 2 integron | | | | |
| <i>dfrA1-sat1-aadA1</i> | <i>E. coli</i> | 99.8% homology with 902 G-A, 2219 A-G, and 2285 C-T | AB211124 | Su et al. (2006) |
| | <i>P. aeruginosa</i> | 99.7% homology with 902 G-A, 910 A-C, 2075 A-T, 2219 A-G, and 2285 C-T | Not deposited | Xu et al. (2009) |

setting (Table 2). It is noteworthy that in enterococci strains, prevalent array cassettes such as *dfrA12-orfF-aadA2*, *aadA2*, and *dfrA17-aadA5*, also dominated in other species in FAHJU (Shi et al., 2006; Su et al., 2006; Xu et al., 2007, 2008a, 2008b, 2009). The observance of identical integrons in genomes of phylogenetically distant bacteria and the similarity of prevalent arrays in diverse clinical organisms strongly suggest intergeneric horizontal transfer of genetic cassettes in the hospital setting. Several classes of integrons spread among Gram-negative bacteria, with 1 example being the transfer of class 1 integron via plasmid between *E. faecalis* (Clark et al., 1999). However, horizontal transfer and dissemination of integrons between Gram-positive and Gram-negative organisms still remained unclear and required further investigation.

This is the first evidence of class 2 integron in *E. faecalis* and class 1 integron in *E. faecium*, representing the first detection of class 2 integron outside of the Gram-negative organisms. Furthermore, it is also the first identification of clinical *E. faecalis* carrying class 1 and 2 integrons simultaneously.

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