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Mobile genetic elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*

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Abstract

Mobile genetic elements (MGEs) including plasmids and transposons are pivotal in the dissemination and persistence of antimicrobial resistance in *Enterococcus faecalis* and *Enterococcus faecium*. Enterococcal MGEs have also been shown to be able to transfer resistance determinants to more pathogenic bacteria such as *Staphylococcus aureus*. Despite their importance, we have a limited knowledge about the prevalence, distribution and genetic content of specific MGEs in enterococcal populations. Molecular epidemiological studies of enterococcal MGEs have been hampered by the lack of standardized molecular typing methods and relevant genome information. This review focuses on recent developments in the detection of MGEs and their contribution to the spread of antimicrobial resistance in clinically relevant enterococci.

Keywords: antimicrobial resistance, enterococcus, insertion sequence, mobile genetic elements, plasmid, review, transposon

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Introduction

Enterococci are important nosocomial pathogens [1]. They are uniquely armed for the antibiotic era and express intrinsic reduced susceptibility to major classes of antimicrobial agents and biocides [2,3]. The high propensity of enterococci to acquire and express new resistance determinants further enhances their ability to sustain antibiotic selection, promoting gastrointestinal colonization and nosocomial infections by antibiotic-resistant enterococci [4].

Transferable antimicrobial resistance in enterococci was first described in the early 1970s [5–7]. The detection and molecular clarification of transferable high-level vancomycin resistance in *Enterococcus faecium* in the late 1980s have further fuelled our interest in the mechanisms and routes to antimicrobial resistance in enterococci [8–11]. Importantly, as residents of human and animal bowel flora, enterococci are in a position to acquire resistance genes from other commensals, which may subsequently proceed to other more pathogenic bacteria [12,13].

Molecular biological studies have elucidated complex functional properties of important mobile genetics elements (MGEs) involved in transferable resistance in enterococci [10,14,15]. However, there is a considerable gap in our knowledge on the molecular epidemiology of MGEs, their genetic content and composition, as well as their relative contributions to the spread of defined antimicrobial resistances. Recent progress in molecular typing methods and enterococcal genome information has provided new tools and necessary insights for filling this gap. This review focuses on MGEs involved in the spread and expression of clinically important antimicrobial resistance in enterococci and their potential contribution to the spread of hospital-adapted clonal lineages of *E. faecium* and *Enterococcus faecalis*.

Plasmids as Important Vehicles for Genetic Information in Enterococci

By definition, plasmids are semi-autonomously replicating extrachromosomal genetic elements. Differences in replica-

tion strategies and modular structures profoundly affect plasmid properties, such as size, copy number, host dependence and host range [16]. The essential backbones for successful plasmids include genetic modules encoding self-replication, stable inheritance and the ability to transfer between bacteria. Accessory plasmid content is integrated in between functional plasmid backbone modules and represents a huge reservoir of genetic variability, often with unknown functions, that is shared among different bacterial genera through horizontal gene transfer.

There are several criteria to classify plasmids in general and plasmids related to Gram-postive bacteria in particular. The mode of replication has been used to distinguish rolling circle replication plasmids and theta-replicating plasmids. In addition, plasmids that fail to co-reside in the same cell are grouped in incompatibility (Inc) groups [17,18]. Inc18 plasmids constitute a large group of enterococcal/streptococcal plasmids with a broad host range [19,20]. Pheromone-responsive plasmids represent a unique group of self-transferable (conjugative) narrow host range plasmids mostly described in *E. faecalis* [21]. pAD1, pAM373 and pCF10 are well-known examples of pheromone-responsive plasmids, where the conjugative process is initiated as a response to short peptide pheromones produced by pheromone-responsive plasmid-free recipient strains mediating intercellular aggregation and high-frequency DNA transfer. Recently, Weaver *et al.* [22] proposed a new family (RepA_N) of broadly distributed plasmids in Gram-positive bacteria encompassing pheromone-responsive plasmids of *E. faecalis*, as well as pRUM of *E. faecium*. Detailed sequence comparisons of the replication initiator protein suggest that the replicons have evolved along with their specific host, explaining their relatively narrow host range.

Plasmid replicon modules (replicons) have recently been used as targets of more simplistic methods for typing and epidemiological tracing of plasmids conferring antimicrobial resistance (R-plasmids). Other essential gene sets for plasmid survival, such as mobilization regions, have also been suggested as targets [23]. Given the modular evolution and genetic plasticity of plasmids, it is of note that schemes based on different core elements may not be congruent [16]. Carattoli *et al.* [18] developed a PCR-based plasmid typing method based on the replication regions from various plasmid incompatibility groups occurring in Enterobacteriaceae. A similar approach was recently described for the detection of plasmids from enterococci and other Gram-positive bacteria [24]. On the basis of 111 published sequences from Gram-positive bacteria, 19 replicon families (*rep*-family) and several unique replicons were identified. Using this PCR-based typing system, pCF10 (rep_9)-, Inc18 (rep_1 and rep_2)- and pUSA02 (rep_7)-related replicons were identified as being most prevalent in *E. faecalis* strains (n = 28), whereas Inc18 (rep_2)-, pRI1 (rep_{14})- and pRUM (rep_{17})-related replicons dominated in *E. faecium* strains (n = 51) of human and animal origin [24]. However, approximately 30% of the strains tested did not support any *rep*-detection, indicating the presence of unidentified *rep*-types.

The enterococcal-specific parts of the *rep*-detection system described by Jensen *et al.* [24] have been used by others. A recent study of an epidemiologically diverse collection of *E. faecium* strains (n = 93) revealed a high prevalence of Inc18 (rep_2)-, pRUM (rep_{17})- and pHTβ (rep_{unique})-related replicons [25]. The actual enterococcal typing scheme accounted for approximately 60% of the total number of plasmids visualized by S1-nuclease analyses. Interestingly, strains belonging to hospital-adapted clades (CC17-related) yielded a significant higher number of *rep* types and pRUM (rep_{17})-related replicons in particular, indicating a role in accessory plasmid DNA for promoting hospital adaptation. *Rep*-typing of extended *E. faecalis* strain collections has so far shown a dominance of pheromone-responsive plasmid ($rep_{8\ and\ 9}$)-, pS86 (rep_6)- and Inc18 ($rep_{1\ and\ 2}$)-related replicons (J. Sun, S. Xiaobo, T. Mikalsen, J. U. Ericson Sollid, A. Sundsfjord, unpublished observations). Other comprehensive studies include vancomycin-resistant *E. faecium* and *E. faecalis* strains causing hospital outbreaks in five continents, from 1986 to date (A. R. Freitas, M. V. Francia, L. Peixe, C. Novais, L. B. Jensen, R. J. Willems, F. Baquero, T. M. Coque, unpublished observations). Among *E. faecium*, mostly CC17-related, a high diversity of *rep* types could be identified; small [pB82 (rep_{11}), pRI1 (rep_{14}), pEF418 (rep_{18}), pCIZ2 (rep_{unique})] or medium to large plasmids [Inc18 ($rep_{1\ and\ 2}$), pRUM (rep_{17}), pHTβ (rep_{unique})], with *vanA* linked to Inc18- and pRUM-like plasmids in most cases. Vancomycin-resistant *E. faecalis* isolates belonging to major clonal complexes (CC2, CC9 and CC87) contained a lower diversity of plasmids, which were mostly associated with the narrow host pheromone-responsive pAD1 (rep_{9}) and Inc18-type ($rep_{1\ and\ 2}$) plasmids.

Linkage of clinically important resistance determinants to specific replicon types in enterococci is of interest for predicting potential transfer to other bacterial genera by conjugative broad host range plasmids. The application of pulsed-field gel electrophoresis of S1-nuclease-digested enterococcal DNA has proved very useful for the identification and sizing of enterococcal plasmids because they appear as linearized bands (5–400 kb) on a faint genomic background [25–27]. Physical linkage between defined plasmid *rep* types and resistance determinants can be visualized by co-hybridization analysis of linearized plasmid DNAs [25]. Co-hybridization