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Mini review

Antibiotic resistant enterococci—Tales of a drug resistance gene trafficker

Guido Werner[a,*], Teresa M. Coque[b], Charles M.A.P. Franz[c], Elisabeth Grohmann[d], Kristin Hegstad[e,f], Lars Jensen[g], Willem van Schaik[h], Keith Weaver[i]

[a] National Reference Centre for Stapyhlococci and Enterococci, Division of Nosocomial Pathogens and Antibiotic Resistances, Robert Koch Institute, Wernigerode Branch, Burgstr. 37, 38855 Wernigerode, Germany
[b] Servicio de Microbiología, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Unidad de Resistencia a Antibióticos y Virulencia Bacteriana asociada al CSIC, Madrid, Spain
[c] Max Rubner-Institut, Department of Safety and Quality of Fruit and Vegetables, Karlsruhe, Germany
[d] Division of Infectious Diseases, University Medical Centre Freiburg, Freiburg, Germany
[e] Reference Centre for Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway
[f] Research Group for Host-Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway
[g] Division of Microbiology and Risk Assessment DTU Food, Technical University of Denmark, National Food Institute, Søborg/Lyngby, Denmark
[h] Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands
[i] Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, SD 57069, USA

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ABSTRACT

Enterococci have been recognized as important hospital-acquired pathogens in recent years, and isolates of *E. faecalis* and *E. faecium* are the third- to fourth-most prevalent nosocomial pathogen worldwide. Acquired resistances, especially against penicilin/ampicillin, aminoglycosides (high-level) and glycopeptides are therapeutically important and reported in increasing numbers. On the other hand, isolates of *E. faecalis* and *E. faecium* are commensals of the intestines of humans, many vertebrate and invertebrate animals and may also constitute an active part of the plant flora. Certain enterococcal isolates are used as starter cultures or supplements in food fermentation and food preservation. Due to their preferred intestinal habitat, their wide occurrence, robustness and ease of cultivation, enterococci are used as indicators for fecal pollution assessing hygiene standards for fresh- and bathing water and they serve as important key indicator bacteria for various veterinary and human resistance surveillance systems. Enterococci are widely prevalent and genetically capable of acquiring, conserving and disseminating genetic traits including resistance determinants among enterococci and related Gram-positive bacteria. In the present review we aimed at summarizing recent advances in the current understanding of the population biology of enterococci, the role mobile genetic elements including plasmids play in shaping the population structure and spreading resistance. We explain how these elements could be classified and discuss mechanisms of plasmid transfer and regulation and the role and cross-talk of enterococcal isolates from food and food animals to humans.

Introduction

Enterococci have constituted a unique taxonomic entity since the mid-1980s when results of DNA–DNA hybridization experiments suggested their separation into a new bacterial genus called *Enterococcus* spp. from the former genus *Streptococcus* spp. (Schleifer and Kilpper Balz, 1987). Enterococci display a number of characteristics shared with some or all Streptococcus species such as a number of type-specific enzyme activities (LAP, leucine aminopeptidase), hemolysis (alpha, beta) on blood agar plates and production of a Lancefield group D antigen (shared with *S. bovis*) (Kohler, 2007; Murray, 1990). In contrast, a number of key reactions are typical for members of the genus *Enterococcus* only, such as PYRase activity, growth at 6.5% NaCl and at high gall concentrations and temperatures. Whereas most prominent streptococcal species have a nasopharyngeal, skin or mucosal habitat, enterococcal species, such as *E. faecalis* and *E. faecium* have a primarily intestinal reservoir in humans, many vertebrate and invertebrate animals and may also constitute an active part of the vegetative flora (not only as a fecal pollutant). A number of enterococcal isolates have a long standing tradition as starter cultures or supplements in food fermentation and food preservation, the latter associated with their production of bacteriocins (Franz et al., 2007, 2011; Hammerum et al., 2010). A pharmaceutical preparation

* Corresponding author. Tel.: +49 3943 679 210; fax: +49 3943 679 207 317.
E-mail address: wernerg@rki.de (G. Werner).

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designated "Symbioflor" contains a specific *E. faecalis* strain licensed as a probiotic to strengthen the host's immune system and prevent chronic diseases (Domann et al., 2007).

Due to their preferred intestinal habitat, their wide occurrence, robustness and ease of cultivation, enterococci are used as indicators for fecal pollution assessing hygiene standards for fresh- and bathing water (Garcia et al., 2007; Martins da Costa et al., 2006). In addition, enterococci serve as important key indicator bacteria for various veterinary and human resistance surveillance systems (DANMAP, SWEDRES). Enterococci have been recognized as important hospital-acquired pathogens for many years. Isolates of *E. faecalis* and *E. faecium* are the third- to fourth-most prevalent nosocomial pathogen worldwide (ECDC, 2011). Acquired resistances, especially against penicillin/ampicillin, aminoglycosides (high-level) and glycopeptides are reported in increasing numbers and limit the therapeutic spectrum tremendously. Therapeutic alternatives to treat infections with multi- and vancomycin-resistant enterococci (VRE) are limited to antibiotics of last resort (quinupristin/dalfopristin, linezolid, tigecycline, daptomycin). However, they are only approved for certain indications, and resistances to those drugs have already been reported (Arias et al., 2011; Werner et al., 2002, 2008b).

From a medical perspective acquired resistance to glycopeptides (vancomycin/teicoplanin; genotypes *vanA-N*) is the key resistance trait in enterococci, as is the case for methicillin resistance in *S. aureus* (Courvalin, 2006; Werner, 2012). Vancomycin resistance is the resistance trait assessed in many surveillance schemes targeted at multidrug-resistant nosocomial pathogens. Infection control and prevention programmes as well as intervention measures aim at reducing especially VRE frequencies (but less enterococcal infections in general) in health care settings. Vancomycin resistance is mediated via different mechanisms; the so-called VanA but also the VanB resistance types are by far the most prevalent in Europe. The reservoir of transferable *vanA*-type (and partly *vanB*-type) resistance in human medicine and other habitats is *E. faecium*. Consequently, increasing rates of VRE in several European countries are due to an increasing prevalence of VR-*E. faecium*. Ampicillin- and/or vancomycin-resistant *E. faecalis* are still rare. Other therapeutically important chemotherapeutics include penicillins and aminoglycosides such as gentamicin and streptomycin showing a synergistic effect in combination with cell-wall active substances such as ampicillin and/or glycopeptides (Murray, 1990).

Strains of *E. faecium* show a certain level of host association (Willems et al., 2011). *E. faecium* strains from distinct ecological backgrounds such as poultry, calf and cattle grouped into clonal complexes as based on DNA sequence typing (MLST) and phylogenetic analyses (eBURST). In contrast, colonizing strains from humans and swine showed similar MLST types and grouped into a single large clonal complex. Defined clonal groups of *E. faecium* and *E. faecalis* show an enhanced capacity to disseminate in the nosocomial setting and are thus called epidemic, hospital-acquired or High Risk enterococcal clonal complexes (HiRECC) (van Schaik and Willems, 2010; Willems et al., 2011). These strains can be grouped to distinct clonal complexes, in *E. faecium* formerly known as clonal complex CC17 (see chapter 1).

Phylogenetic analysis of a larger set of *E. faecalis* and *E. faecium* strains revealed a comparably high rate of recombination and horizontal gene transfer among these two species suggesting that genomic variability appears at a population level less by SNP mutations and more by horizontal gene spread and recombination events. However, this may be limited within single clonal complexes and subgroups below the species level, as shown recently for hospital-associated strain types (Willems et al., 2012). Thus, lateral gene transfer has a great impact on the genomic constitution and composition of the genus *Enterococcus*, particularly in *E. faecalis* and *E. faecium*. This genus is not similarly naturally competent as some streptococcal species are. Knowledge about phages in *Enterococcus* is very limited and only a few studies described phages and phage transduction in *Enterococcus* (see chapter 2). As far as we know now, genetic exchange among enterococci is mainly realized by conjugative gene transfer, relying either on conjugative and mobilizable plasmids or by composite and conjugative chromosomal elements (integrative and conjugative elements; "ICE").

A number of recent book chapters and review articles contain large tables and listings of resistance genes and mutations acquired, conserved and distributed by *Enterococcus* spp. strains and will thus not be listed here in detail. We mainly aimed at describing the corresponding mechanisms and carriers involved in making enterococci a "resistance gene trafficker" for Gram-positive bacteria.

Population biology and genomics of enterococci

Studies on the population structure of enterococci have been performed to discern whether structure can be identified among enterococcal populations that may be linked to certain ecological niches (Willems et al., 2011). Specifically, considerable research has been done to discern whether clinical isolates of *E. faecalis* and *E. faecium* are genetically distinct from strains that inhabit the intestinal tract of animals or healthy humans. Currently, sequence-based methods, particularly Multi Locus Sequence Typing (MLST) have become the standard for epidemiological studies on enterococci (Willems and van Schaik, 2009). In MLST schemes, fragments of housekeeping genes are sequenced and each sequence is assigned an allele, resulting in a 'barcode' consisting of seven allele numbers, which can be assigned a sequence type (ST). MLST data can be deposited in a freely accessible database and because of the large number of sequences that are available in these databases, the data allow a truly global view of the population structure of these bacteria (Maiden, 2006). While MLST has provided important insights into the population structure of *E. faecalis* and *E. faecium*, its resolution is inherently limited by the small number of housekeeping genes that are assayed. Consequently, MLST is currently being superseded by full genome sequencing which allows an analysis of bacterial population structure at an unsurpassed level of detail (van Schaik and Willems, 2010). Here we will discuss recent studies in which sequence-based methods (MLST and genome sequencing) were applied to obtain insights into the population structure of *E. faecalis* and *E. faecium*.

A total of three MLST schemes exist for *E. faecalis*, with the number of loci that are sequenced ranging from three to seven (Chowdhury et al., 2009; Nallapareddy et al., 2002; Ruiz-Garbajosa et al., 2006), with the scheme developed by Ruiz-Garbajosa et al. (2006) being the most widely applied. Genotyping by MLST showed that a relatively limited number of clonal complexes (a group of closely related STs) are responsible for the majority of clinical infections (Freitas et al., 2009; Kawalec et al., 2007; Kuch et al., 2012). These high-risk enterococcal clonal complexes (or HiRECCs), such as CC2, CC9, and CC87, are not exclusively limited to clinical settings as illustrated by studies in which strains belonging to these HiRECCs could also be identified in pigs (Freitas et al., 2011) and healthy babies (Solheim et al., 2009). A notable feature of *E. faecalis* is that antibiotic resistance and carriage of genes with a proposed role in virulence are not exclusively confined to clinical isolates but are also found in *E. faecalis* strains from a wide variety of niches (McBride et al., 2007; Solheim et al., 2009). However, a recent study into the gene content of CC2 *E. faecalis* showed that mobile genetic elements and genes encoding cell surface structure are overrepresented in this HiRECC (Solheim et al., 2011).

The first complete genome sequence of an *E. faecalis* strain was generated for strain V583, a vancomycin-resistant clinical isolate

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