Distribution of Four Capsular Serotypes of *Enterococcus faecalis* among Clinical Isolates from Different Geographical Origins and Infection Sites

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Abstract

Background: Enterococci possess capsular polysaccharide antigens that are the targets of opsonic antibodies. These antibodies are potential candidates for development as immunotherapy.

Material and Methods: The present study analyzes the distribution of four capsular serotypes within a collection of 157 isolates of *Enterococcus faecalis* from four countries with different sites of clinical infection.

Results: By using a capsular polysaccharide-specific ELISA, 42% of the isolates were grouped into one of four serogroups, and another 9% showed cross-reactivity between two serotype-specific sera. Heterogeneity of serotype distribution by both geographical origin and infection site was observed.

Conclusion: Half of the strain collection could be typed with four serotype-specific sera. No serotype from a given country or infection site clearly predominated.

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Introduction

Enterococci are a leading cause of nosocomial infections, especially in immuno-compromised patients, and are often untreatable because of bacterial resistance to multiple antibiotics [1]. Up to 25% of enterococcal hospital isolates are resistant to glycopeptide antibiotics [2]. When newer antibiotics, such as linezolid and quinupristin-dalfopristin, have been introduced, enterococci have developed resistance to these agents within a short time [3].

In 1999, *Huebner* et al. identified a capsular polysaccharide in a clinical *Enterococcus faecalis* isolate as well as in a vancomycin-resistant *Enterococcus faecium* strain. They showed this antigen to be a teichoic acid and the target of opsonic antibodies [4]. Antibodies raised to this antigen protected mice in an experimental model of systemic enterococcal infection [5], thereby demonstrating potential for the use of this antigen as an active and/or passive immunotherapy. Studying the diversity of capsular polysaccharides in a small collection of *E. faecalis* strains, *Hufnagel* et al. used four type-specific sera raised to polysaccharide antigen that had been extracted from cell walls of four *E. faecalis* prototype strains, named CPS-A to CPS-D [6]. They were able to assign 55% of the 29 strains tested in a capsular polysaccharide-specific ELISA to one of the four serotypes. These findings suggested that the number of *E. faecalis* capsular serotypes needing to be incorporated into a broadly active immunotherapeutic agent would be limited.

The current investigatory study explored the distribution of the four different serotypes in a larger collection of 157 clinical and laboratory *E. faecalis* isolates. These isolates were associated with different enterococcal infections from four countries. The aim of the study was to determine whether the four serotypes represent the majority of serotypes among the 157 isolates as well as whether any single prototype strain from either a country or a given clinical infection site could be shown to be predominant.

Materials and Methods

Strains, Culture, and Sera

In addition to the described 29 *E. faecalis* isolates [6] originating from the U.S. and Japan, other clinical isolates from patients in the U.S., Germany and Italy were used. Twenty-six of the isolates came from Brigham and Women's Hospital, Beth Israel/Deaconess Hospital, and Children's Hospital (all in Boston, MA, USA).

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Fifty-six isolates were from different regions of Germany (collected by the National Reference Center for Streptococci, Institute for Medical Microbiology, Aachen), and 46 isolates originated from various hospitals in Italy (collected by the Istituto Superiore di Sanità, Rome). Isolates were randomly collected over several years to prevent the inclusion of isolates from clonal outbreaks. Patient information was gathered from the laboratory request form. Urinary tract infections by enterococci were only included when isolated in single culture.

E. faecalis isolates were grown without agitation at 37°C for the capsular polysaccharide-specific ELISA (CPS-ELISA) in Columbia Broth (CB, Difco Laboratories, Detroit, MI, USA) with the addition of 0.5% glucose. Four type-specific rabbit sera, named αCPS-A to αCPS-D, were used as previously described [6].

CPS-ELISA

The capsular polysaccharide-specific ELISA described previously [6] was used to test all 157 *E. faecalis* isolates for their immunoreactivity with the four serotype-specific sera. Crude polysaccharide extracts were prepared by extraction with 10% trichloroacetic acid for 18 to 24 h at 4°C on a rotor rack. Each isolate was tested at least in duplicate, and positive and negative controls were included on all plates. In accordance with published results, an *E. faecalis* isolate was assigned to one of the four serotype-specific sera when the OD_{405} was at least 70% of the value obtained with the prototype strain [6].

Results

Distribution of Capsular Serotypes among 157 *E. faecalis* Isolates

The assignment criteria described above allowed for the unambiguous classification of 42% (66/157) of the isolates into one of the four serotypes: 3% (5/157) CPS-A, 20% (32/157) CPS-B, 10% (15/157) CPS-C, and 9% (14/157) CPS-D. An additional 13 isolates (8%) showed cross-reactivity with the CPS-C-specific and CPS-D-specific sera; one isolate reacted with both CPS-A-specific and CPS-C-specific sera; and 49% of the *E. faecalis* isolates (77/157) were not be able to be assigned to one of the four serotypes.

Distribution of Capsular Serotypes from Different Geographic Locations

The distribution of the different serotypes among isolates from four different countries (USA, Japan, Italy, and Germany) is shown in figure 1. All four serotypes were present in all four countries, but the geographical distribution of CPS-B and CPS-D strains showed differences. In comparison to their presence in the U.S. and Japan (9% and 10% of isolates, respectively), CPS-B strains were over-represented in Germany (30% of isolates) and Italy (22% of isolates). CPS-D strains were found more frequently among the Italian isolates (20%) than among those from the USA, Germany, and Japan (3%, 4%, and 10%, respectively).

Distribution of Capsular Serotypes from Different Infection Sites

For 107 of the isolates, information was available about the specimen from which the bacteria were originally isolated. Isolates were grouped into five categories: invasive infections (i.e., sepsis, meningitis and endocarditis) (n = 32, with eight endocarditis isolates); urinary/genital infections (n = 32); foreign-body infections (n = 16); wound infections (n = 16); and gallbladder infections (n = 11). The serotype distribution of the isolates with known infection sites (including invasive isolates) was identical to the overall distribution (Table 1). For foreign-body infections, CPS-B and CPS-D strains were over-represented in comparison to the overall distribution of isolates. In contrast to the percentage among all isolates, there were fewer non-typeable isolates among foreign-body infections. For

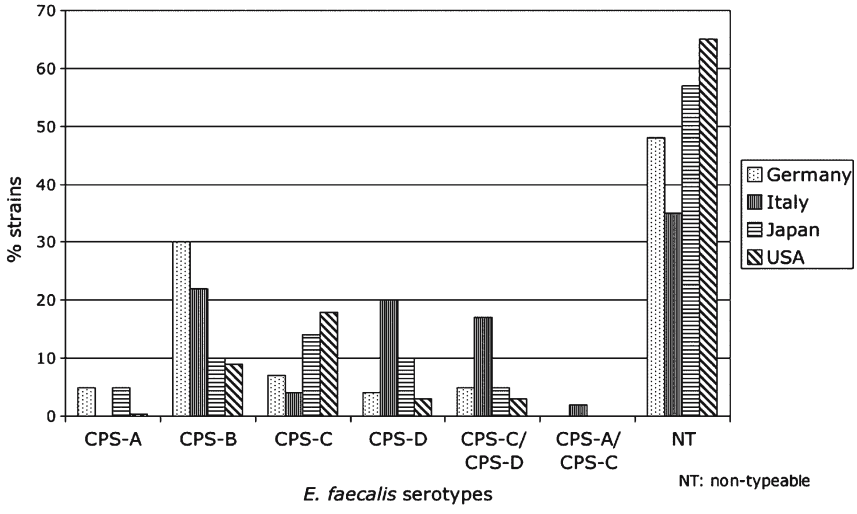

Figure 1. Geographic distribution of four serotypes among 157 *E. faecalis* isolates from four different countries.

Table 1
Distribution of 156 *E. faecalis* serotypes isolated from specific infection sites (one CPS-A/-C strain is not shown in this table).

Infection site	n	CPS-A	CPS-B	CPS-C	CPS-D	CPS-C/-D	NT
Foreign body infections	16	0	5(31%)	2	4(25%)	1	4(25%)
Gallbladder infections	11	0	0	0	1	4(36%)	6
Invasive infections	32	1	4	2	4	4	17
Urinary/genital infections	32	2	10(31%)	2	1	1	16
Wound/drainage isolates	16	0	5(31%)	0	1	1	9
All 107 strains with known infection site	107	3(3%)	24(22%)	6(6%)	11(10%)	11(10%)	52(49%)
All 156 strains	156	5(3%)	32(20%)	15(10%)	14(9%)	13(8%)	77(49%)

NT: non-typeable

gallbladder infections, only CPS-D strains and isolates with reactivity to both the CPS-C- and CPS-D-specific sera were found. Urinary/genital infections and wound isolates were more often associated with CPS-B strains.

Discussion

The data presented confirm previously published results, as obtained through the original panel of 29 *E. faecalis* strains [6]. While 55% of the 29 strains were classified into one of the four serotypes, one strain reacted with both the CPS-C-specific and the CPS-D-specific sera, and 41% of the strains were unable to be classified with the CPS-ELISA. The overall distribution of the four serotypes among the 29 *E. faecalis* strains is similar to the distribution among the larger panel of clinical and laboratory isolates in this study. This indicates that the original panel of 29 *E. faecalis* strains represents the existing capsular serotypes among *E. faecalis* strains. The serotype distribution among isolates from healthy persons colonized with enterococci so far has not been determined. The observed cross-reactivity between the CPS-C- and CPS-D-specific sera may be explained by the chemical and/or structural similarity of different polysaccharides – a situation not uncommon for polysaccharide antigens from other bacteria [7]. Based on identical lipoteichoic acids, preliminary structural data for the two polysaccharides support the relatedness of the two prototype strains (unpublished observation).

The nature of the non-typeable isolates is not yet known. So far, all isolates examined by immune electron microscopy have shown a capsule-like structure using ruthenium red staining. These non-typeable isolates most likely represent unknown serotypes. By using a serotype-specific serum raised against one of the non-typeable strains, five isolates of a random sample of non-typeable isolates reacted with that serum (unpublished observation). Another possibility may be that non-typeable isolates express their polysaccharide antigens under different growth conditions than the ones used in our assay.

Although every serogroup was present in all the geographic areas tested, some serogroups were more prevalent in certain countries, as has been observed with serogroups of other gram-positive bacteria [8].

Because of the development and subsequent spread of multiply resistant enterococci, few antibiotics remain for treatment of these infections [9]. Alternative strategies for their treatment and prevention are critical to controlling these potentially lethal infections. The development of passive and/or active immunotherapy based on capsular polysaccharides appears promising as a therapeutic and/or prophylactic option [10]. From the present data, we conclude that the four serogroups described here represent a major portion (approximately 50%) of clinically relevant *E. faecalis* isolates. However, a prospective study with a larger collection of isolates is needed to confirm the hypothesis that a limited number of enterococcal capsular serotypes exists. While all four serogroups were present in all geographic areas, no single serotype predominated in a specific infection site. These findings indicate that geographic- and infection site-specific considerations are probably of minor importance for vaccine development. The likelihood that the number of serotypes is limited suggests that the development of a broadly active enterococcal vaccine may be feasible.

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