Epidemiology of *Enterococcus faecalis* Urinary Tract Infection in a Teaching Hospital in London, United Kingdom

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Received 26 December 1991/Accepted 29 April 1992

Enterococcus faecalis is a frequent cause of urinary tract infection in hospitalized patients. Recent reports have suggested that the organism may frequently be acquired by cross-infection from other patients. In this study, we used total DNA restriction patterns to type 135 urine isolates of *E. faecalis* from four sets of patients. Isolates were placed into types (all bands identical) and into groups (most bands identical). Most isolates were discriminated by the typing method, and the results suggested that direct cross-infection occurred rarely if at all. However, two groups of clonally related isolates occurred frequently in the urine specimens and also in feces from hospital-associated patients and were often associated with antibiotic resistance. Isolates from these two groups were found less frequently in feces from people not associated with the hospital.

Enterococci are among the most frequent causes of hospital-acquired infection, yet our understanding of their spread is very incomplete. Resistance to many antibiotics is already common among enterococci. High-level gentamicin resistance (henceforth referred to as gentamicin resistance) in *Enterococcus faecalis* has now become widespread. The more recent findings of β -lactamase production and especially of vancomycin resistance highlight the need to understand routes of infection, in the hope of preventing strains with these properties from becoming endemic in our hospitals.

It was long assumed that enterococcal infections were autogenous, arising from organisms already present in the patient's intestinal flora. This view was supported by a study of enterococcal urinary tract infections that used antibiograms for typing and that revealed no clustering of antibiogram types by location in the hospital (6). However, a more recent study of colonization by gentamicin-resistant *E. faecalis*, typed by plasmid profiles, showed that patients in adjacent beds acquired indistinguishable isolates (26). This finding and other reports of outbreaks (9, 12) have led to speculation that enterococcal infection may frequently be spread between patients, with consequent implications for control methods. The epidemiology of enterococci has been reviewed in detail by Chenoweth and Schaberg (3).

The potential for detailed epidemiological studies has been improved recently by the development of DNA fingerprinting methods for typing enterococci. Pulsed-field electrophoresis of restriction fragments has shown the relatedness of a number of β -lactamase-producing *E. faecalis* isolates across the United States (15), while ribotyping has been used to demonstrate the unrelatedness of vancomycin-resistant *E. faecium* isolates in one hospital in France (2). In our laboratory, a simpler method that uses conventional electrophoresis of restriction fragments was shown to be highly discriminatory for enterococci (7). In the present study, we used this method to type *E. faecalis* urine isolates obtained from four sets of patients at the Royal London Hospital over a 9-month period. The findings are correlated with other

factors, such as antibiotic resistance. A number of fecal isolates were also investigated.

MATERIALS AND METHODS

Description of isolates. All isolates of *E. faecalis* from urine specimens from four sets of patients were selected retrospectively from a survey of enterococci (6a) at the Royal London Hospital, a large teaching hospital in the East End of London. The survey was conducted from January to October 1989, during which all enterococcal isolates were collected. Identification of isolates has been described elsewhere (7). Patients were from an ethnically diverse local population or referred from elsewhere in the country. The sets of patient represented (i) a general surgical ward (43 isolates), (ii) a urology-nephrology ward (30 isolates), (iii) a maternity unit (27 isolates), and (iv) urology outpatients (35 isolates). All replicate isolates from patients were excluded. Seventy six of the 135 cases were considered to have a clinically significant infection, defined as the detection of $>10^5$ CFU/ml of urine, with growth of *E. faecalis* alone or with one other species. For the remainder of the cases, results were reported as mixed growth (probable fecal contamination) or no significant growth (colony count below 10^5 CFU/ml). All urine isolates were tested for resistance to gentamicin, streptomycin, erythromycin, tetracycline, and chloramphenicol. MICs of erythromycin, tetracycline, and chloramphenicol were determined by conventional agar dilution methods (24). Isolates for which MICs were ≥ 16 μ g/ml for erythromycin, ≥ 32 μ g/ml for tetracycline, and ≥ 16 μ g/ml for chloramphenicol were considered resistant to those antibiotics. In addition, the isolates were screened for high-level aminoglycoside resistance on agar containing 1,000 μ g of gentamicin or streptomycin per ml. All isolates were also characterized by their ability to lyse horse erythrocytes.

Fecal isolates were obtained from specimens submitted to the diagnostic laboratory for other investigations during the summer of 1991. These specimens were either from hospital-associated patients (including outpatients) or from patients attending a general practice in the local area (considered community specimens). A sample of feces was plated di-

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rectly on kanamycin-esculin-azide agar (Oxoid, Basingstoke, United Kingdom). Colonies with a dark halo were Gram stained and tested for group D agglutination with latex grouping reagent D (Oxoid). *E. faecalis* was distinguished from other enterococci by hydrolysis of pyruvate. Thirty isolates were obtained from hospital-associated patients, and 46 were obtained from community specimens. Fecal isolates were screened for gentamicin resistance by the disk diffusion method with 100- μ g disks.

Statistical analysis. Chi-square tests were performed with SPSS software (SPSS Inc.) or EpiInfo software (Centers for Disease Control-World Health Organization). Results were considered significant when P was <0.05 .

DNA fingerprinting. DNA was extracted by the method of Pitcher et al. (18) exactly as described elsewhere (7). DNA was digested with *Sst*I and separated by electrophoresis in 0.9% agarose with TBE buffer at 120 V by standard procedures (20). Ethidium bromide-stained gels were photographed with Polaroid 665 (positive-negative) film. The photographed patterns of all urine isolates were compared with each other by eye by superimposing negatives on a light box. Fragments between the 1.6- and 8-kb molecular size markers were considered in the comparison. When patterns of two or more isolates were possibly identical, the DNAs were run on adjacent lanes of a gel. Patterns were scored as identical, similar (the majority of fragments in each of two patterns matched in size), or different.

Gentamicin probe. An oligonucleotide representing 30 bases from the 6'-aminoglycoside acetyltransferase [AAC(6')] coding region (amino acid codons 158 to 167 from Ferretti et al. [5] or nucleotides 1171 to 1200 from Rouch et al. [19]) of the AAC(6')-2"-aminoglycoside phosphotransferase [APH(2'')] gentamicin resistance gene found in staphylococci and enterococci, which had been used previously for studies of methicillin-resistant *Staphylococcus aureus* (11), was used in this study. It was labelled with digoxigenin by use of terminal transferase and a digoxigenin oligonucleotide 3'-end-labelling kit from Boehringer Mannheim. Total DNA from selected isolates was digested, electrophoresed, and transferred by capillary transfer to a Hybond N membrane (Amersham International, Amersham, United Kingdom) by standard procedures (20). The probe was hybridized to blots at 55°C and washed at a maximum stringency of $0.2\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate at 55°C. Hybridization was detected by use of a digoxigenin nonradioactive detection kit from Boehringer Mannheim.

RESULTS

Typing of isolates and relationship to place and time. *E. faecalis* was isolated from single urine specimens from 135 patients: 30 from a urology-nephrology ward, 43 from a surgical ward, 27 from a maternity unit, and 35 who were urology outpatients. When DNA restriction fragments of all the isolates were compared, 94 different patterns were observed. A single band difference in the size range of 1.6 to 8 kb was used as the criterion for a pattern difference; identity was always checked by running potentially identical isolates in adjacent lanes of a gel (Fig. 1). The largest number of isolates in a type was six. Two types were represented by six isolates, 3 were represented by five isolates, 5 were represented by three isolates, 9 were represented by two isolates, and 75 were represented by single isolates (Table 1). Isolates of the same type frequently differed in their profile of resistance to antibiotics (data not shown).

FIG. 1. Examples of *Sst*I restriction fragment patterns. Four pattern types from group A, four pattern types from group B, and examples from three other groups (groups 23, 5, and 11) are shown. Patterns were traced from Polaroid negative images of gels; bands of less than 1.6 kb and of more than 8 kb in length were omitted. Lanes M contain molecular size markers of 1.6, 2, 3, 4, 5, 6, 7, and 8 kb in length (1-kb ladder; Life Technologies, Paisley, Scotland).

The types were related to the four patient sets from which the isolates were derived (Table 1) and to the date of isolation to identify any clustering of cases. Four isolates of one type (Bf; Table 1) were obtained from urology outpatients, one on 9 January, one on 18 April, and two on 4 and 9 May. Only the second and third isolates had the same antibiotic resistance pattern. In 15 instances, two isolates of a single type were obtained from the same patient set (Table 1), and in 6 of these instances, the antibiogram was also the same. One pair was isolated on the same day from the urology-nephrology ward (same antibiogram), a second pair was isolated 2 days apart from the maternity unit (different antibiograms), and the others were all isolated at least 2 weeks apart.

Grouping of isolates and relationship to other factors. When DNA patterns were compared, it was clear that many patterns, while not identical, were very similar to each other. Isolates were therefore placed in pattern groups, such that for any two isolates in a group the majority of bands ($>50\%$) were identical (see examples in Fig. 1). (Within each group most pairs of isolates had at least 75% of bands in common.) With this criterion, two groups of 24 isolates each, two groups of 11 isolates each, and eight groups with between 2 and 6 isolates each were found similar, and the remaining 32 isolates were found unique.

The two most common groups, designated A and B (Fig. 1), contained over one-third of all urine isolates. The association of these two groups with other factors was examined (Table 2). Both groups had a higher frequency of antibiotic resistance than other isolates. Group B had the highest frequency of isolates resistant to all five antibiotics examined. The association of gentamicin resistance with this group was particularly marked. Group A had a high frequency of tetracycline resistance, and resistance to streptomycin, erythromycin, and chloramphenicol was also more frequent in this group than in the other groups. No group A isolates were resistant to gentamicin. The distribution of beta-hemolytic isolates was also examined, and 19 of 31 beta-hemolytic isolates were from group B. Chi-square analysis confirmed that the number of resistant isolates in groups A and B, and other groups differed significantly ($P < 0.01$) from a random distribution for all antibiotics except streptomycin and for beta-hemolysis.

TABLE 1. Distribution of pattern types among patient sets, in order of prevalence of the types

Patient set ^a	No. of isolates of the following type:																			
	Aa	1a	Ad	Bf	Bl	Af	Be	11	17	23	Ba	Bh	1c	3a	5c	5i	16a	16b	19c	UT ^b
URO	1	1	1	0	2	1	0	1	1	0	2 ^c	0	0	2	0	0	1	0	0	17
SURG	2	2	1	1	2	1	0	0	1	0	0	2	1	0	2	1	1	1	2	23
UO	1	2	1	4 ^d	1	1	2	0	0	1	0	0	1	0	0	1	0	0	0	20
MAT	2 ^e	1	2	0	0	0	1	2	1	2	0	0	0	0	0	0	0	1	0	15

^a URO, urology-nephrology ward; SURG, surgical ward; UO, urology outpatients; MAT, maternity unit.

^b UT, unique type not found elsewhere.

^c Isolates obtained on the same day.

^d Two isolates obtained within 5 days of each other.

^e Isolates obtained within 2 days of each other.

The frequency of isolation of the two most common groups from the four sets of patients was compared (Table 3). Group B was isolated less frequently from maternity patients than from the other patients, but this result was not found statistically significant by chi-square analysis.

All isolates of *E. faecalis* from urine specimens were analyzed, but not all specimens were considered to show a clinically significant infection (see Materials and Methods). Fourteen of 24 (58%) group A isolates, 16 of 24 (67%) group B isolates, and 42 of 87 (48%) other isolates came from positive specimens. This distribution was not significantly different from a random distribution.

Frequency of group A and B isolates in fecal specimens. To determine the underlying frequency of groups A and B, we examined *E. faecalis* isolates obtained from fecal specimens. Group A and especially group B patterns were found less frequently in community specimens (Table 4). For hospital-associated patients, the frequency of isolation of these groups was closer to that obtained with urine specimens (Table 4). Chi-square analysis indicated that the probability of the distribution shown in Table 4 occurring at random was 0.0695.

Two of 45 community fecal isolates tested and 2 of 30 hospital fecal isolates tested were gentamicin resistant; in each set of isolates, one of the resistant isolates belonged to group B and the other belonged to a different group.

Further analysis of gentamicin-resistant isolates. *E. faecalis* urine isolates showing resistance to gentamicin (20 in total) were found to have eight different patterns in group B and seven other patterns. Thus, although there was an association of resistance with group B patterns, the isolates did not represent a single identical clone. An alternative hypothesis was that a single plasmid was spreading among organisms. The enzyme most frequently responsible for gentamicin resistance in enterococci is AAC(6')-APH(2''), which is identical to the staphylococcal enzyme with the same activity. A 30-base oligonucleotide was used to probe for the gene encoding gentamicin resistance in restriction digests of total DNA. All resistant isolates hybridized with the probe, and four susceptible isolates did not hybridize. The probe hy-

bridized to *EcoRI* fragments of at least seven different sizes (five isolates had fragments of >20 kb in length that were not well resolved by conventional electrophoresis). Tests of some isolates after digestion of DNA with *EcoRV* (Fig. 2) showed that more differences could be distinguished. *EcoRI* and *EcoRV* do not digest within the resistance gene or within the transposon that carries it (8, 19), so the fragment size differences demonstrated that the DNA sequences flanking the gene were different.

DISCUSSION

Lack of evidence for clustering of types by ward or time. We have demonstrated elsewhere (7) that digestion of total DNA from *E. faecalis* with *SstI* is a simple and discriminatory typing method for distinguishing isolates. Furthermore, the patterns produced appear to be very stable, with no differences being detected after repeated subculturing (7) (25 times) or upon later isolation from the same patient (7).

In the present study, 135 urine isolates from different patients were typed and compared. A total of 94 types were obtained, with no type containing more than six isolates. The patients were from three different wards or were urology outpatients. Isolates of the same type were not generally clustered in any ward or patient set (Table 1), nor were they obviously clustered in time. In three instances, a pair of isolates of the same type was obtained from patients in the same set within less than 1 week. Of these, only one pair also had the same antibiotic resistance profile. All other isolates of the same type and from the same patient set were obtained at least 2 weeks apart, and most were obtained more than 1 month apart. Furthermore, in more than half of these cases, pairs of isolates with identical DNA patterns had different antibiograms, suggesting differences in plasmid carriage.

The large number of different types obtained and the lack of clustering of isolates of the same type suggest that direct patient-to-patient spread is not a frequent occurrence. The same conclusions are reached whether isolates that may have come from fecal contamination or low-level colonization were excluded.

TABLE 2. Frequencies of resistance to antibiotics and of beta-hemolysis among isolates from groups A and B and all other groups combined

Group (no. of isolates)	% (no.) of isolates resistant to:					% (no.) of beta-hemolytic isolates
	Gentamicin	Streptomycin	Erythromycin	Tetracycline	Chloramphenicol	
A (24)	0 (0)	17 (4)	21 (5)	75 (18)	29 (7)	4 (1)
B (24)	54 (13)	29 (7)	54 (13)	79 (19)	50 (12)	79 (19)
Other (87)	8 (7)	10 (9)	18 (16)	33 (29)	13 (11)	13 (11)

TABLE 3. Frequency of isolates in group A or B or other groups among the four patient sets

Patient set ^a (no. of patients)	% (no.) of isolates in group:		
	A	B	Other
URO (30)	13 (4)	20 (6)	67 (20)
SURG (43)	19 (8)	19 (8)	63 (27)
UO (35)	20 (7)	23 (8)	57 (20)
MAT (27)	19 (5)	7 (2)	80 (20)

^a See Table 1, footnote a.

The findings do not, of course, rule out the possibility that cross-infection can occur. Specific instances of transfer between patients have indeed been reported (26), as have clusters or outbreaks of infection by indistinguishable organisms (9, 12, 25). However, other suggestions of cross-infection based, for example, on the increased frequency of aminoglycoside resistance, have not been supported by adequate typing. It is noteworthy that in an apparent outbreak of vancomycin-resistant *E. faecium* isolates in a pediatric hospital, the isolates were found by DNA typing to be genetically diverse (2). It may well be that gradual changes in populations of fecal enterococci occur during extended hospital stays, through colonization with new strains and selection of strains with characteristics such as antibiotic resistance. Transfer of resistance determinants among strains and species may also occur in this context. Thus, subsequent endogenous infections with organisms acquired or selected in the hospital may occur. (Prior antibiotic treatment, especially with multiple or broad-spectrum agents [1, 10, 25], and extended hospital stays [1, 25] have been recognized as risk factors for infection by gentamicin-resistant enterococci.) A low level of spread of a number of different strains may well not be detected as clustering of types by location and time but rather as differences in the prevalence of certain strains inside and outside the hospital (see Results). Our findings in this regard are consistent with those of Patterson et al. (16) for β -lactamase-producing *E. faecalis* strains in a Connecticut hospital; they concluded that there was a strain endemic to the hospital but did not find evidence of direct spread among patients.

Location of the gentamicin resistance determinant. It has been observed widely that the frequency of enterococcal resistance to gentamicin has increased in recent years (e.g., reference 22; see also reviews in references 13, 14, and 17). This increase could be due to the spread of resistant organisms, the spread of plasmids bearing the resistance determinants, or the spread of the resistance determinants to new plasmids and organisms. The gene encoding AAC(6')-APH(2'') aminoglycoside-modifying activities is frequently detected in gentamicin-resistant *E. faecalis* (10, 23). All 20 of the gentamicin-resistant urine isolates examined in this study carried this gene, as detected by an oligonucleotide probe.

TABLE 4. Frequency of isolates in group A or B or other groups among specimens from various sources

Source	% (no.) of isolates in group:		
	A	B	Other
Hospital urine	18 (24)	18 (24)	64 (87)
Hospital feces	17 (5)	13 (4)	70 (21)
Community feces	9 (4)	4 (2)	87 (40)

FIG. 2. Total DNA from six gentamicin-resistant isolates showing different patterns, digested with *EcoRV* and probed with an oligonucleotide representing the AAC(6')-APH(2'') gene. Lanes 1 to 4 contain isolates of other groups, and lanes 5 and 6 contain group B isolates. All hybridizing fragments were >10 kb in length.

The isolates were of 15 different genomic types, and the gene occurred in more than seven different restriction fragments. Therefore, there are many different organisms carrying the gentamicin resistance determinant in the Royal London Hospital, and no single plasmid is responsible. When resistant isolates were of the same genomic type, the determinant was on a fragment of the same size, but isolates of the same genomic type could be resistant or susceptible to gentamicin. The AAC(6')-APH(2'') gene has only been found on a transposon (5, 8, 19), so it is possible that the determinant transposes quite frequently to different locations on the DNA. Our results are also in accord with the previous observation that gentamicin resistance is carried on many different plasmids (17). Nevertheless, there is a striking association of resistance with one clonally related group of isolates, as discussed below.

Recognition of groups of clonally related isolates and their characteristics. When restriction patterns of different isolates were compared, we found distinct groups of related patterns in which the majority of bands were common. Many isolates could be placed into groups, as described in Results. Over one-third of all isolates fell into either group A or group B. The isolates within each group were clonally related, since the majority of the restriction sites observed were conserved among them. Equations derived by Upholt (21) suggest that isolates sharing more than one-half of restriction fragments in common would have more than 95% conservation of DNA sequence overall.

The possibility of an association between the two largest groups and other features was examined. Isolates from both groups A and B were more frequently resistant to antibiotics than isolates from other groups (Table 1). Resistance to erythromycin, tetracycline, chloramphenicol, and streptomycin was most frequent in group B but was also more frequent in group A than in other groups. Tetracycline resistance in particular was frequent in group A. Resistance to gentamicin was associated with group B but not at all with group A. The ability to lyse horse erythrocytes was also associated with group B but not group A. It is interesting that a recent study by Huycke et al. (10) of *E. faecalis* bacteraemia also revealed, on the basis of pulsed-field electrophoresis of chromosomal DNA, a clone of gentamicin-resistant, hemolytic organisms. The genetic determinants of both antibiotic resistance and hemolysis are frequently encoded

on plasmids. One possible explanation for the association of these characteristics with isolates of some groups of organisms more than others is the influence of chromosomal genes on plasmid uptake and propagation. For example, in *E. faecalis* the transfer of certain plasmids is promoted by sex pheromones secreted by the recipient (4).

Prevalence of group A and B isolates in fecal specimens. The common occurrence of group A and B isolates in urine specimens led us to question the frequency of such organisms in the general population and whether they were found only in the hospital or were widespread in the community. Only two group B isolates were obtained from maternity patients (Table 3), most of whom have only a short-term connection with the hospital and no prior antibiotic treatment. However, the total number of isolates in this patient set was low. *E. faecalis* was therefore isolated from fecal specimens submitted for other investigations and obtained from both hospital-associated patients and general-practice (community) patients (Table 4). Group A and B isolates were almost as frequent in feces from hospital-associated patients as in urine specimens. Both groups were rare in fecal specimens from the community, although this result was not statistically significant. (Since hospital isolates included those from outpatients and day-care patients, the association of groups A and B with the hospital may have been underestimated.)

The findings of this study suggest that direct cross-infection was not the source of most urinary tract infections at the time of this investigation. On the other hand, they are consistent with the proposition that the fecal flora of hospital-associated patients changes through the acquisition or selection of specific strains. A subsequent autogenous infection could then involve hospital-associated enterococci and an increased likelihood of antibiotic resistance problems.

ACKNOWLEDGMENTS

We are grateful to Rosamund Williams and J. D. Williams for help and support on this project. We thank David Beighton and Nick Banatvala for help with statistical analysis and Esther Simpson for providing some of the fecal isolates.

This study was funded by a grant from the Special Trustees of The Royal London Hospital Trust.

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