Frequent Transmission of Enterococcal Strains between Mechanically Ventilated Patients Treated at an Intensive Care Unit

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The objectives of this investigation were to study the respiratory tract colonization and transmission of enterococci between 20 patients treated with mechanical ventilation at an intensive care unit (ICU), to compare genotyping with phenotyping, and to determine the antibiotic susceptibilities of the isolated enterococci. Samples were collected from the oropharynx, stomach, subglottic space, and trachea within 24 h of intubation, every third day until day 18, and thereafter every fifth day until day 33. Enterococcal isolates (n = 170) were analyzed by pulsed-field gel electrophoresis and with the PhenePlate (PhP) system. The antimicrobial susceptibilities to five agents were determined. Seventeen of the 20 subjects were colonized with enterococci in the respiratory tract; 12 were colonized in the lower respiratory tract. Genotype analyses suggested that 13 patients were involved in a transmission event, including all patients intubated more than 12 days. In conclusion, colonization of resistant enterococci in the respiratory tract of intubated patients treated at an ICU was common. Transmission of enterococci between patients occurred frequently. Prolonged intubation period seems to be a risk factor for enterococcal cross-transmission.

Some reports have proposed that the intensive care unit (ICU) is the part of the hospital with the highest frequency of nosocomial infections (11, 23). Pneumonia is the most common type of nosocomial infection occurring at an ICU (19, 24). According to a recent review by Morehead et al. the incidence of ventilator-associated pneumonia was 9 to 24% for patients intubated longer than 48 h (14).

The main organisms causing nosocomial infections at ICUs in Europe are Staphylococcus aureus, Pseudomonas aeruginosa, coagulase-negative staphylococci, yeasts, and enterococci (19) but may vary between different geographic locations. Enterococci are an increasing nosocomial problem worldwide, especially in severely ill and immunocompromised patients, mainly due to intrinsic and acquired antimicrobial resistance. Exogenous acquisition of virulence determinants might also increase the pathogenicity of a certain strain (5). Their inherent tolerance toward unfavorable conditions renders them prone to survive for long periods in the hospital setting even on dry surfaces (17, 25) and thus further facilitates transmission between patients. Although transmission of bacteria between patients does not necessarily lead to infection, it is nevertheless an indication that infection control measurements can be improved. Measuring the rate of nosocomial infections will underestimate the true number of bacterial exchanges between patients and hospital settings.

The aims of the present study were to study the colonization of enterococci in the gastric and respiratory tract of mechanically ventilated patients at an ICU using molecular and biochemical fingerprinting methods and to study if transmission of enterococcal clones occurs between patients. The antibiotic susceptibility pattern of the isolated enterococcal strains was also investigated.

MATERIALS AND METHODS

Study design and subjects. The study was conducted from April to August in 1998. Twenty-six consecutive patients, in two periods, admitted to the multidisciplinary ICU at Huddinge University Hospital, Stockholm, Sweden, entered the study. Inclusion criteria were adult patients requiring mechanical ventilation for a probable period of 3 days or more. Relatives’ informed consent was collected before patients entered the trial. The study was approved by the Local Ethics Committee at Huddinge University Hospital.

Collection of samples. Samples were collected within 24 h after intubation, every third day until day 18, and then every fifth day until day 33. The sampling locations were the oropharynx, the stomach, the subglottic space, and the trachea. Oropharyngeal samples were taken from both the tonsils and the pharynx wall using a sterile swab and a charcoal transport medium and transferred to glycerin-containing broth prior to freezing. Gastric secretions were collected by aspiration of approximately 2 ml via the gastric tube directly into a sterile tube. The first gastric sample was collected directly after application of the gastric tube, and subsequent samples were collected 3 h after enteral feeding ceased. Subglottic secretions were aspirated via a special suction device in the endotracheal tube and preceded by disinfection of the outer channel surface with 70% isopropanyl. Tracheal secretions were aspirated with a sterile suction catheter via the tracheal tube (Evac Hi Lo; Mallinckrodt, Athlone, Ireland) or a tracheostoma cannula directly into sterile tubes. The samples were transported in transport medium to the Department of Clinical Bacteriology, Huddinge University Hospital, within 30 min of collection and stored at −70°C until analyzed.

Microbiological analyses. All the samples were cultured quantitatively and qualitatively, and enterococci were identified by colony morphology, Gram staining, and biochemical tests, as previously described (6). When necessary, subglottic and tracheal secretions were lysed by addition of Sputolysin containing dithiothreitol (ICN Biomedicals Inc., Aurora, Ohio). One enterococcal isolate of each type of colony morphology from each site and time point was stored in glycerin-containing broth at −70°C until further analyzed.

Genotyping by PFGE. All collected enterococcal isolates (n = 170) were further analyzed by pulsed-field gel electrophoresis (PFGE). Chromosomal DNA from the enterococcal isolates was prepared as described by De Lencastre et al. (4) with some modifications as previously described (12). The DNA-containing disks were restricted overnight with Smal (Promega Corporation, Madison, Wis.) at +37°C and loaded in a gel run for 20 h at +14°C in a contour-clamped homogeneous electric field apparatus (GenePathsystem; Bio-
Rad Laboratories, Hercules, Calif.). From each gel, at least one representative of isolates that were visually judged to have identical banding patterns within the patient was rerun on a new gel, which was then further analyzed regarding genetic relatedness. Calculation of similarity matrices and creation of dendrograms was done by aid of Molecular Analyst Software program (Bio-Rad Laboratories) using the unweighted pair group method using arithmetic averages. The similarity coefficients were calculated according to the method of Dice. This gave rise to dendrograms consisting of 32 Enterococcus faecalis and 27 Enterococcus faecium isolates, respectively. The average number of bands per lane was 16. Computing a three-band difference for probably genetically related strains and a four- to six-band difference for possibly related strains has previously been recommended by Tenover et al. (20). Blind duplicate samples were run in the software analysis to check the appropriateness of the dendrogram created.

**Phenotyping with PhP system.** All 170 enterococcal isolates were also analyzed according to their phenotype with the PhP system using PHP-FS plates (PhPlate Microplate Techniques AB, Stockholm, Sweden) designed for typing enterococci (10). This biochemical typing system is based on the reactions of 23 biochemical tests, plus one negative control, which are read quantitatively with a microtiter plate reader, three times during the incubation period. Thus, the results of the measurements reflect the kinetics of the biochemical reactions. The PhP system consists of biochemical tests, which give diverse results within a species (13). With the PhP system hundreds of isolates can be analyzed simultaneously. Dendrograms were created with the PhP software, PhPWIN (PhPlate Microplate Techniques AB) using the unweighted pair group method using arithmetic averages for clustering. By running duplicate identical reference strains in each round of analysis and subsequently clustering these, the recommended identity level of 97.5% was confirmed.

**Determination of antibiotic susceptibility.** The MICs of ampicillin (Sigma, St. Louis, Mo.), gentamicin (Biochrom, Berlin, Germany), imipenem (Merck, Rahway, N.J.), moxifloxacin (Bayer, Elberfeld, Germany), and vancomycin (Eli Lilly, Stockholm, Sweden) for 169 enterococcal isolates were determined using the agar dilution method performed as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (15). *E. faecalis* ATCC 29212 was used as a reference strain. The break points for resistance were set in accordance with NCCLS recommendations (16). Isolates were considered intermediate resistant or resistant (I+R) to ampicillin when the MIC was ≥16 mg/liter, I+R to imipenem and vancomycin when the MIC was ≥8 mg/liter, and high-level resistant to gentamicin when the MIC was ≥500 mg/liter. Recommended break-points for moxifloxacin are currently lacking. The level of resistance (I+R) was set at ≥2 mg/liter since this breakpoint is recommended for the related compound ciprofloxacin according to the NCCLS (16).

**RESULTS**

**Subjects and collection of samples.** A total of 26 patients were initially included in the study. Six patients were excluded due to death (*n* = 2) or extubation or allocation to another hospital (*n* = 4) within 3 days of inclusion. Thus, data from a total of 20 patients (median age, 58.5 years; range, 34 to 82 years; median APACHE-II score, 24 [range, 9 to 40]) were available for analyses. The median length of intubation was 4.5 days; five patients were intubated for ≥12 days (patient 6, 8, 15, 17, and 21). The total number of samples collected was 243, deriving from a total of 75 sample occasions.

**Microbiological analyses.** The quantitative and qualitative results of the total microflora of the respiratory tracts will be published elsewhere. Seventeen of the included 20 patients were on one or more occasions colonized with enterococci. Sixteen subjects had detectable amounts of enterococci at the first sampling occasion. Twelve patients were, on at least one occasion, colonized with enterococci in the lower airway, i.e., subglottis and/or trachea, with nine already colonized at the onset of intubation. A total of 170 isolates of enterococci were collected for further analysis with PFGE. Ninety-seven of these were identified as *E. faecalis* distributed in 15 patients. Sixty-one isolates, from nine individuals, were considered to be *E. faecium*. Twelve isolates, all from one subject, were classified as *Enterococcus durans*.

**Genotypic analyses with PFGE.** (i) **Colonizing patterns within patients.** The first site where a specific enterococcal genotype could be detected was the oropharynx in seven instances, the stomach in three instances, the subglottic space in two instances, and the trachea in five instances. The route of colonization could not be verified for 12 strains since the initial detection of these strains occurred at several sites simultaneously. Ten patients harbored only one enterococcal genotype. In three of five patients intubated for at least 12 days, more than one enterococcal genotype or enterococcal species could be detected. In these individuals the enterococcal diversity increased with time (Table 1). All *E. durans* isolates belonged to the same genotype.

(ii) **Transmission of enterococci between patients.** The dendrograms revealed that seven strains were shared between two or more patients. These genotype analyses suggested that 13 of the 20 patients were involved in an enterococcal transmission event. According to the *E. faecalis* dendrogram, patient 17 had a strain related to one in patient 16 and another strain related to isolates from patients 13, 21, and 22. *E. faecalis* isolates from patients 8 and 12 also appeared to be possibly genetically related. The intubation period of the patients harboring a common *E. faecalis* clone mostly overlapped each other (data not shown). The genotyping of *E. faecium* isolates revealed that patients 5 and 17 and patients 15 and 17 were colonized pairwise by probably related genotypes (B:1 and B:2), respectively. These two genotypes were also considered possibly genetically related to each other. Patients 9 and 10 also had a genotype in common. Gel images of genetically related *E. faecalis* and *E. faecium* strains isolated from more than one patient and their corresponding dendrogram based on the phenotype are shown in Fig. 1.

**Phenotypic analyses with PhP system.** According to the phenotyping results, patients 1, 13, 17, 21, and 22 shared *E. faecalis* strains of the same phenotype. Patients 6, 8, and 15 were suggested to have phenotypically identical *E. faecalis* strains in common, as well as patients 16 and 17. Three *E. faecium* phenotypes were isolated from more than one patient each. Indistinguishable *E. faecium* phenotypes were shared by patients 17 and 24, patients 6 and 15, and patients 5, 9, 10, and 17, respectively. The cophenetic correlation of the *E. faecalis* and *E. faecium* dendrogram was 0.84 and 0.92, respectively.

**Comparison of phenotyping with genotyping.** Phenotypic analyses of enterococcal isolates yielded larger clusters, includ-

**TABLE 1. Sequence of detection of strains in specimens from three patients harboring multiple enterococcal clones**

<table>
<thead>
<tr>
<th>Day</th>
<th>Detection of enterococcal clone(s) in specimen from patient:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>I, II</td>
</tr>
<tr>
<td>3</td>
<td>I, II, III</td>
</tr>
<tr>
<td>9</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td>12</td>
<td>I, II, V</td>
</tr>
<tr>
<td>15</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>18</td>
<td>2, 5</td>
</tr>
<tr>
<td>23</td>
<td>3</td>
</tr>
</tbody>
</table>
ing isolates from more patients, than were yielded by PFGE. Strains clustered by PFGE were also clustered with the PhP system with few exceptions. \textit{E. faecalis} isolates from patients 8 and 12 were considered possibly genotypically related and had a phenotypic similarity of 85% according to the original dendrogram, which included all 97 \textit{E. faecalis} isolates. Similarly, probably genotypically related \textit{E. faecium} isolates from patients 15 and 17 and patients 6 and 24 had a phenotypic similarity of 95.4 and 91.7%, respectively, according to the original dendrogram including all \textit{E. faecium} isolates. The dendrogram in Fig. 1 illustrates the phenotypic relation between the isolates that were genotypically clustered and the corresponding gel image of each isolate. The dendrogram in Fig. 1 has a cophenetic correlation of 0.94.

**Antibiotic susceptibility of enterococcal isolates.** The results of the MIC determination are shown in Table 2. The resistance to ampicillin and imipenem was highest among the \textit{E. faecium} isolates, with rates of 67 and 58%, respectively. The corresponding figures for \textit{E. faecalis} were 0 and 18%. All tested enterococcal isolates were susceptible to vancomycin. One \textit{E. faecalis} isolate and 10 \textit{E. durans} isolates were highly resistant to gentamicin (resistance defined as a MIC of $\geq 500$ mg/liter). Moxifloxacin resistance was generally high in all three species. Multiple resistances were most common in \textit{E. faecium} where 50% of all isolates displayed resistance to ampicillin, imipenem and moxifloxacin. The occurrence of multiple resistant strains did not increase with time.

**DISCUSSION**

Colonization with enterococci in the respiratory tract was common in the studied patient group. The fact that 16 of the 20 patients (80%) were colonized with enterococci already at time of intubation and that 12 (60%) were colonized by enterococci in the lower airway on at least one sampling occasion reflects the poor health status of these patients. Treatment in hospital prior to intubation might also influence this coloniza-

![Dendrogram based on phenotype and Banding pattern from PFGE]

**FIG. 1.** Dendrogram of \textit{E. faecalis} and \textit{E. faecium} isolates, genetically related according to PFGE analysis, created with PhPWIN software based on analysis of results with the PhP system. The dotted line at 97.5% illustrates the suggested identity level. The corresponding banding pattern after \textit{SmaI} digestion and subsequent PFGE is shown for each isolate. Strains are regarded as being of the same PFGE type and thereby probably genetically related when band differences between them are three or fewer. Strains within a given PFGE type are denoted with the same capital letter, e.g., A. Strains are regarded as being of related PFGE types, thereby possibly genetically related, when the band difference between them are four or more or six or fewer. Strains with related PFGE types are termed, e.g., B:1 or B:2. Abbreviations: \textit{fs}, \textit{E. faecalis}; \textit{fm}, \textit{E. faecium}.

**TABLE 2. Activities of various drugs against enterococci in this study\(^a\)**

<table>
<thead>
<tr>
<th>Organism (no. of strains)</th>
<th>\begin{tabular}{c} \text{MIC (mg/liter)}^b \ \text{Range} \text{ 50%} \text{ 90%} \end{tabular}</th>
<th>\text{Rate of resistance (I+R)}</th>
<th>\text{No.}</th>
<th>\text{%}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{E. faecalis} (97)</td>
<td>\begin{tabular}{c} \text{Ampicillin} \ \text{Imipenem} \ \text{Vancomycin} \ \text{Gentamicin} \ \text{Moxifloxacin} \end{tabular}</td>
<td>\begin{tabular}{c} 0.5–2 1 2 0 0 \ 0.5–8 4 8 17 18 \ 1–2 1 2 0 0 \ 1–$\geq 512$ 4 128 1 1 \ 0.125–16 8 16 58 60 \end{tabular}</td>
<td>\text{40}</td>
<td>\text{67}</td>
</tr>
<tr>
<td>\textit{E. faecium} (60)</td>
<td>\begin{tabular}{c} \text{Ampicillin} \ \text{Imipenem} \ \text{Vancomycin} \ \text{Gentamicin} \ \text{Moxifloxacin} \end{tabular}</td>
<td>\begin{tabular}{c} 0.5–16 16 16 40 67 \ 1–$\geq 8$ 8 8 35 58 \ 0.5–2 0.5 1 0 0 \ 1–16 4 8 0 0 \ 0.5–16 4 8 52 87 \end{tabular}</td>
<td>\text{10}</td>
<td>\text{83}</td>
</tr>
<tr>
<td>\textit{E. durans} (12)</td>
<td>\begin{tabular}{c} \text{Ampicillin} \ \text{Imipenem} \ \text{Vancomycin} \ \text{Gentamicin} \ \text{Moxifloxacin} \end{tabular}</td>
<td>\begin{tabular}{c} 1–2 2 2 0 0 \ 1 1 1 0 0 \ 1 1 1 0 0 \ 2–$\geq 512$ 512 512 10 83 \ &gt;4 4 4 12 100 \end{tabular}</td>
<td>\text{12}</td>
<td>\text{100}</td>
</tr>
</tbody>
</table>

\(^a\) \text{Activities of a \beta-lactam (ampicillin), a carbapenem (imipenem), a glycopeptide (vancomycin), an aminoglycoside (gentamicin), and a new methoxyquinolone (moxifloxacin) against \textit{E. faecalis}, \textit{E. faecium}, and \textit{E. durans} group strains.} \\
\(^b\) \text{50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.}
tion pattern, since enterococci are known to be a nosocomial problem. Few data are available for comparison. Bonten et al. (2) demonstrated in a study from 1995 that 16% of patients admitted to an ICU were colonized with *E. faecalis* in the oropharynx and/or trachea at admission. The higher initial rate of *E. faecalis* colonization in the present study (55%) might be due to the fact that the time point for admission and intubation sometimes do not coincide. However, it is important to be aware that colonization patterns differ between different countries and different wards.

The fact that the oropharynx was the first site of isolation for seven of the genotypes might indicate that the route of colonization of enterococci in the respiratory tract starts in the oropharynx. In a study by Saberia-Leal et al. (18) it was suggested that gastric colonization of *E. faecalis* could serve as a reservoir of enterococci involved in nosocomial infections. In the present study, enterococci were first isolated also from the other test sites; the preference for oropharynx might, therefore, be coincidental. In 12 subjects enterococci were first detected in more than one site simultaneously, which may be explained by the time lapse between the sampling occasions.

A majority of the patients colonized with enterococci only exhibited one enterococcal genotype each. A possible reason for this may be short intubation periods. Only five of the patients were intubated for a period longer than 12 days. In three of these individuals the number of enterococcal genotypes increased with time. This might suggest an increased risk for divergent colonization with prolonged intubation period. Since only one isolate of each colony morphology type, site, and time point was analyzed, there is a risk for missing a particular isolate at a certain sampling occasion. Noteworthy is that all five patients intubated more than 12 days were involved in an enterococcal transmission event (Fig. 1). This indicates an increased risk for cross-transmission with prolonged intubation.

Some previous studies regarding transmission of enterococci between patients in ICUs have focused on nosocomial infections (9, 11) or colonization of vancomycin-resistant enterococci (1, 3, 9). This is relevant from a therapeutic point of view but will underestimate the true transmission rate between patients. From a hospital infection control aspect, every transmission of bacteria between patients must be regarded as undesirable. Here we demonstrate frequent transmission of potentially pathogenic bacteria. Enterococci may be considered relevant indicators of bacterial transmission since their potential pathogenicity renders them difficult to control in a healthcare environment. If enterococcal transmission can be reduced, cross-infection of other bacteria is also likely to decline.

Most isolates of the same PFGE type were recovered from patients whose intubation periods at the ICU overlapped each other, which supports the notion that a direct transmission between these patients may have occurred. There were two exceptions to this. According to the genotypic analyses, both patient 6 and patient 24 seemed to have isolates belonging to the same PFGE type (Fig. 1), although patient 6 was discharged 3 months prior to the admission of patient 24. The three-band difference between these two isolates might be explained by the long period between their isolation. If these two isolates are genetically related, the strain from patient 6 must have resided elsewhere in the ward before colonizing patient 24. This is probably also the case for the isolates with identical banding patterns from patients 5 and 17, since the time span between the discharge of patient 5 and the admission of patient 17 was approximately 1 month. This might imply that the extent of cross-transmission of these strains between different patients is greater than the results show.

PFGE is a well-accepted epidemiological tool for determining strain relatedness. However, PFGE is a laborious method requiring special equipment and may be inconvenient for identifying large numbers of isolates. The PhP system poses an interesting alternative, since it is relatively quick to use compared to PFGE. Enterococci are suitable for phenotyping since they are metabolically diverse within their species. Results from the PhP system revealed larger clusters, consisting of strains from more patients than PFGE revealed. All strains suggested to be probably genetically related by PFGE were either phenotypically identical or highly related (>90% similarity) according to the PhP system. The PhP system may therefore be a suitable method for screening clonal relations between large numbers of isolates. Clones suggested by the PhP system can be further analyzed by PFGE as previously suggested by Kühn et al. (10).

The species with the highest frequency of antibiotic resistance was *E. faecium*, both with regard to solutide and multiple resistances. The level of resistance is lower in the present study than in a recent study by Hallgren et al. (7). However, in that study antibiotic susceptibilities of enterococci isolated from patients with infections at Swedish ICUs were tested and could thus represent another more-virulent subpopulation of enterococci. No vancomycin-resistant enterococci were detected, which is in accordance with previous studies reporting low levels of glycopeptide resistance in Sweden (7, 8, 22). Reports of vancomycin-resistant enterococci outbreaks in Sweden are still sparse (21).

In conclusion, mechanically ventilated patients at a Swedish ICU were commonly colonized with enterococci in the upper and lower respiratory tract already at the time of intubation. Prolonged intubation seemed associated with an increased risk for cross-transmission and acquisition of multiple enterococcal genotypes. Transmission of enterococci between the intubated patients occurred frequently, indicating an increased risk of nosocomial infections and stressing the importance of optimal hygienic precautions in this group of severely ill patients.

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**REFERENCES**


