BRIEF COMMUNICATION

Resistance in Clinical Isolates of *Enterococcus faecalis* Encountered at the University Hospital of the West Indies, Jamaica

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ABSTRACT

Enterococcus faecalis *isolates were examined by an automated identification and susceptibility system. Almost all of the 97 isolates were ampicillin susceptible (n = 86) and tetracycline resistant (n = 89). All were nitrofurantoin susceptible. About a third of isolates showed high level resistance to the aminoglycosides streptomicin and gentamicin and this was usually associated with ciprofloxacin resistance (n = 34). Seven isolates were vancomycin resistant, including one that was ampicillin resistant. Most forms of resistance described elsewhere were found.*

Resistencia en los Aislados Clínicos de *Enterococcus Faecalis* Encontrada en el Hospital Universitario de West Indies, Jamaica

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RESUMEN

Aislados de enterococcus faecalis *fueron examinados mediante un sistema automatizado de identificación y susceptibilidad. Casi todos los 97 aislados examinados fueron susceptibles a la ampi-cilina (n = 86) y resistentes a la tetraciclina (n = 89). Todos fueron susceptibles a la nitrofurantoína. Alrededor de una tercera parte de los aislados mostró un alto nivel de resistencia a los aminoglicósidos, por regla general asociada con la resistencia a la ciprofloxacina (n=34). Siete aislados resultaron resistentes a la vancomicina, incluyendo uno que fue resistente a la ampicilina. Se encontraron la mayoría de las formas de resistencia descritas en otras partes.*

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INTRODUCTION

Enterococcus faecalis is the most frequently isolated species of the genus Enterococcus. The species is a common component of normal faecal flora and spread from an intestinal source is a common route of infection. Urinary tract infection and peritonitis or abscess formation following intestinal perforation are typical presentations. Despite a low level of intrinsic resistance to penicillins, monotherapy with ampicillin is usually adequate therapy, in conjunction with urinary catheter replacement or surgical drainage, as appropriate (1–3).

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The organism is an uncommon cause of subacute bacterial endocarditis (SBE), causing 5–15% of cases, but in SBE, bacteriocidal rather than bacteriostatic therapy is preferred. Combination with streptomycin (despite low level intrinsic resistance) was shown to be more reliably bactereriocidal, in the mid 1940s (1). With the emergence of high-level streptomycin resistance that abolished the synergy with penicillin, empiric therapy for SBE was changed to penicillin with gentamicin (1).

Ampicillin resistance in *E faecalis* due to transferable beta-lactamase production was first encountered in the mid-1980s (4, 5). Vancomycin resistance was first described in the late 1980s (6, 7). Ciprofloxacin was introduced at this time, and resistance was soon described (8, 9).

In this report, the authors review the results of susceptibility testing of *E faecalis* isolates from the Microbiology laboratory at the University Hospital of the West Indies that used an automated method (Vitek, BioMerieux, Marcy-

I'Etoile, France) (10). It also includes an assessment of high level aminoglycoside resistance.

MATERIAL AND METHODS

The laboratory has an established manual protocol for the identification of group D streptococci. Essentially, when a catalase negative Gram positive coccus gives hydrolysis of aesculin on a bile-aesculin plate, the organism is considered a group D streptococcus. Training of technologists to use the Vitek system followed a "cascade" process, with authorized users to pass on their expertise to younger members of staff. The reasons for introducing the automated Vitek system (BioMerieux, L'Etoile, France) was to cope with increasing workload by increasing efficiency and capacity. Although the manual method does give less information, the results from this are generally sufficient for routine clinical practice. The isolates in this study are therefore an arbitrarily selected small sample (about 25%) of the total number of group D streptococci isolated in the laboratory.

Laboratory logbooks for the nine months January to September 2004 were used to identify all isolates of group D streptococci and from this list, the database of the Vitek system was interrogated to identify isolates that had been examined using the automated system.

The additional information that the Vitek system gives includes speciation, susceptibility to tetracycline, ciprofloxacin, nitrofuratoin and vancomycin and measures of high level resistance to streptomycin and gentamicin. During this period, one blood culture isolate was investigated using the Vitek system. Other isolates came from urine, including catheter samples and catheter tips, wounds and abscesses and tips of intravenous and intra-peritoneal catheters. The study was not designed to try and distinguish community from hospital acquired isolates and was also not intended to distinguish isolates derived from colonization or from infection.

RESULTS

Ninety-seven isolates were identified. The overall rate of resistance to individual antibiotics is shown in Table 1.

Table 1: Enterococcus resistance observed

Antibotic	µg/ml	number (%)
Ampicillin	$16	1 (1)
Tetracycline	$16	89 (92)
Ciprofloxacin	$16	35 (36)
HLR[a] streptomycin	$2000	35 (36)
HLR[a] gentamicin	$500	36 (37)
Nitrofuratoin	$128	0 (0)
Vancomycin	$32	7 (7)

HLR[a] = high level resistance observed for aminoglycoside suggesting that synergy between a beta-lactam and the aminoglycoside will not occur.

Resistance to individual antibiotics are shown as percentages in the last column. The patterns of susceptibility that were found are shown in Table 2 with their frequencies.

Table 2: Patterns of resistance and their frequency

Resistant to tetracycline only (n = 44)
Resistant to tetracycline, ciprofloxacin, HLR[a] streptomycin, HLR[a] gentamicin (n = 31)
Resistant to no antibiotic tested (n = 7)
Resistant to tetracycline and HLR[a] streptomycin (n = 3)
Resistant to tetracycline and HLR[a] gentamicin (n = 2)
Resistant to tetracycline, ciprofloxacin and HLR[a] gentamicin (n = 2)
Resistant to tetracycline and vancomycin (n = 2)
Resistant to teracycline, ciprofloxacin and HLR[a] streptomycin (n = 1)
Resistant to tetracycline, HLR streptomycin and HLR[a] gentamicin (n = 1)
Resistant to vancomycin only (n = 1)
Resistant to vancomycin and tetracycline (n = 1)
Resistant to vancomycin, ampicillin and tetracycline (n = 1)
Resistant to vancomycin, ciprofloxacin and tetracycline (n = 1)
Resistant to vancomycin, tetracycline, HLR[a] streptomycin, HLR[a] gentamicin (n = 1)

HLR[a] = high level resistance observed for aminoglycoside suggesting that synergy between a beta-lactam and the aminoglycoside will not occur.

All except one isolate were ampicillin susceptible and most (92%) were tetracycline resistant. No nitofurantoin resistance was identified.

Ciprofloxacin resistance was seen in about a third of isolates, and tended to be associated with high level aminoglycoside resistance. Most isolates with high level aminoglycoside resistance showed resistance to both streptomicin and gentamicin, but there were a few isolates with high level resistance to only one of these antimicrobials.

Seven isolates were vancomycin resistant and one of these was also ampicillin resistant, without evidence of beta-lactamase expression.

DISCUSSION

For group D streptococci, the manual method is easier and cheaper to perform than use of the automated Vitek system (BioMerieux, Marcy l'Etoile, France). As a consequence, the authors have found that only technologists trained to use, and having a particular enthusiasm for using the Vitek system, were likely to use it for the identification and susceptibility of enterococci. There were also several periods of system failure.

E faecalis poses an increasingly difficult therapeutic challenge due to acquired resistance. Although this study sample cannot be considered representative, the finding of 14 different patterns of resistance suggests that patterns of resistance (or susceptibility) will not be predictable for isolates of *E faecalis.* Acquisition of genes coding for high level streptomycin resistance has made the use of synergistic combination treatment with ampicillin for subacute bacterial endocarditis (SBE) uncertain (1). In the present study, a substantial proportion of isolates (33%) showed high level resistance to both streptomicin and gentamicin. High level resistance to gentamicin alone, seen in a few isolates, has been described (11) and provides the rationale for testing both gentamicin and streptomycin.

More recently, the emergence of high level penicillin resistance mediated by beta-lactamase production (or presumed alteration in penicillin binding protein affinities) has resulted in treatment failure of infections that were previously effectively treated with ampicillin (12, 13). Although only one isolate was found resistant to ampicillin in the present study, emergence of further ampicillin resistance has been reported (1, 14). However, for the isolates, ampicillin appears to be a commonly effective agent. No determination of the presence of a beta-lactamase was available, such as use of the chromogenic cephalosporin, nitrocefin (15).

The MICs for ciprofloxacin found in *E faecalis* are close to the levels obtained in serum, so that resistance was expected to emerge (8). Over a third of the isolates in the present study were ciprofloxacin resistant, compared to the 10% found elsewhere (9). Because the isolates were chosen arbitrarily, this finding would have to be substantiated with an appropriate collection, but testing for quinolone susceptibility should be added to the manual method. Uniform susceptibility to nitrofuratoin suggests a useful place in the treatment of enterococcal cystitis (1), once this has been identified.

Finally, the emergence of vancomycin resistance (6) has presented the prospect of untreatable enterococcal infection. The identification of several vancomycin resistant isolates in this study suggests the need for ongoing surveillance, with the addition of vancomycin to the manual method of susceptibility testing. It has been established that Vitek may miss Van B vancomycin levels of resistance, so that a screening plate composed of BHI containing 6µ g/ml has been advised (16). One vancomycin resistant isolate demonstrated high-level aminoglycoside resistance, which has been noted by others (17).

The association of ampicillin and vancomycin resistance in one isolate has been described in *E faecium* but not, to the authors' knowledge, in *E faecalis* (16).

Possession of specific virulence factors in some strains of *E faecalis* that enhance causation of SBE in animal models (19, 20) suggests that bacteraemic *E faecalis* may differ from other strains – a systematic study of resistance in the blood culture isolates in this study is most certainly required. For those laboratories that do not have Vitek or equivalent systems, then the likelihood of beta-lactam-aminoglycoside synergy may be obtained using high content discs, commercially available (21).

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