ENTEROCOCCI HIGHLY RESISTANT TO PENICILLIN: CHARACTERIZING ISOLATES FROM SINGAPORE HOSPITALS

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ABSTRACT

Enterococci are increasing in importance as nosocomial pathogens and causes of severe sepsis in immunocompromised patients. From September to November 1989, a survey of 898 enterococcal isolates showed that 52 had acquired high-level resistance to penicillin and ampicillin (MIC>100 mg/l). These were all Enterococcus faecium, did not produce beta-lactamase and showed high-level resistance to gentamicin and streptomycin as well. The majority were urinary isolates, but a few caused bacteraemia in severely ill patients. The potential spread of these highly-resistant enterococci would limit the therapeutic options for systemic infections.

Keywords: Enterococci, resistance, penicillin, ampicillin

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INTRODUCTION

Enterococci, including many organisms formerly classified as "Group D Streptococci", are commensals of the human intestinal tract. In hospitals, they are frequent colonizers of superficial wounds and bedsores. However, they may cause significant infections of the urinary tract and hepatobiliary system; this may lead to generalized sepsis. Enterococci are most dangerous when they cause meningitis and infective endocarditis.

The increasing importance of enterococci in the hospital is due to the growing population of immunocompromised patients susceptible to generalized sepsis caused by these hitherto relatively innocuous organisms. Enterococci are also responsible for a substantial proportion of nosocomial infections[1].

Penicillin and ampicillin are the drugs of choice for enterococcal infections and this is often combined synergistically with an aminoglycoside in cases of infective endocarditis[2].

Over the past two years, our laboratory has noticed an increasing number of isolates resistant to penicillin. The disc diffusion method used for routine sensitivity tests determines resistance at a level equivalent to a Minimal Inhibitory Concentration (MIC) of 16 mg/l[3], and does not distinguish 'moderate' from 'high'-level resistance viz. MIC > 100 mg/l. We decided to characterize these resistance isolates as they would be likely to cause further clinical problems in the future.

METHOD

Enterococci were identified by Gram stain morphology, bile aesculin hydrolysis and tolerance to 6.5% sodium chloride. Speciation was done by the scheme proposed by Facklam[4]. All strains with resistant zone sizes to penicillin/ampicillin by the Kirby-Bauer method had their MICs determined by agar dilution using blood agar plates with an inoculum size of 10,000 organisms[5]. Where available, case notes were examined retrospectively. Tests for high-level aminoglycoside resistance were performed by disc diffusion using 120mcg gentamicin and 300mcg streptomycin discs. Penicillinase production was tested using Nitrocefin discs.

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RESULTS

During the period from September to November 1989, 898 enterococcal isolates were identified, of which 52 were found to be penicillin resistant. Isolates from the same patient were counted once. In the disc diffusion test, the criterion of a zone size less than 15 mm was more than met, and all strains in fact showed no zone of inhibition around a 10mcg penicillin disc; this first suggested to us that high-level resistance might be present.

The penicillin MICs of all these isolates were more than 128 mg/l, except for one strain with an MIC of 64 mg/l. None produced penicillinase. All showed high level resistance to gentamicin and streptomycin (corresponding to MIC ≥ 2000 mg/l)[6]. All belonged to the species *Enterococcus faecium*.

The isolates came from a variety of sources (Table I), the distribution of which was similar to that of non-resistant enterococcal isolates.

Table I
Sources of Isolates of Penicillin-resistant Enterococci: Sep to Nov 1989

Urine	35
Pleural Fluid	1
Perineum/Bedsore	4
Surgical Drain/Abdominal Wound	4
Amputation Stump/Diabetic Ulcer	2
Biliary Mud	1
Arterial Line Tip	1
Blood	4
Total	52

Twelve case notes were examined. Most of the patients had previous antibiotic treatment - most often with ceftriaxone - and many of them were long-stay inpatients with serious underlying conditions (Table II).

Table II
Clinical Profile of 12 Patients with Penicillin-resistant Enterococci

SEX : 9 Females, 3 Males	
AGE : 27-87 years, Mean : 56.6 years	
CLINICAL DIAGNOSIS (Most have more than one)	
Haematologic malignancy	2
Other malignancies	3
Hepatobiliary disease	5
Pancreatitis	1
Diabetes mellitus	3
Nephrotic syndrome	1
Urinary tract infection	2
Septicaemia	3
PREVIOUS ANTIBIOTICS (Within past 2 weeks)	
Ceftriaxone	10
Gentamicin	7
Metronidazole	4
Penicillin/Ampicillin	2
LENGTH OF STAY BEFORE ENTEROCOCCUS ISOLATED:	
1 to 24 days, Mean : 13.6 days	
SOURCE OF ISOLATES	
Blood	2
Urine	8
Pleural Fluid	1
Abscess	1

DISCUSSION

Enterococci, compared to streptococci, have intrinsic relative resistance to penicillin and ampicillin, with usual MICs of 1 to 4 mg/l about 10- to 100- fold higher than streptococci (<0.25 mg/l). However, penicillin and ampicillin can still be effective as high doses can usually be safely given to achieve concentrations above the MIC. In addition, there can be synergism when given with aminoglycosides.

In 1983, a penicillinase-producing *Enterococcus faecalis* was described. Concurrently, *E. faecium* strains with high-level penicillin resistance (MIC > 100 mg/l) not due to penicillinase production were also found. Since 1988, there has been an increase in the number of reports of penicillin-resistant enterococci, especially in the United States[7,8].

The emergence of penicillin-resistant enterococci may be due to several factors. The penicillinase-bearing plasmid of *E. faecalis* is likely to be derived from resistant *Staphylococcus aureus*; *E. faecium* resistance is due to an altered penicillin binding protein[2,9]. Prolonged hospital stay, a larger pool of immunocompromised patients and the widespread use of third generation cephalosporins, which are ineffective against enterococci and therefore exert a positive selective pressure, all contribute to the persistence of these enterococci.

Antimicrobial pressure, however, cannot fully explain the emergence of penicillin resistance, as many patients have had no previous treatment with the penicillins. Once present, however, penicillin-resistant enterococci might spread by cross-infection, potentially generating a pool of asymptomatic carriers from which susceptible patients might acquire significant infection.

A survey of 49 enterococcal isolates in our laboratory uncovered only 3 *E. faecium* strains, the rest being mostly *E. faecalis* (Lin 1990, unpublished data). This would seem to suggest that problems with high-level penicillin resistance would be confined to only a minority of enterococcal infections. However, systemic infections due to these strains tend to occur in the very ill patients, and as the MICs are expected to be greater than 128 mg/l, this means that effective concentrations of penicillin would not be readily attained. Moreover, a recent report describing high-level penicillinase-negative *"E. faecium* type" of resistance in a strain of *E. faecalis*[10] is an ominous portent of the possible spread of such resistance to the majority of enterococcal isolates, as *E. faecalis* form the majority of species in most studies.

The association of all our resistant *E. faecium* isolates with high-level aminoglycoside resistance narrows the therapeutic options to single agent therapy with vancomycin. The recent emergence of vancomycin resistance in enterococcal isolates elsewhere may eliminate this last option in the future[11,12]. The promise of the antibiotic era remains unfulfilled in this age of widespread antibiotic use, a growing pool of immunocompromised patients and bacteria which continue to adapt, survive and infect.

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