Gentamicin-resistant enterococci and endocarditis

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Summary: We report a case of endocarditis associated with a highly gentamicin-resistant enterococcus that failed to respond to conventional antibiotic combination therapy. Clinical resolution was achieved following cardiac surgery and antimicrobial treatment dictated by *in vitro* testing of the patient's organism. Laboratories should test blood culture isolates of enterococci for high level gentamicin resistance. Management of such cases requires individual assessment and extensive laboratory investigation.

Introduction

The established antibiotic regimen for the treatment of enterococcal endocarditis is penicillin (or vancomycin in the presence of B-lactam allergy) given with an aminoglycoside. Faecal streptococci may show tolerance to the action of penicillin or vancomycin but the synergistic combination of either of these with an aminoglycoside provides an effective therapeutic option. We report a case of endocarditis due to a highly gentamicin-resistant enterococcus and discuss the resultant problems in achieving adequate antimicrobial therapy.

Case report

A 69 year old man underwent transurethral resection of the prostate for an adenocarcinoma in another hospital. Because of known aortic and mitral valve disease he was given cephadrine (500 mg 6 hourly for 18 hours) perioperatively. This was followed by radiotherapy and over the following 3 months he received 3 courses of antibiotics for recurrent urinary tract infections.

Four months after operation he was admitted in severe cardiac failure which responded to bed rest and diuresis. He was afebrile and otherwise well. Echocardiography confirmed regurgitation of the aortic and mitral valves but also detected vegetations on the aortic valve.

Six sets of blood cultures drawn over a 3-day period all grew a faecal streptococcus. Following the diagnosis of infective endocarditis, therapy was initiated with benzyl penicillin (2 mega-units every 4 hours) and

gentamicin (dosage determined by serum levels) both given intravenously. Despite the patient's improved clinical condition, *in vitro* tests of his serum bactericidal levels proved unfavourable. Blood cultures taken after 7 days of antibiotic treatment grew the faecal streptococcus once again. After extensive bacteriological testing, therapy was changed to a combination of intravenous ampicillin 2 g four hourly and ciprofloxacin 200 mg twice daily. Seven days later the diseased aortic valve was removed at operation because of gross haemodynamic decompensation. At surgery, vegetations were noted on the left and non-coronary cusp and there was a large perforation in the centre of the non-coronary cusp. There was evidence of an aortic root abscess and the mitral valve appeared normal. A Carpentier-Edwards valve was inserted.

A faecal streptococcus was grown from culture of the aortic vegetations. Antibiotic therapy continued for a further 5 weeks. The patient remained well in review 3 months later.

Bacteriological methods

The patient's blood was cultured in thioglycolate broth and by means of a modified Castenada biphasic medium. Subcultures on cysteine and heated blood ('chocolate') agar were incubated anaerobically and in an atmosphere of 5% carbon dioxide in air respectively. Material taken from the aortic vegetations was inoculated on to blood, chocolate and MacConkey agar which were incubated aerobically and in an atmosphere of 5% carbon dioxide in air. Anaerobic cultures were performed on blood agar containing neomycin (70 mg/l) and cysteine (0.05%) as well as on blood agar containing cysteine alone. The remaining

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tissue was added to thioglycolate broth, incubated overnight, then subcultured onto chocolate and cysteine blood agar and incubated in 5% carbon dioxide in air and under anaerobic conditions respectively. All isolates were identified by routine laboratory methods¹ and their identity confirmed by Dr G. Colman at the Public Health Laboratory Service Streptococcal Reference Unit.

Bacteriostatic and bactericidal activity of the patient's serum was determined by two-fold dilution of the serum in nutrient broth and inoculation with approximately 10^5 CFU/ml of the patient's own isolate. After overnight incubation, aliquots were cultured on blood agar. Antibiotic susceptibility testing was carried out using the comparative disc diffusion method² on DST agar (Oxoid Ltd, Basingstoke, Hants). Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of various antibiotics were determined by a macrodilution technique.³

Different antibiotic combinations were assessed using the half chessboard and full chessboard titration techniques and the tambour transfer method.³

Results

Blood cultures taken before therapy and during penicillin with gentamicin treatment, all grew a Gram-positive coccus provisionally identified as a group D streptococcus. A similar organism was isolated from the aortic valve vegetations removed at operation. All isolates were later identified as *Streptococcus faecalis* serotype 1 by the PHLS Streptococcus Reference Unit. Serum antibacterial studies performed on samples taken after 4 days of penicillin with gentamicin therapy showed both peak and trough samples were bacteriostatic only at a dilution of 1:2 and not bactericidal. Samples taken after 4 days treatment with ampicillin and ciprofloxacin were not fully bactericidal. However, the peak sample of serum was bacteriostatic at a dilution of 1:64 while the trough serum was static at 1:16.

Using the comparative disc diffusion method, the patient's isolate was found to be resistant to gentamicin, netilmicin, amikacin, trimethoprim, clindamycin, rifampicin and fucidin. Reduced susceptibility was recorded for ampicillin, erythromycin and ciprofloxacin. MIC and MBC values determined for several antibiotics are presented in Table I. The organism was found to be tolerant to the action of penicillin, ampicillin and vancomycin and highly resistant to gentamicin. MIC values were consistent with results obtained at the PHLS Antimicrobial Reference Laboratory. Although never fully bactericidal (99.9% reduction of inoculum) penicillin and ampicillin showed greatest killing at the concent-

Table I Antibiotic susceptibility of the clinical isolate (*Streptococcus faecalis* serotype 1)

	MIC	(mg/l)	MBC
Penicillin	2		>32
Ampicillin	1		>16
Vancomycin	3		>50
Gentamicin	>1000		—
Ciprofloxacin	1.0		8

ration of the MIC and lesser cidal activity at higher concentrations (Eagle's phenomenon⁴).

Antibiotic combination assessment by the half chessboard method failed to demonstrate synergy between erythromycin, vancomycin, imipenem, rifampicin, fucidin or amikacin. No combination was fully bactericidal. Ampicillin and ciprofloxacin tested by the full chessboard technique showed only indifference with no demonstrable synergy or antagonism of action. Using the tambour transfer method, ampicillin, ciprofloxacin, erythromycin, rifampicin, fucidin, imipenem, vancomycin and gentamicin all failed to show synergism in trials of combined action.

Discussion

Group D enterococci are intrinsically resistant to aminoglycosides (MIC 16–64 µg/ml). However synergy can be achieved when penicillin is given in combination with an aminoglycoside, probably due to enhanced uptake of the aminoglycoside.⁵

In recent years high level resistance to streptomycin and kanamycin (MIC > 1000 µg/ml) has been noted in the USA, with up to 40% of clinical isolates resistant in some centres.⁶ With these organisms it was not possible to achieve synergy using penicillin and streptomycin,⁷ but they were, however, sensitive to gentamicin.

Since 1980 there have been reports worldwide of high level resistance to gentamicin (MIC > 1000 µg/ml).^{8–15} Resistance is mediated by aminoglycoside modifying enzymes, often multiple, and is transferable on a conjugative plasmid.⁹ There is a possibility that gentamicin-resistant genes were acquired from staphylococci.^{9,16} Zervos¹⁷ found that patients with highly gentamicin-resistant enterococci were more likely to have received aminoglycosides and cephalosporins in the previous 3 months. Usually there is cross resistance to all the aminoglycosides with the occasional exception of streptomycin. Initially these isolates showing high level gentamicin resistance were sporadic but Zervos¹⁷ recently reported 13% resistance among clinical isolates of enterococci in Michigan and similar figures have been published by others in the USA.

In the UK there have been no clinical reports of high level gentamicin resistance as far as we are aware and this is the first reported case of endocarditis caused by such an organism. So what are the therapeutic options? It is well espoused that endocarditis should be treated with bactericidal antibiotics, preferably in combination.¹⁸ This patient had already failed on penicillin with gentamicin (despite the acceptable MIC of penicillin and lack of demonstrable antagonism between the two antibiotics) and ampicillin alone was unlikely to be preferable.^{14,19} Eagle's phenomenon⁴ was noted on determination of the MICs of ampicillin and penicillin, i.e., the killing effect of penicillin or ampicillin was maximum at about the MIC and decreased as the concentration of the B-lactam was increased. The clinical significance of these findings is uncertain. The addition of gentamicin at various concentrations did not achieve synergy. Characteristically, enterococci are tolerant to vancomycin²⁰⁻²² and our organism fulfilled these criteria. The addition of gentamicin did not provide a synergistic combination.²³

Careful synergy testing by a variety of techniques did not provide a reliably bactericidal combination against this organism. Laboratory testing revealed a MIC of 1 µg/ml for ciprofloxacin and stasis was achieved on synergy testing with ampicillin and ciprofloxacin. Grimm²⁴ described a synergistic bactericidal effect when ampicillin and ciprofloxacin are used against enterococci in combination. In our patient, 48 hours after starting ciprofloxacin and ampicillin, blood cultures became negative, serum static levels were more acceptable and, following

surgery, the patient improved markedly and remained well 3 months after treatment was completed.

This case illustrates the significant role the laboratory can play in the management of serious infection. It was the positive blood cultures and poor serum bactericidal levels which precipitated the change in treatment and contributed to the decision to operate.

In conclusion, high level resistance to gentamicin in enterococci is already well established in the USA and elsewhere and is likely to become more common in the United Kingdom. When enterococci are isolated from blood cultures it is recommended that they be tested with a high content gentamicin disc (or an MIC performed)²⁵ It may be prudent to barrier nurse cases as they arise¹² and to consider the value of early surgery. The choice of appropriate antibiotics is difficult and we suggest each case be assessed individually and bactericidal antibiotics used if possible. There is great need for new antibiotics with activity against such organisms.

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