Neomycin blood agar as a selective medium for vancomycin resistant *Enterococcus faecium*

P R Chadwick, B A Oppenheim

Abstract

Neomycin blood agar is commonly used as a selective medium for the isolation of vancomycin resistant enterococci from faeces; however, not all isolates are recovered using this medium, perhaps because the neomycin concentrations are too high. To test this hypothesis, the neomycin minimum inhibitory concentration (MIC) was determined for 27 vancomycin resistant *Enterococcus faecium* isolates, 14 from patients with leukaemia and 13 from patients on the renal unit. A further eight isolates that had been recovered from the faeces of patients on the renal unit on neomycin agar were also studied. Eleven of the 14 isolates from the patients with leukaemia showed equal recovery on neomycin agar and blood agar and had MICs >64 mg/l. In three other isolates there was this hospital. However, these conclusions do not necessarily apply generally. A recent survey of this hospital’s bacterial flora showed that its antimicrobial susceptibility patterns were stable and very predictable; gentamicin resistant Gram negative rods and methicillin resistant strains of *S aureus* were very rare. In hospitals with higher rates of antimicrobial resistance, where empirical treatment is less likely to be effective, earlier processing of specimens with more rapid availability of sensitivity data could well be justified.

Detection of vancomycin resistant *E. faecium* using neomycin blood agar

were used in the determination of the neomycin minimum inhibitory concentration (MIC). Isolates were maintained in a glycerol bead system at -20°C and then in nutrient agar stabs.

RECOVERY OF *E. FAECIUM* ON AGAR MEDIA

Organisms were grown overnight in nutrient broth and then serial decimal dilutions were made in maximum recovery diluent (Oxoid, Basingstoke, UK). A surface drop method was used to assess recovery on blood agar (Columbia agar base, and Oxoid agar with whole horse blood) and on 50 mg/l neomycin agar (Wilkins–Chalgren anaerobe agar, Oxoid with whole horse blood and neomycin). Then, 20 μl of each dilution was dropped in duplicate onto agar plates which were incubated for 48 hours at 37°C. Colonies were counted and recovery of organisms per ml of broth culture calculated.

METHODOLOGY ON AGAR

MIC determination on agar

Organisms were grown overnight in nutrient broth and then diluted 1 in 50 in water to produce a final dilution of about 10⁶ per ml. Dilutions of neomycin (Selectatab, Mast, Bootle, UK) were made in water and incorporated into DST agar (Oxoid) plates to give agar dilutions of 0-03–64 mg/l. Plates were inoculated using a multipoint inoculator (Mast), incubated for 18 hours at 37°C and then examined for growth.

**Results**

Of the 14 isolates from patients with leukaemia, 11 showed equal recovery on the two media and had MICs >64 mg/l. In three other isolates there was a 4 log₁₀ reduction in recovery on neomycin agar and the neomycin MIC was 8 mg/l. Only two of the non-selected isolates from the renal unit were recovered equally on the two media, the other 11 isolates showed a 4–5 log₁₀ reduction in recovery and were relatively susceptible to neomycin (table). By contrast, all eight faecal isolates recovered from patients on the renal unit on neomycin agar were highly resistant to neomycin (MIC >64 mg/l).

**Discussion**

We have been using our routinely prepared neomycin blood agar to screen for enterococci in faeces. We considered this to be a simple and suitable medium for the isolation of vancomycin resistant enterococci when incubated aerobically with a 5 μg vancomycin disc in the well of the plate. Indeed, we have recovered many vancomycin resistant enterococci in this way, but recently noted that some strains did not seem to grow on the selective medium despite growth on blood agar (also used for surveillance of faeces in patients with leukaemia). We were uncertain whether this was an inoculum effect or whether neomycin agar was inhibiting some strains of enterococci.

Over the past 20 years, enterococci have risen from a position of relatively minor significance to one of notoriety and are now among the commonest bacteria isolated from nosocomial infections. While they are still considered to be of low pathogenicity, increasing antimicrobial resistance and, in particular, glycopeptide resistance, has provided the impetus for intensive epidemiological study. Vancomycin resistant enterococci, particularly *Enterococcus faecium*, have caused outbreaks of infection and colonisation throughout Europe and North America. However, it is not clear at a local level how best to detect and control these organisms. Vancomycin resistant enterococci have been present in South Manchester since 1992 and for some time we have been using a selective medium, neomycin blood agar, for their isolation from faeces. Recently, we became suspicious that not all isolates were being recovered on this medium and evaluated the agar against vancomycin resistant *E. faecium*.

**Methods**

**BACTERIAL ISOLATES**

Fourteen vancomycin resistant *E. faecium* isolates from patients with acute leukaemia and 13 isolates from patients on the renal unit were studied. These isolates were recovered from blood, urine, peritoneal fluid, faeces, wound, throat, pus, or intravenous line tips on non-selective media. A further eight isolates that had been recovered from the faeces of patients on the renal unit on neomycin agar were also studied. Control strains of *E. faecalis* (NCTC 775) and *Staphylococcus aureus* (NCTC 6571) were used in the determination of the neomycin minimum inhibitory concentration (MIC). Isolates were maintained in a glycerol bead system at -20°C and then in nutrient agar stabs.

**MIC DETERMINATION ON AGAR**

Organisms were grown overnight in nutrient broth and then serial decimal dilutions were made in maximum recovery diluent (Oxoid, Basingstoke, UK). A surface drop method was used to assess recovery on blood agar (Columbia agar base, and Oxoid agar with whole horse blood) and on 50 mg/l neomycin agar (Wilkins–Chalgren anaerobe agar, Oxoid with whole horse blood and neomycin). Then, 20 μl of each dilution was dropped in duplicate onto agar plates which were incubated for 48 hours at 37°C. Colonies were counted and recovery of organisms per ml of broth culture calculated.

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In this study a reduction in recovery of at least $4 \log_{10}$ was demonstrated for some of the leukaemia unit isolates and most of the renal unit isolates on 50 mg/l neomycin agar compared with blood agar. This difference was attributable to differing susceptibility to neomycin. Neomycin blood agar has been used by other authors for the detection of vancomycin resistant enterococci, but the concentration of antibiotic used was not specified. It is clear that we may have missed faecal colonisation in some of our patients, at least in those on the renal unit. This has obvious implications for the control of infection, as strict application of barrier precautions for colonised patients may be necessary to limit an outbreak due to vancomycin resistant enterococci.

Several approaches are possible to improve the detection of faecal carriage of these organisms. Firstly, we have reduced the concentration of neomycin in our medium and this will be evaluated. Secondly, alternative selective media could be used such as cephalaxin-aztreonam-arabinose agar or campylobacter blood agar. Thirdly, an enrichment phase could be used to maximise the inoculum onto a selective agar. Whichever approach is used, careful assessment of antibiotic concentrations will be required, together with a full evaluation of results. If we had relied on the NCTC E faecalis (MIC 64 mg/l) for quality control of our media, we could have assumed, wrongly, that other enterococci would have been adequately recovered. As vancomycin resistant enterococci become more prevalent nosocomial pathogens, comparative studies of screening media are urgently needed.


Gold granuloma after accidental implantation

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Abstract
A case, in a 66 year old man, of a florid granulomatous reaction to gold dental alloy presenting about 20 years after accidental implantation in the oral mucosa of the lip is reported. Subsequent energy dispersive analysis confirmed the presence of a high nobility gold dental alloy. Florid granulomatosis has only rarely been reported in association with gold. Possible explanations for the delay in presentation include alteration of immune status or the development of hypersensitivity with components of the gold dental alloy acting as hapten.

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Keywords: Gold dental alloy, florid granulomatosis, oral mucosa.

Gold has been widely used as a dental restorative material, largely because of its inert biological nature. Gold deposition has been reported in a variety of sites, usually as a result of chrysotherapy. Cox et al⁵ and Keen et al⁶ have reported cases of gold deposition in the dermis following chrysotherapy. Landas et al⁷ have described gold deposition in the liver in rheumatoid arthritis. However, gold is an uncommon finding in oral lesions. Levison et al⁸ analysed particulate matter from 222 oral lesions and gold was identified in one case only. Experimental studies carried out by Matsui et al⁹ and Nagem-Filho et al⁹ showed that subcutaneous implantation of gold (24 K) and gold alloy in rats caused only a mild tissue reaction compared with other dental restorative materials, inducing relatively few inflammatory cells.

Case report
A fit and otherwise healthy 66 year old man presented with an 18 month history of painless oral swellings. Examination showed three pale mucosal nodules on the inner aspect of the right upper lip and both sides of the inner lower lip, each measuring approximately 1 cm in diameter. An incisional biopsy of one lesion was