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

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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...
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 2 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.

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 10^6 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 log_{10} 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 log_{10} reduction in recovery. Comparatively, the non-selected Enterococcus species were isolated from 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.

Keywords: Enterococcus faecium, neomycin blood agar, vancomycin resistance.

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.

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Results
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Discussion
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Gold granuloma after accidental implantation

F R Scott, A P Dhillon, J F Lewin, W Flavell, I M Laws

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 hapitens.

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 al.¹ and Keen et al.² have reported cases of gold deposition in the dermis following chrysotherapy. Landas et al.³ have described gold deposition in the liver in rheumatoid arthritis. However, gold is an uncommon finding in oral lesions. Levison et al.⁴ analysed particulate matter from 222 oral lesions and gold was identified in one case only. Experimental studies carried out by Matsui et al.⁵ and Nagem-Filho et al.⁶ 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...