Colonial variation in vancomycin resistant Enterococcus faecium

L W J Baillie, J J Wade, M W Casewell

Abstract
Vancomycin resistant enterococci are increasingly being isolated from inpatients. This report describes the colonial variation present in most isolates of vancomycin resistant Enterococcus faecium obtained at this hospital. Colonial variants within the same culture were indistinguishable by antimicrobial susceptibility, biochemical reactions, and ribotyping. Failure to appreciate this colonial variation will lead to pure cultures being regarded as contaminated or mixed.

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In recent years enterococci have emerged as an increasingly important cause of hospital acquired infection.1 Innately resistant to many antimicrobial drugs, the acquisition by enterococci of resistance to agents that are required for treatment is a cause of concern.13 Following what seemed to be the world's first outbreak of vancomycin resistant enterococci at a nearby hospital,4 we began to screen patients in our liver unit. We found, and recently reported, the isolation of strains of E faecium which were resistant both to vancomycin and high concentrations (>2000 mg/l) of gentamicin.5 During this screening programme, we observed that most clinical isolates of vancomycin resistant E faecium yielded, on subculturing single colonies, a mixture of two colonial types.

Methods
Single colonies of vancomycin resistant isolates of E faecium from screening swabs taken from patients with liver disease were subcultured on 7% horse blood agar (CM 271, Oxoid, Basingstoke) and were incubated at 37°C in air overnight. Morphologically distinct colonies were identified as Enterococcus faecium by the API 20 STREP system (BioMerieux UK, Basingstoke). A standard controlled disc-diffusion method was used to determine the susceptibility of each colonial type to the following antimicrobial drugs: amoxycillin (10 μg), erythromycin (5 μg), tetracycline (10 μg) and streptomycin (300 μg). Ribotyping was kindly performed by Mr Donald Morrison of the Division of Hospital Infection at the Central Public Health Laboratory, London.

Results
On 7% horse blood agar two distinct colonial types were seen, apparently without exception, for all vancomycin resistant E faecium isolated from several hundred screening specimens (figure). The predominant type consisted of grey, low convex, matt colonies characteristic of E faecium; the second type appeared as white, glossy, domed colonies, similar to those of Staphylococcus epidermidis. After reincubation for 2 to 3 days the grey colonies developed white molar teeth-like surfaces with multiple surface protrusions which then developed into discrete white circular colonies. Repeated subculture of a single colony of either the grey or white colony types on to blood agar reproduced the mix of...
Measurement of serum arabinosylitol by gas-liquid chromatography: Limitations for detection of systemic candida infections

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Abstract

The measurement of serum arabinosylitol in the diagnosis of systemic candida infections was evaluated using a gas-liquid chromatography technique in a cohort of at risk patients. The prevalence of seropositivity was low and did not correlate with evidence of infection. This technique is unlikely to achieve acceptance because it does not discriminate between patients with and without infection; it requires specialised equipment and is expensive.

Methods

All the patients were potentially immunocompromised. Most had leukaemia or were from intensive therapy units, and had a fever of unknown origin. Blood samples were sent from 213 adults and 65 children, 22 of whom were neonates. Thirty three patients had repeat sera taken within 10 days of the original specimen.

Serum arabinosylitol was measured by a GLC technique. Briefly, this entailed removal of serum protein (usually 200 µl) with 2 volumes of acetone containing a standard amount of mannitol. The supernatant was dried by evaporation and reconstructed in a reagent mix which sialilated the sugars and sugar-alcohols (Sylon HTP kit containing hexamethydisilazane, trimethylsilylolsilane, and pyridine; Supelco Inc., Bellefonte Philadelphia, USA) which rendered them volatile. The reaction mix was dried, reconstructed in 100 µl diethyl ether and 3 µl were applied to GLC apparatus (Sigma 3 gas chromatograph, Perkin-Elmer Ltd, Bucks, England). The arabinosylitol peak was identified by its relative mobility compared with the peak corresponding to mannitol, and was quantified by their relative heights.

There is considerable clinical value in suitable procedures for the rapid testing for deep seated candida infections. D-arabinosylitol is a major metabolite of the clinically important Candida species, and previous work has suggested that measurement of this pentitol in serum by gas-liquid chromatography (GLC) is rapid, specific, and suitable for use in the diagnostic laboratory of a larger general hospital. To examine this claim we undertook a retrospective assessment (1987–90) of 246 measurements of serum arabinosylitol in 213 patients.

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