Short reports

Colonial variation in vancomycin resistant *Enterococcus faecium*

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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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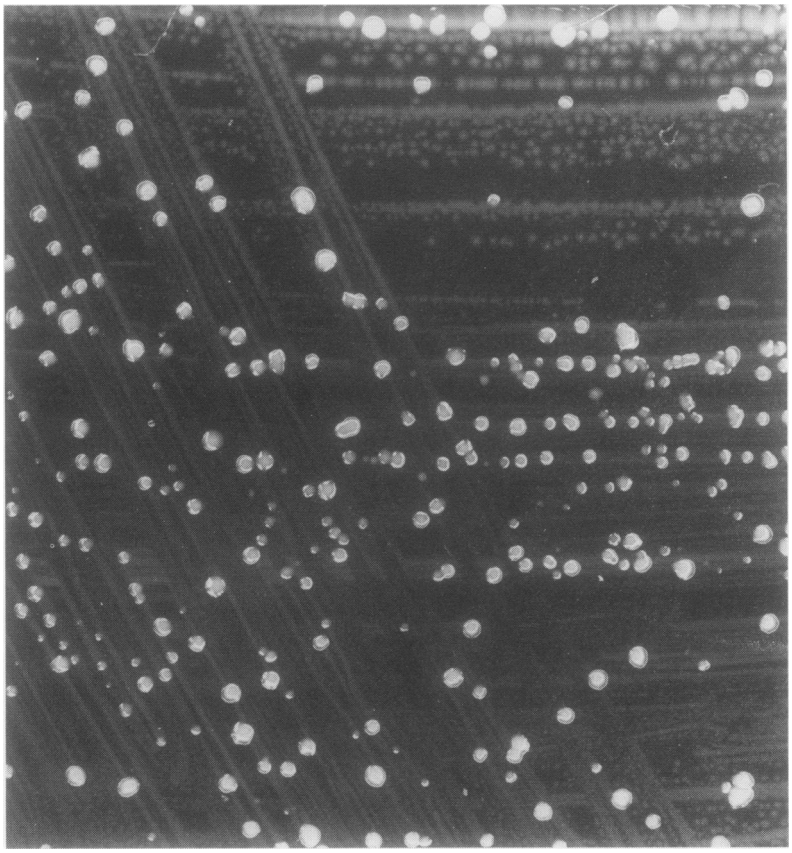
In recent years enterococci have emerged as an increasingly important cause of hospital acquired infection.[1] Innately resistant to many antimicrobial drugs, the acquisition by enterococci of resistance to agents that are required for treatment is a cause of concern.[2 3] Following what seemed to be 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.



Two distinct colonial types are visible for all vancomycin resistant E faecium isolated from several hundred screening specimens.

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), tetracyline (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

both types. Similarly, subculture of broth cultures derived from single colonies of either colonial type, yielded both types of colony on blood agar.

The carbohydrate fermentation and other biochemical tests included in API STREP did not reveal any biochemical differences between the two colonial types of any isolate tested. Furthermore, using a standard controlled disc-diffusion method, we found no differences in antimicrobial susceptibilities between the two colonial types of any isolate tested. Ribotyping did not distinguish between the two colonial types of *E faecium* E227, an isolate from the bile of a liver transplant recipient.

Discussion

The explanation for this variation in morphology is not clear. It may be related to the alteration in cell-wall structure that occurs with vancomycin resistance,[6] as we have yet to see such colonial variation in any vancomycin sensitive isolates of *E faecium*. This phenomenon may assume particular importance when interpreting culture results. Pure cultures of a vancomycin resistant *E faecium* from clinical specimens may appear mixed which may detract from the importance of the culture results. A considerable amount of time and effort may also be wasted in attempting to isolate pure cultures when the "contaminant" is, in reality, the second colonial type.

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Measurement of serum arabinitol by gas-liquid chromatography: Limitations for detection of systemic candida infections

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Abstract

The measurement of serum arabinitol in the diagnosis of systemic candidosis 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 it is expensive.

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There is considerable clinical value in suitable procedures for the rapid testing for deep seated candidosis. D-arabinitol 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.[1 2] To examine this claim we undertook a retrospective assessment (1987–90) of 246 measurements of serum arabinitol in 213 patients.

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 arabinitol was measured by a GLC technique.[1] Briefly, this entailed removal of serum protein (usually 200 μl) with 2 volumes of acetone containing a standard amount of mannitol. The supernatant fluid was dried by evaporation and reconstituted in a reagent mix which sililated the sugars and sugar-alcohols (Sylon HTP kit containing hexamethyldisilazane, trimethylcholorosilane, and pyridine; Supelco Inc., Bellefonte Philadelphia, USA) which rendered them volatile. The reaction mix was dried, reconstituted in 100 μl diethyl ether and 3 μl were applied to GLC apparatus (Sigma 3 gas chromatograph, Perkin-Elmer Ltd, Bucks, England). The arabinitol peak was identified by its relative mobility compared with the peak corresponding to mannitol, and was quantified by their relative heights. The quality control of the procedure was assessed by the satisfactory

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