## Isolation of Mycoplasma hominis using the BACTEC 9000 series blood culture system

### Table 1

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Inoculum (cfu/ml)</th>
<th>BHI broth</th>
<th>BACTEC (aerobic)</th>
<th>BACTEC (anaerobic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>70</td>
<td>1</td>
<td>No growth</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>260</td>
<td>1</td>
<td>No growth</td>
<td>7</td>
</tr>
<tr>
<td>C</td>
<td>150</td>
<td>1</td>
<td>No growth</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>1</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>1</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

BACTEC (aerobic) = BACTEC Plus Aerobic/F; BACTEC (anaerobic) = BACTEC Plus Anaerobic/F.

of BACTEC bottles. The subcultures, taken by needle and syringe, were plated onto Columbia blood agar and were incubated anaerobically for 48 hours before examination. The undisturbed BACTEC pairs were terminally subcultured on the seventh day of incubation.

### Results

The five test strains of *M. hominis* grew after incubation for one day in the BHI broth. None of the BACTEC bottles gave a positive growth response—that is, growth was not detected by the fluorescence system during the seven day incubation period. Growth was not detected by daily subculture of the BACTEC Aerobic Plus/F bottles during the incubation period. However, three of the strains grew in the BACTEC Anaerobic Plus/F medium. One strain was detected by subculture after six days' incubation and a further two after seven days' incubation (table 1).

### Discussion

The results of this study are similar to the findings of other investigators in that *M. hominis* may grow poorly on some blood culture media and this may be attributable to the presence of SPS. Even at sub-bactericidal levels SPS may retard the speed of growth.

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### Heat tolerance of vancomycin resistant Enterococcus faecium

**S Panagea, P R Chadwick**

**Abstract**

The heat tolerance of 27 *Enterococcus faecium* isolates in water was studied. Stationary phase cultures including vancomycin resistant and sensitive clinical and food isolates were exposed to heat at 60°, 65°, 71°, and 80°C for one, three, 10, and 30 minutes and the log₁₀ reductions in bacterial counts were determined. Exposure at 71° and 80°C resulted in >6 log₁₀ reduction in viable counts for all isolates. Seven (24%) isolates survived (<5 log₁₀ reduction) heat at 65°C for 10 minutes. The *E faecium* isolates were more resistant to heat than the two *E faecalis* reference strains. No differences in heat tolerance were observed between vancomycin sensitive and resistant strains or between isolates of human and animal origin.


Keywords: *Enterococcus faecium*, heat, antibiotics, glycopeptide, disinfection methods.
Enterococci, particularly *Enterococcus faecium*, are increasingly being isolated from hospitalised patients. The mode of spread has not been fully elucidated; however, recent reports have demonstrated that patient to patient transmission of these organisms, including vancomycin resistant enterococci (VRE), can occur via hands of personnel, contaminated surfaces or patient care equipment. Enterococci are relatively heat tolerant bacteria and there has been concern that some strains may be able to survive the temperature/time parameters currently recommended for disinfection processes.

### Methods

Twenty nine isolates were studied, 27 of which were of *E faecium* comprising 10 vancomycin resistant clinical isolates (six VanA and four VanB phenotype glycopeptide resistance), eight isolates from uncooked meat (VanA phenotype), and nine vancomycin sensitive isolates from infected patients. Vancomycin sensitive *E faecalis* reference strains NCTC 775 and ATCC 29212 were also tested. All VRE were considered to be different strains as determined by pulsed field gel electrophoresis (Chadwick PR, unpublished data).

Isolates were cultured in nutrient broth overnight at 37°C and held at room temperature for another 24 hours to achieve stationary phase of growth. Viable counts were determined before heating by serial 10-fold dilutions and cultivating 0.1 ml volumes on blood agar plates. Aliquots (0.1 ml) of each culture were added to 0.9 ml sterile water in glass bijoux and the bijoux were incubated in a water bath at 60°C for 30 minutes, at 65°C for three and 10 minutes, and at 80°C for one, three and ten minutes. The temperature was monitored in a dummy bijou containing 1 ml sterile water and timing was started after the water in the bijou had reached the required temperature ±1°C (approximately five minutes). Rapid cooling followed exposure at the above temperatures/holding times and the number of organisms surviving in each bijou was estimated by subculturing 0.1 ml on blood agar plates. The remaining 0.9 ml were added to 9 ml nutrient broth for enrichment. Agar plates were incubated at 37°C for 48 hours and the broths were subcultured after 24 hours on blood agar plates. The killing effect was quantified by calculating the log_{10} reduction in the number of viable organisms before and after exposure to heat. The limit of our detection system was 10 cfu/ml nutrient broth.

### Results

Viable counts of initial cultures were found to range from 9 × 10^7 to 7.5 × 10^7 cfu/ml. Complete kill (no viable organisms) was achieved for all isolates after heating at 71°C for 10 minutes and 80°C for one, three and 10 minutes. Three (10.3%) isolates were recovered after heating at 71°C for three minutes, but the reduction in viable count was >6 log_{10} for one and >7 log_{10} for the other two. After heating at 71°C for one minute, nine (31%) isolates were recovered (two human VanA phenotype, two human VanB phenotype, two of animal origin, and three human vancomycin sensitive), but bacterial counts were reduced by >6 log_{10} for three and by >7 log_{10} for six. After exposure at 65°C for 10 minutes, 21 (93.1%) isolates were recovered (including the two *E faecalis* strains) and seven showed <5 log_{10} reduction in viable count. All 29 isolates survived heating at 65°C for three minutes and at 60°C for 30 minutes (<5 log_{10} reduction in viable count). No differences were observed between VRE and sensitive strains or between isolates of human and food origin (table 1). Eleven isolates were tested in duplicate and no differences in results were observed.

### Discussion

Enterococci have long been recognised as relatively heat tolerant bacteria, surviving at 60°C for 30 minutes. Concern has been expressed that some strains may be able to survive the temperature/time parameters recommended by the Department of Health for the disinfection of used and infected linen (65°C for 10 minutes or 71°C for three minutes), or the British Standard for heat disinfection of bedpan washers (80°C for one minute). None of the isolates in the present study survived exposure to heat at 80°C for one minute and it is unlikely that washer disinfectors pose a risk of cross infection, provided that they are used and maintained properly. Exposure to heat at 71°C resulted in a notable reduction in bacterial counts for all isolates (>6 log_{10}). Although very low number of organisms were recovered from a few strains, we consider such a reduction in viable counts to be effective disinfection. However, the observation that at 65°C for 10 minutes, seven (24.1%) isolates survived (viable counts were reduced by <5 log_{10}) raises some concerns about the efficiency of one recommended standard for the

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**Table 1** Log_{10} reduction in bacterial counts after exposure at different temperature/time parameters

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>Glycopeptide resistance phenotype</th>
<th>65°C for 10 minutes</th>
<th>71°C for one minute</th>
<th>71°C for three minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;5 log_{10}</td>
<td>5-6 log_{10}</td>
<td>&gt;6 log_{10}</td>
</tr>
<tr>
<td>Human</td>
<td>10</td>
<td>Van A/B</td>
<td>3 (30)</td>
<td>1 (10)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Human</td>
<td>9</td>
<td>Sensitive</td>
<td>1 (11)</td>
<td>4 (44.4)</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>Animal</td>
<td>8</td>
<td>Van A</td>
<td>3 (37.5)</td>
<td>3 (37.5)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>E faecalis</td>
<td>2</td>
<td>Sensitive</td>
<td>0</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td>7 (24.1)</td>
<td>8 (27.6)</td>
<td>14 (48.2)</td>
</tr>
</tbody>
</table>

At 60°C for 30 minutes and at 65°C for three minutes, all isolates showed <5 log_{10} reduction in viable counts.

At 71°C for 10 minutes and at 80°C for one, three and 10 minutes, all isolates showed >6 log_{10} reduction in viable counts.
disinfection of infected linen (65°C for 10 minutes). It must be emphasised that our tests assessed the effect of heat in the absence of a cleaning effect, which in practice will very much reduce the load of bacterial contamination. Recent in situ studies failed to demonstrate survival of enterococci in a modern hospital laundry system. Nevertheless, it may be appropriate to use the higher (71°C) temperature for the laundry process whenever possible. *E. faecium* isolates were more resistant to heat than the two *E. faecalis* control strains. Overall, we found *E. faecium* isolates to be less heat tolerant than others have suggested. These differences are almost certainly due to a combination of variations in methodology and interpretation of log reductions, rather than major strain variations in heat tolerance. These methodologies take no account of the washing effect of medical disinfection processes and we consider that current disinfection guidelines are satisfactory.

Isolates of VRE were provided by Dr B A Oppenheim, Manchester Public Health Laboratory. We thank Dr H Panigrahi for his support in carrying out this study.

The hidden increase in histopathologists' workload

D M Parham

Abstract

The informational content of histopathological reports generated between 1985 and 1995 was assessed. This showed an exponential rise over the past five years. It is postulated that this has arisen from developments in medical science and demands from clinical colleagues. This increase in workload is not addressed by present methods of measurement and has major resource implications.

Keywords: workload, histopathology.

Histopathologists, like other doctors, are under increasing pressure of work. This includes not only their routine work but also teaching, research, continuing medical education, quality assurance, management, administration, and audit. In histopathology the routine work can be crudely quantified by the number of requests. A more formal unit of measurement is the Welch system. This measures the technical requirements in processing the specimen but does not assess a pathologist's input in interpreting or reporting the specimen. This study has examined the increase in the amount of information contained in histopathological reports over the past decade to determine what contribution this has made to the increased workload.

Methods

In East Dorset there is a combined histopathological department serving Poole and Royal Bournemouth Hospitals. Between 1985 and 1995 histopathological reports for all specimens received at the Royal Bournemouth Hospital during the first two complete weeks (Monday to Friday) in February in selected years were examined. All reports were handwritten by the pathologists prior to typing. The number of items of information recorded were quantified. A point of information included details of the nature of the specimen, measurement, histological description, diagnosis, grade, individual prognostic factors, excision margins, etc. During the weeks of the study in 1985, 1990 and 1992 there were three reporting consultant histopathologists. Two of these subsequently retired and their positions were filled by younger consultants. In 1993 there were four and five consultant histopathologists.

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S Panagea and P R Chadwick

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