Vancomycin heteroresistance of *Staphylococcus capitis*

bloodstream isolates

**Running title:** Vancomycin heteroresistance of *Staphylococcus capitis*

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Abstract

Nine *Staphylococcus capitis* from blood cultures of newborns were examined for resistance to vancomycin. Minimum inhibitory concentrations were within the susceptible range, but population profiling revealed a resistant sub-population. Only isolates with the largest subpopulation were identified as heteroresistant to vancomycin by E-test. This finding may have therapeutic implications.
Recent reports indicate the possible emergence of *Staphylococcus capitis* as a significant pathogen causing late-onset sepsis in very low birth weight (VLBW) infants (<1500 g) (6, 16, 18, 20). Reduced susceptibility to vancomycin has been reported in several species of coagulase-negative staphylococci (1, 2, 4, 9, 10, 17); however, there is very little information on the levels of such resistance in *S. capitis*. Reduced susceptibility to vancomycin occurring in methicillin-resistant staphylococci translates into limited treatment options, particularly in newborn infants.

Heteroresistant *S. capitis* may escape detection because MICs of vancomycin of \( \leq 4 \) \( \mu \)g/ml are generally interpreted as susceptible (5). Agar-based screening tests for detecting heteroresistance in staphylococci are simple to perform (14, 15, 19); however sensitivity and specificity of E-test strips are superior (22). Population analysis profiling (PAP) is the most reliable method for detecting heteroresistance, but is time consuming and fails to provide results in a clinically relevant time frame (19). Data on the prevalence and level of vancomycin resistance is essential for assessing clinical relevance and treatment options for infants infected with *S. capitis*. This study examines a collection of nine *S. capitis* isolates obtained from blood cultures of VLBW infants in the Neonatal Intensive Care Unit (NICU) at the Royal Women’s Hospital, Melbourne, Australia between 1998 and 2002 (3). Pulsed-field gel electrophoresis (PFGE) combined with Southern blot analysis and probing with IS256 showed they were closely related yet unique, except for two isolates from the same infant (3). The reference strains used were: *S. capitis* ATCC 27840, *S. aureus* Mu3 (ATCC 700698) and Mu50 (ATCC 700699) (11, 12, 13).
Isolates were screened for vancomycin heteroresistance on brain heart infusion agar (BHIA) (Oxoid Ltd., Hampshire, England) containing 4 µg/ml of vancomycin (VAN 4) (Sigma-Aldrich Pty. Ltd., Sydney, Australia) (12). MICs were determined by conventional methods and by vancomycin and teicoplanin E-test strips (AB Biodisk, Solna, Sweden) (8), taking care to include small colonies within the clear zone. The PAP profiles were interpreted by calculating area under the curve ratios (AUC_{test}/AUC_{Mu3}) with the aid of GraphPad Prism 5 software (San Diego, CA, USA) (19, 21). Since the AUC is affected by the size of the initial inoculum, CFUs were standardized to match the initial inoculum of Mu3 for each replicate. All tests were performed on at least three separate occasions.

The various methods differed in their ability to detect resistant subpopulations of S. capitis; micro broth dilution (CLSI) was the least sensitive, detecting only one resistant isolate, the E-test detected three resistant isolates, while VAN 4 screening and PAP analysis detected resistant subpopulations in all isolates (Table 1). Three isolates produced variable results on the VAN 4 screening plates indicating instability of their heteroresistant phenotype. Visual examination of the PAP graphs showed heterogeneous resistance with strain-dependent differences in the size of the resistant subpopulation (Fig. 1). For the three most resistant strains (Mu50, isolates 15 and 22), there was complete agreement between the results of PAP-AUC analysis, VAN 4 screens and the E-test. Isolate 6 had a very high PAP-AUC value, produced variable screening results and was interpreted as non-heteroresistant by the E-test. The discrepant PAP-AUC value could be explained by the unusual shape of the PAP graph, reflecting high colony counts on VAN 2 followed by a sharp drop on VAN 3 plates (Fig. 1). All other isolates and
reference strains showed PAP-AUC values close to those of Mu3, but were not heteroresistant according to the E-test. Ranking of isolates according to the size of the resistant subpopulation was generally similar by PAP-AUC analysis and MICs generated by E-tests.

These results suggest that a vancomycin heteroresistant subpopulation is present in all isolates of *S. capitis*. They confirm the unreliability of conventional MICs except for the most resistant isolates and show that the E-test and the VAN 4 screening tests detect only the most resistant isolates, but fail to detect isolates with smaller resistant subpopulations, which could be enriched during vancomycin therapy.

This report is, to the best of our knowledge, only the second to describe a cluster of vancomycin heteroresistant *S. capitis* among VLBW infants in a NICU. Van Der Zwet *et al.* (18) demonstrated variable proportions of resistant subpopulations in *S. capitis* isolates with closely related or identical genetic profiles. These data suggest that heteroresistant *S. capitis* strains, which are undetectable by standard MIC measurement, could be emerging pathogens in NICUs.

The origin of these nine closely related vancomycin-heteroresistant isolates, present in the NICU for at least 5 years, is enigmatic. It is possible that frequent vancomycin use in the unit led to enrichment of resistant cells present in a common ancestor, resulting in subpopulations of variable size. Although the outcome for *S. capitis* septicaemia in VLBW infants is generally good in our unit, it is of concern that further increases in vancomycin resistance could occur. Our observation of a resistant subpopulation in all *S. capitis* examined, including ATCC 27840, which was deposited in 1975, suggests that heteroresistance to vancomycin could be an intrinsic property of *S. capitis*. Although
more isolates of *S. capitis* need to be examined, given that the relationship between vancomycin heteroresistance and treatment failure is still uncertain (7), it would be wise to consider all isolates as potentially resistant and to monitor clinical responses to vancomycin very carefully, particularly with more deep-seated infections such as osteomyelitis or infections where antibiotic penetration is an issue, such as endocarditis and meningitis. There is an urgent need for more data on the clinical relevance of vancomycin heteroresistance in staphylococci, in particular *S. capitis*, and for the development of reliable, rapid and inexpensive methods to detect such resistance (7).

This work was supported by internal funding from the School of Applied Sciences, RMIT University, Australia and the Department of Microbiology and Infectious Diseases, Royal Children’s and Royal Women’s Hospitals, Australia.
REFERENCES


Legend to Table 1.

a Mean (SE) area under the curve (AUC) determined by population analysis profiling (PAP); \( \frac{AUC_{\text{test}}}{AUC_{\text{Mu3}}} \): a ratio of \( \leq 0.90 \) was interpreted as susceptible (S), 0.90 to 1.3 as heteroresistant (H), and \( \geq 1.3 \) as intermediate (I) (18).

b Number of colonies on BHIA containing 4 µg/ml vancomycin after 48 h. CG, confluent growth on at least 3 of 4 replicates; V (n), variable results, R = potentially resistant, H = possible heteroresistant.

Isolates 8a and 8b were from the same infant, collected on different occasions.
Fig. 1. Population analysis profiling of *S. capitis* strains isolated from blood cultures of infants. Dotted line = Mu3, standard line = heteroresistant, heavy line = intermediate. The means of three separate investigations and standard errors are presented.
Table 1. Vancomycin resistance of *S. capitis* isolates.

<table>
<thead>
<tr>
<th>Isolate or strain number</th>
<th>PAP-AUC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Vancomycin screen (4µg/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CLSI Vancomycin/Teicoplanin E-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC&lt;sub&gt;TEST&lt;/sub&gt;/MU3</td>
<td>Interpretation</td>
<td>No. of colonies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. capitis</em> 6</td>
<td>1.7 (0.15)</td>
<td>I</td>
<td>7, 86, CG</td>
</tr>
<tr>
<td><em>S. capitis</em> 22</td>
<td>1.7 (0.12)</td>
<td>I</td>
<td>CG</td>
</tr>
<tr>
<td><em>S. capitis</em> 15</td>
<td>1.3 (0.08)</td>
<td>I</td>
<td>CG</td>
</tr>
<tr>
<td><em>S. capitis</em> 17</td>
<td>1.2 (0.04)</td>
<td>H</td>
<td>4</td>
</tr>
<tr>
<td><em>S. capitis</em> 8a</td>
<td>1.1 (0.03)</td>
<td>H</td>
<td>2</td>
</tr>
<tr>
<td><em>S. capitis</em> 9</td>
<td>1.1 (0.03)</td>
<td>H</td>
<td>0, 2, CG</td>
</tr>
<tr>
<td><em>S. capitis</em> 16</td>
<td>1.1 (0.03)</td>
<td>H</td>
<td>1</td>
</tr>
<tr>
<td><em>S. capitis</em> 8b</td>
<td>1.0 (0.04)</td>
<td>H</td>
<td>12</td>
</tr>
<tr>
<td><em>S. capitis</em> 18</td>
<td>1.0 (0.05)</td>
<td>H</td>
<td>0, 36, CG</td>
</tr>
<tr>
<td><em>S. capitis</em> ATCC 27840</td>
<td>1.0 (0.01)</td>
<td>H</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> Mu 3</td>
<td>1</td>
<td>H</td>
<td>14</td>
</tr>
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</table>
FIG. 1. Population profile analysis of *Staphylococcus capitis*