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Project Title: Glycopeptide heteroresistance in coagulase-negative staphylococcus: clinical implications, risk factors, and surveillance

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Summary (250 words max single spaced):

Coagulase negative *Staphylococci* (CoNS) remain the leading cause of line-associated bacteremia in adults and of late-onset sepsis in neonatal patients. As vancomycin is commonly employed to treat such infections, there are emerging concerns associated with glycopeptide resistance among CoNS. Of additional concern is the development of heteroresistance to glycopeptides - the growth of a small subpopulation of resistant bacteria in a larger population that is susceptible. The risk factors for the development of heteroresistance and the clinical course associated with it for neonatal patients are poorly studied, and such features for adult inpatients have yet to be explored. Our study began with a pre-existing cluster of heteroresistant CoNS cases at the Health Sciences Centre neonatal ICU and explored the clinical impact and risk factors associated with heteroresistant CoNS bacteremia in neonatal patients. To assess the scope of the phenomenon, other inpatient wards were surveyed for heteroresistant CoNS, including the neurosurgery ward and the dialysis unit. Our study reaffirms the increased morbidity and mortality observed in NICU patients affected by heteroresistant CoNS and identified factors amongst adult inpatients that may prompt for appropriate laboratory screening for heteroresistance, including: previous exposure to vancomycin, significant underlying comorbidity, advanced age, and prolonged use of central vascular catheters. Due to the often significant comorbidity experienced by adult patients affected by heteroresistant CoNS, a definitive description of the clinical impact and outcome of these infections remains unclear.

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Introduction and Background

Coagulase-negative staphylococci (CoNS) include a large number of species of human pathogens that can have a highly variable impact on human health. Among neonatal patients, they are also the leading cause of late-onset neonatal sepsis and also contribute significantly to early morbidity and extended length of stay in hospital (1, 2). By contrast, in adult patients CoNS have traditionally been associated with low virulence and relatively low risk of morbidity and mortality, even in the absence of appropriate therapy. That being said, CoNS have been identified as a leading cause of hospital-acquired bloodstream infections, and are associated with prolonged hospital stays in the adult population (3). The prolonged use of central lines has been shown to increase the risk of bacteremia, with CoNS routinely ranking within the most common causative pathogens (4).

CoNS are frequently resistant to first-line antibiotics, as such vancomycin is commonly used in treating CoNS bacteremia. Unfortunately, there are mounting concerns about vancomycin resistance (5, 6). A specific issue is the recent identification of intermediate resistance to glycopeptide antibiotics among CoNS (5). This intermediate resistance is brought about by the synthesis of a thickened cell wall with numerous free D-ala-D-ala residues that promote trapping of the glycopeptide within the cell wall, thus preventing its action. This is thought to be accomplished by either increased peptidoglycan synthesis or reduced turnover of peptidoglycan (6). Detection of intermediate resistance in CoNS can be difficult in the clinical laboratory, especially when it manifests under the guise of heteroresistance. Heteroresistance refers to a phenomenon where a strain of bacteria appears to be susceptible to an antibiotic, but contains a small subpopulation with reduced susceptibility to that antibiotic (1). This is of concern, there is a relative paucity of information on the clinical significance of heteroresistance, which raises concerns regarding potentially increased morbidity from inadequate antibiotic treatment.

A cluster of *Staphylococcus capitis* demonstrating heteroresistance to vancomycin (hVISC) was detected in a local tertiary care neonatal intensive care unit in 2013. An investigation revealed this cluster had been active from 2008 to 2013. While hVISC have been reported in other NICUs (1, 7, 8), there is limited literature to define their clinical impact. We present here a retrospective review of the clinical features of infants infected with hVISC compared to infants infected with vancomycin susceptible CoNS. Additionally, we investigated other areas in the same health care facility to assess the scope of potential heteroresistance and assess their clinical impact in adults.

Materials and Methods

Case Selection

The initial cluster of 14 infants affected by hVISC was identified as part of clinical care activities. Since all *S. capitis* samples within the study period were found to be heteroresistant, a further ten isolates containing other CoNS species were selected for further analysis. For the non-NICU surveillance aspect of this project, 50 isolates of CoNS were selected from positive blood and cerebrospinal fluid (CSF) cultures obtained from Health Sciences Centre patients between January 2010 and December 2012 inclusive. Of these, 10 isolates were chosen from the dialysis unit; 10 isolates were chosen from an inpatient neurology ward while the remaining 30 isolates came from other wards. Particular focus was placed on dialysis and neurology patients due to the high usage of invasive devices (e.g. central venous catheter, lumbar drains) on these units. Isolates were selected semi-randomly with every third patient being chosen.

Heteroresistance Testing

Selected isolates had been stored at -80°C in skim milk. Each isolate was subcultured twice from frozen stock onto blood agar and incubated in ambient air at 35°C for 24 hours. Each isolate was then tested for susceptibility to vancomycin using a standard Etest® as well as a macrodilution method Etest®.

For the standard Etest®, isolated colonies from the blood agar plates were chosen and inoculated into 3 mL of 0.45% saline in sufficient quantity to equal a 0.5 McFarland turbidity standard. This inoculum was then swabbed onto Mueller Hinton agar plates in order to generate a uniform lawn of bacterial growth. After drying, a vancomycin Etest® strip was applied. Plates were examined after 24 hours of incubation in ambient air at 35°C and were compared to a vancomycin-intermediate *Staphylococcus aureus* (VISA) standard (Mu50) as well as a heteroresistant VISA (hVISA) standard (Mu3). Areas of clearing were carefully examined for haze and isolated colonies, and the minimum inhibitory concentration (MIC) was recorded from the Etest® strip accordingly. To be interpreted as intermediately resistant to vancomycin, isolates must have demonstrated a MIC of 4-8 µg/mL.

The macrodilution method Etest® procedure was also carried out on each isolate and controls using the method described by Wootton et al (7). In brief, isolated colonies were chosen from previously incubated blood agar plates as described for the standard Etest® procedure. These colonies were inoculated into Mueller Hinton broth in sufficient quantity to equal a 2 McFarland turbidity standard. Inoculates were then swabbed onto 90 mm brain-heart infusion (BHI) agar plates (manufactured by BD™) to generate a uniform lawn of growth and the plate was allowed to dry. To each plate, a vancomycin Etest® strip and a teicoplanin Etest® strip were applied. Plates were then incubated in ambient air for 48 hours at 35°C. Plates were examined twice – once at 24 hours and a second time at 48 hours of incubation – to determine MICs with respect to vancomycin and teicoplanin. Isolates were defined as heteroresistant if either 1) MICs for both vancomycin and teicoplanin were both ≥ 8 µg/mL or 2) if the teicoplanin MIC was ≥ 12 µg/mL irrespective of the result for vancomycin.

Confirmatory testing of those isolates designated as heteroresistant will be carried out by population analysis profiling using area under the curve (PAP-AUC), as has been previously described as the gold standard for identification of heteroresistant strains (7). This testing had been performed on the 14 previously investigated heteroresistant NICU cases.

Assessing Relatedness of Heteroresistant Isolates

Isolates identified as heteroresistant by the macrodilution method were tested for same-species relatedness using pulsed field gel electrophoresis (PFGE). Isolates were incubated in brain-heart infusion (BHI) broth overnight, then embedded in low-melt agarose plugs. These plugs were treated with a Tris-HCL-NaCl-EDTA-deoxycholate-N-lauroylsarcosine lysis buffer for one hour at 37°C followed by treatment with an EDTA-N-lauroylsarcosine-Proteinase K buffer for one hour at 37°C to facilitate cell lysis and protein digestion, respectively. Gel plugs containing DNA of interest were digested with a *Sma* restriction enzyme and were mounted along with two *Salmonella* serotype Braenderup plugs to act as standards. *Salmonella* plugs were digested with an *Xba* restriction enzyme. PFGE was run on a CHEF-DR® III electrophoresis apparatus for 19 hours. The resulting gel was stained with SYBR® Green and banding patterns were captured via Alphamager® and analyzed for similarity with BioNumerics®.

Clinical Data Collection

The patient records corresponding to the samples identified in the laboratory were reviewed and abstracted. The information captured included: patient gender; patient age at admission; dates of admission and discharge; dates and results of initial and follow-up blood or cerebrospinal fluid cultures; results of antibiotic sensitivity testing; duration of usage of central venous or arterial catheters; description of clinical deterioration including usage and duration of vasopressors and/or intubation; dates and durations of antibiotic therapy; antibiotic agents used; serum vancomycin levels during and up to 1 month prior to CoNS bacteremia; evidence of recurrence of infection; duration of treatment of recurrent infections and agents used for therapy; and evidence of mortality due to CoNS bacteremia. The results of the Etest® procedures for each case were also recorded on the data capture sheets. Each case was assigned a random 4-digit number as an identifier. Aside from date of birth, no other patient identifiers were included in the data capture sheets. All data capture sheets were stored on a BitLocker® encrypted USB drive.

Data Analysis

Statistical analysis was performed using SPSS. Each clinical variable was assessed for normality using a Shapiro-Wilk test, and then comparisons between heteroresistant and non-heteroresistant cases were conducted by use of two-sample t test in cases of normally-distributed variables or by Mann-Whitney U test in cases of skewed distributions. All comparisons of categorical data employed Fisher's exact test. Testing for normality and tests of comparison were conducted as previously described.

Approval and Support

This project has been granted ethics approval by the University of Manitoba Bannatyne Campus Research Ethics Board. Research funding and stipendiary support were provided by a Health Science Centre Medical Staff Research Award and by an endowment from the Manitoba Medical College Foundation, respectively.

Results

Isolate Screening and Susceptibility Testing

All 14 isolates of *S. capitis* from the NICU during the study period screened positive for heteroresistance. To ensure that the test was performing correctly in addition to the Mu3 and Mu50 control strains, 10 *S. capitis* isolates from another Winnipeg hospital were tested. The controls behaved as expected and all of the other strains screened negative. Of the 10 non-*S. capitis* NICU isolates, none screened positively for heteroresistance to either of vancomycin or teicoplanin.

Of the 50 chosen isolates for adult patients 8 (13%) were identified from the macrodilution method Etest® as being heteroresistant. All 8 isolates met or exceeded the established cut-point of a MIC ≥ 12 $\mu\text{g}/\text{mL}$ for teicoplanin, and none of the isolates met or exceeded the cut-point of a MIC ≥ 8 $\mu\text{g}/\text{mL}$ for vancomycin. Of these isolates, 2 (25%) were obtained from patients attending the HSC dialysis unit, 1 (12.5%) was obtained from a HSC adult emergency department patient, and 5 (62.5%) were obtained from a variety of inpatient wards, including one from the neurosurgery ward. The oncology ward was the source of 2/8 (25%) heteroresistant isolates.

A number of different species of CoNS were screened positive as heteroresistant, including (number of isolates in parentheses): *S. epidermidis* (3), *S. hominis* (1), *S. haemolyticus* (3), and *S. pettenkoferi* (1).

Antibiotic Susceptibility of Isolates

Refer to Table 1 below, which summarizes average MIC values from standard microdilution and macrodilution Etest® methods for heteroresistant and non-heteroresistant isolates obtained for all cases.

Relatedness of Isolates – Adult Inpatient Cases

Of the 8 isolates identified as heteroresistant by the macrodilution Etest® method, there were two pairs of isolates that presented virtually identical banding patterns on PFGE. One pair consisted of two *S. epidermidis* isolates that were collected in 2010 and 2012. The other pair consisted of an isolate of *S. epidermidis* and an isolate of *S. hominis* both collected in 2010.

Clinical Impact of Glycopeptide Heteroresistance – NICU Cases

Patient Demographics

NICU cases involving heteroresistant CoNS and non-heteroresistant CoNS were compared in terms of proportion of genders, length of hospital stay, central line days prior to the first positive blood culture, and duration of exposure to antimicrobial therapy prior to the first positive culture. These data are summarized in Table 2. No significant differences were found in these factors save for prior exposure to vancomycin, which was significantly different between the heteroresistant group (mean 4 days, ranging from 0 to 19 days) and the non-heteroresistant group, which had only a single case of prior vancomycin use lasting 3 days.

Clinical Consequences

Cases with heteroresistant CoNS and cases lacking heteroresistant CoNS were compared for clinical course on the basis of duration of bacteremia, mention of clinical deterioration in the chart, usage of vasopressors, intubation or increases in ventilation, and evidence of gut dysfunction. Though 7 heteroresistant and 7 non-heteroresistant cases involved multiple positive blood cultures, no significant differences were found in terms of duration of bacteremia (6.4 days versus 7.4 days; $p = 0.609$ by two-sample t-test). No significant differences were found in the proportion of cases with clinical deterioration ($p = 0.163$ by Fisher's exact test), cases involving vasopressor usage ($p = 0.204$ by Fisher's exact test), or intubation or increases in ventilation ($p = 0.635$ by Fisher's exact test). Only NICU patients in the heteroresistant group showed any evidence of gut dysfunction during bacteremia, with 9 of 14 cases citing such dysfunction. Although the proportion of cases involving vasopressor use was not significantly different, the mean duration of vasopressor usage was significantly different (11.3 versus 3.6 days for non-heteroresistant cases; $p = 0.044$ by two-sample t test).

Outcomes

Clinical outcomes were compared on the basis of recurrent infection and mortality due to sepsis or other causes. None of the 10 infants in the non-heteroresistant group experienced a recurrence of infection with the same organism, while 3 infants in the heteroresistant group did have recurrence. The difference in proportions was not significant ($p = 0.229$ by Fisher's exact test). There was 1 mortality in the non-heteroresistant group, while there were 3 mortalities in

the heteroresistant group. Of these, 2/3 of the mortalities in the heteroresistant group were attributed to sepsis. There was no significant difference in the proportion of mortalities between the two groups ($p = 0.604$ by Fisher's exact test), nor in the proportion of mortalities attributed to sepsis ($p = 1.00$ by Fisher's exact test).

Therapy

Treatment of bacteremia was compared between heteroresistant and non-heteroresistant cases on the basis of mean days of antimicrobial therapy as well as mean serum vancomycin levels before and after resolution of bacteremia (as determined by a negative blood culture). There was no significant difference between the duration of treatment between these groups ($p = 0.123$ by Mann-Whitney U test). There was a significant difference between the groups for both pre-negative culture serum vancomycin levels (7.8 mg/L versus 19.3 mg/L in the non-heteroresistant group; $p = 0.000$ by Mann-Whitney U test) and post-negative culture serum vancomycin levels (9.4 mg/L versus 18.7 mg/L in the non-heteroresistant group; $p = 0.001$ by Mann-Whitney U test).

Clinical Impact of Glycopeptide Heteroresistance – Adult Inpatient Cases

Patient Demographics

Of the 50 selected isolates, the corresponding clinical records could only be obtained for 39 patients; the excluded patients were the 10 sampled from the HSC dialysis unit as well as one additional inpatient case for whom a record could not be obtained. As such, only 6 of the 8 identified heteroresistant cases were analyzed. Cases were compared in terms of proportion of genders, mean age at admission, mean length of stay, mean number of central line days prior to the first positive blood or cerebrospinal fluid (CSF) culture, and mean duration of antimicrobial exposure prior to the first positive blood or CSF culture. These data are summarized in Table 3.

There were no significant differences between heteroresistant and non-heteroresistant cases in any of these demographic or exposure factors. It should be noted that the majority (4 of 6) of the heteroresistant cases involved the use of one or more central lines prior to the first positive culture. Furthermore, half of these cases involved some exposure to vancomycin prior to the first positive culture.

Clinical consequences

Adult inpatient cases involving heteroresistant CoNS were compared to those not involving heteroresistant CoNS in terms of clinical course. This comparison was based on the mean duration of bacteremia, proportion of cases involving clinical deterioration as noted in the chart, as well as proportion of cases requiring vasopressors, intubation, or increases in ventilation.

Of the six cases analyzed that yielded heteroresistant CoNS, 3 involved multiple positive blood cultures. However, there was no significant difference in the duration of bacteremia between the heteroresistant and non-heteroresistant groups (3.8 days versus 4.9 days; $p = 0.846$ by Mann-Whitney U test). In terms of clinical course, only 1 of the 6 patients in the heteroresistant isolate group was noted to have clinical deterioration. There was no significant difference in the proportion of patients who experienced clinical deterioration ($p = 1.00$ by Fisher's exact test), required use of vasopressors ($p = 0.287$ by Fisher's exact test), duration of vasopressor use

(one patient from each group required 1 day of vasopressors), or required intubation or increases in ventilator support ($p = 0.287$ by Fisher's exact test) between groups.

Clinical Outcomes

Clinical outcomes were compared on the basis of recurrent infection and mortality due to sepsis or other causes. There is a statistically significant difference in the proportion of mortalities between the heteroresistant cases and non-heteroresistant cases ($p = 0.036$ by Fisher's exact test). However, the difference in proportion between mortalities thought to be caused by sepsis versus other causes was not significant ($p = 1.00$ by Fisher's exact test). Additionally, while sepsis was considered a contributory cause of mortality in these patients, it was not cited as a sole cause. Only one case experienced recurrence of infection with the same organism, which was not heteroresistant.

Therapy

Treatment of bacteremia was compared between heteroresistant and non-heteroresistant cases on the basis of mean days of antimicrobial therapy as well as mean serum vancomycin levels before and after resolution of bacteremia. Duration of treatment considered vancomycin separately from other antimicrobial agents.

Difference in the duration of vancomycin therapy between heteroresistant and non-heteroresistant cases approached significance (4.5 ± 4.5 days versus 12.8 ± 13.8 days; $p = 0.063$ by Mann-Whitney U test). Similarly, difference in duration of other antimicrobial therapy also approached significance between these groups (2.7 ± 2.4 days versus 10.5 ± 11.1 days; $p = 0.076$ by Mann-Whitney U test). There was no significant difference between pre-negative culture serum vancomycin levels (16.9 mg/L versus 15.5 mg/L for non-heteroresistant cases; $p = 0.673$ by two-sample t test) or post-negative serum vancomycin levels (14.5 mg/L versus 15.9 mg/L; $p = 0.661$ by two-sample t test) between these groups.

Pooled Heteroresistant Organism Results - Adult Inpatient and NICU Cases

In total, 20 clinical cases involving heteroresistant CoNS bacteremia were investigated – 14 from the NICU and 6 from various HSC adult wards. Of these cases, 10 involved prolonged bacteremia (defined as the receipt of more than one consecutive positive culture), while 9 involved clearance on bacteremia on a follow-up culture. The mean duration of bacteremia for the cases of prolonged bacteremia was significantly different from the mean duration of bacteremia in the non-prolonged cases (9.1 days versus 1.9 days; $p < 0.001$ by two-sample t-test).

Mean length of stay for those cases involving prolonged bacteremia was 77.4 days (ranging from 24 to 131 days), while mean length of stay for cases without prolonged bacteremia was 69.4 days (ranging from 1 to 138 days). There was no significant difference between lengths of stay of these groups ($p = 0.700$ by two-sample t test). Additionally, there was no significant difference between the number of central line days occurring before the first positive culture in either group (11.5 ± 2.1 days versus 30.6 ± 22.2 days in those patients with prolonged bacteremia; $p = 0.412$ by Mann-Whitney U test). Moreover, there was no significant difference in the proportion of patients who showed evidence of clinical deterioration ($p = 1.00$ by Fisher's exact test) or required intubation or increases in ventilator support ($p = 1.00$ by Fisher's exact

test). All patients requiring vasopressor use had prolonged bacteremia, with a mean duration of 8.8 ± 7.2 days.

In terms of prior antibiotic exposure, 8 of the patients without prolonged bacteremia were exposed to vancomycin (mean 3.1 days) or other antibiotic therapy (mean 8.3 days) in the month preceding CoNS bacteremia, while 9 of the patients with prolonged bacteremia were exposed to vancomycin (mean 3.8 days) or other antibiotics (mean 9.3 days) over a similar period, with no significant difference in this duration ($p = 0.743$ and 0.888 for vancomycin and other antibiotic exposure, respectively, by Mann-Whitney U test).

The mean treatment durations between patients with prolonged bacteremia (18.7 ± 17.3 days) and without prolonged bacteremia (8.8 ± 3.5 days) were not significantly different ($p = 0.277$ by Mann-Whitney U test). Moreover, the mean vancomycin levels during treatment for the prolonged bacteremia group were not significantly different than that for the other cases (9.5 mg/L versus 8.7 mg/L, respectively; $p = 0.808$ by two-sample t test), nor were the mean vancomycin levels that were obtained after resolution of the bacteremia though this difference did approach significance (12.1 mg/L versus 7.9 mg/L for the non-prolonged bacteremia group; $p = 0.065$ by two-sample t test).

While bacteremia evidently persisted longer in one group of patients than in another despite no significant difference in serum vancomycin levels, there was no significant difference in the cases of prolonged bacteremia in terms of mean standard Etest® vancomycin MIC (3.2 versus 3.0 $\mu\text{g}/\text{mL}$; $p = 0.536$ by Mann-Whitney U test), macrodilution vancomycin MIC (10.2 versus 8.3 $\mu\text{g}/\text{mL}$; $p = 0.837$ by Mann-Whitney U test), or macrodilution teicoplanin MIC (22.2 versus 26.3 $\mu\text{g}/\text{mL}$; $p = 0.758$ by Mann-Whitney U test).

In terms of outcomes, there was no difference in the proportion of patients with prolonged bacteremia who experienced a recurrence of infection ($p = 1.00$ by Fisher's exact test). However, recurrences were quite rare in this sample, overall with only 3 recurrent infections noted, only 1 of which was originally associated with prolonged bacteremia. Finally, there was no difference between these groups in terms of the proportion of mortality ($p = 0.294$ by Fisher's exact test). Of the patients who did not experience prolonged bacteremia, none had mortality attributed to sepsis, and of the 4 mortalities in the group of patients with prolonged bacteremia, only 2 were attributed to sepsis.

Discussion

Though the risk factors and clinical course associated with heteroresistant CoNS are areas still requiring elucidation, existing studies have begun to address this topic, at least with respect to NICU cases. Our study aimed to further investigate these factors with respect to NICU patients, but also to include a similar investigation of adult inpatients presenting with heteroresistant CoNS bacteremia. Our findings suggest a difference in the impact and prevalence of this phenomenon with this population. As well, prior studies have mainly focused on heteroresistant *Staphylococcus capitis* (1, 8). As *S. capitis* is rarely associated with adult inpatient cases (8), our study included other species in an attempt to gauge the prevalence of heteroresistance in other inpatient settings. Finally, there are few indications suggested in the literature for heteroresistance screening, with persistent bacteremia despite optimal treatment and removal

of central lines being one of the only existing recommendations (5). We offer additional considerations for screening based on the clinical courses of the heteroresistant cases analyzed.

As our study considered all species of CoNS, it may be that glycopeptide heteroresistance is a more common phenomenon amongst certain species. Given our finding that *S. capitis* represented the majority of heteroresistant cases, particularly when including NICU patients, it could be that *S. capitis* is more prone to demonstrate heteroresistance because of the degree of usage of glycopeptide antibiotics in the NICU setting. Alternatively, *S. capitis* may simply have some intrinsic predisposition to developing heteroresistance by a mechanism yet to be investigated, as has been previously suggested (8). Of the adult inpatient cases, *S. epidermidis* and *S. haemolyticus* made up the majority (6 of 8) of the heteroresistant isolates. This somewhat echoes a previous study focused on glycopeptide heteroresistance in CoNS, which also found that *S. haemolyticus* and *S. epidermidis* made up the majority of the heteroresistant isolates (6), as well as previous observations of *S. haemolyticus* developing vancomycin resistance (1, 6). It could be that the ubiquity of these organisms, alone, accounts for their likelihood to develop heteroresistance in the face of antibiotic exposure and/or consistent use of central lines or other invasive devices. Further testing of other CoNS in the neonatal population, as well as acquisition and testing of additional isolates from adult inpatients is necessary to clarify if such tendency – if any – exists.

Despite assessing 60 banked isolates of CoNS collected over 3 years, only 13% of cases yielded a heteroresistant organism. This would appear to suggest that the phenomenon of glycopeptide heteroresistance in CoNS is uncommon overall. Heteroresistant CoNS appears to be associated with inpatients at the extremes of age or with marked immunosuppression. Given that several of the cases studied involved use of central lines for dialysis or treatment of hematologic malignancy, it is likely that the use of indwelling or invasive devices are also important risk factors for the introduction of heteroresistant CoNS in both adult and neonatal patients. This may simply be secondary to the increased risk of CoNS bacteremia in such cases (8), compounded by the additional use of vancomycin for treatment of CoNS bacteremia, at least in the NICU setting (5).

While our study shows that the clinical course associated with glycopeptide heteroresistant CoNS bacteremia is not necessarily more severe or complicated than similar infections by non-heteroresistant CoNS in adults, the PFGE findings do suggest that these organisms may be more persistent, an observation of some strains made previously in the literature (1). In our study, one pair of *S. epidermidis* isolates provided banding patterns on PFGE that were virtually identical despite having been collected two years apart and from distinct locations within HSC. This may suggest a sort of trade-off between virulence and glycopeptide resistance, perhaps due to the expense incurred by synthesis of a markedly thickened cell wall (6). Nevertheless, it remains unclear why some strains persist while others do not.

Heteroresistant CoNS has been associated with vancomycin usage at least one week prior to the isolation of a heteroresistant organism (1, 5, 9). The NICU cases analyzed in our study seem to support this finding. Further, it has been previously suggested in the literature that glycopeptide heteroresistance is associated with prolonged bacteremia and increases in morbidity and mortality in NICU patients (5). While several cases of heteroresistant CoNS did

show prolonged bacteremia in our study, so too did several non-heteroresistant cases, suggesting that there are, perhaps, other factors responsible for this discrepancy. Nevertheless, our study does support the existing finding that persistent bacteremia despite appropriate vancomycin therapy should prompt consideration of a heteroresistant CoNS (5).

While recent vancomycin exposure is cited as an independent risk factor for development of heteroresistance in neonatal patients (1, 5, 9), our study could show no significant difference between the duration of prior vancomycin usage in heteroresistant and non-heteroresistant cases for adult inpatients. In contrast, prior exposure to vancomycin was a statistically significant risk for development of heteroresistant bacteremia in NICU patients. This difference suggests that additional factors beyond vancomycin exposure are likely at play. These could include the presence of a persistent heteroresistant in the clinical care environment, presence of a central line, impaired immunity, or other yet to be defined factors.

The risk factors and course associated with glycopeptide heteroresistant CoNS remain less clear for adult inpatients. Currently, it is suggested that vancomycin heteroresistant staphylococci are more likely to be hospital-acquired and to be associated with intravascular catheters (9). Indeed, our findings also suggest that the majority of heteroresistant cases are associated with central line use and were isolated during an inpatient stay. However, two of the heteroresistant isolates were taken from patients prior to admission to an inpatient ward, thus further consideration may need to be given to risk factors that would be present outside of the hospital setting.

It has also been suggested that heteroresistance is associated with an increased mortality rate in adults (9), but both the literature and our study have not identified a definite link between heteroresistant CoNS bacteremia and mortality. In our study, the adult inpatient heteroresistance cases showed clinical courses that were not appreciably or significantly different from those associated with non-heteroresistant CoNS, causing no greater proportion of morbidity or mortality based on the information gathered. When mortality did occur, sepsis was cited as a possible contributory factory, but was never the sole cause irrespective of the presence of a heteroresistant organism. It is challenging, however, to make definitive determinations on the degree of mortality attributable to heteroresistant CoNS in the adult patients in our study due to the often considerable morbidity these patients already had. That stands in contrast to findings for neonatal patients (5). Indeed, of the neonatal cases analyzed in our study, only those involving a heteroresistant organism concluded in mortality due to sepsis. As well, we found that neonatal patients persistently infected with a heteroresistant CoNS required more days of vasopressor use during their treatment. It is also of note that all heteroresistant cases that involved vasopressor usage were also those that involved a persistent bacteremia, though it is unclear if persistent bacteremia is an independent predictor of the need for vasopressors in such cases. Additional cases would need to be considered to substantiate this potential link.

An additional concern with glycopeptide heteroresistant CoNS bacteremia is that treatment may be inadvertently suboptimal, leading to insufficient serum antibiotic levels to clear the heteroresistant subpopulation; however, this has not been shown, in neonatal patients, to be a definite cause of prolonged bacteremia or recurrent infection (1). In our study, there was only a single instance of recurrent infection, which did not involve a heteroresistant organism, thus

supporting this finding for adult inpatients. The study's findings for neonatal patients, however, do suggest a higher rate of recurrent infection among those cases involving a heteroresistant organism. As all of these cases involved heteroresistant *Staphylococcus capitis*, it is uncertain if this higher rate of recurrence can be attributed to glycopeptide heteroresistance or to other factors that may enhance the virulence of *S. capitis*.

If recurrence of infection is used as a metric of optimal treatment, however, then there may be some grounds to suggest that, in NICU patients affected by heteroresistant CoNS, typical treatment for CoNS bacteremia may be insufficient. If treatment duration is considered a metric of the severity of infection, then only in the adult population is there a significant difference in treatment duration, though this may be exaggerated by the fact that 3 of the 6 cases involved premature cessation of antimicrobial therapy for reasons of palliative care or death. Thus, while the data suggest that adult inpatient may face a less severe clinical course when a heteroresistant organism is involved, the particulars of the cases studied make such a distinction unclear at this point.

Perhaps an even larger concern, though, is the lack of efficient screening methodologies for glycopeptide heteroresistance, with the phenomenon typically being missed by conventional susceptibility testing (5). Indeed, 7 of the heteroresistant isolates from adult patients were identified as vancomycin susceptible by ViTEK® testing. As the Etest® macrodilution method requires in excess of 48 hours to perform, and the gold standard of PAP-AUC analysis is more time-consuming still, it would be unreasonable to implement facility-wide screening for heteroresistance. Moreover, based on the survey of HSC inpatient wards, there does not seem to be any one location that is arguably more prone to this phenomenon. As well, the literature suggests that, when deprived of vancomycin exposure in long-term storage, heteroresistant strains may lose expression of this phenotype (6), thus the true scope of the phenomenon may not be easily detectable by current screening methods. There are some common elements to the patient cases, however, that yielded heteroresistant organisms and may, thus, be considered factors that could prompt consideration for Etest® macrodilution screening.

Surveillance could be considered in areas where: patients are likely to present at the extremes of age, such as a NICU or a geriatric medicine ward; patients are often in a state of prolonged immune compromise (e.g. an inpatient oncology ward); patients present with either or both of these elements and there is considerable use of internalized foreign bodies such as central venous or arterial catheters, or joint implants. In the extremes of age, it may also be prudent to consider screening for this phenomenon when bacteremia is either prolonged or recurrent despite adequate treatment. While no such recurrences were noted in the adult population studied, these risk factors still bear consideration for NICU patients and support current findings to that end (5). For adult inpatients, some consideration for screening could be given to those patients who possess the aforementioned risk factors and who present with long-standing comorbidities – of the 8 heteroresistant cases identified, 2 involved attendance to a dialysis unit, 3 involved a comorbid hematologic malignancy, and 1 involved a case of infectious endocarditis immediately prior to CoNS bacteremia.

Our study was subject to important limitations. The number of adult inpatients presenting with a heteroresistant CoNS bacteremia was quite small, and it was often not possible to ascertain the exact cause of mortality or to separate the contributions made by underlying disease and the

infecting organism. Further study must be done of such cases in order to make this determination. A major limitation of our study is the relative scarcity of heteroresistant cases to analyze, particularly among adult inpatients. This makes it challenging to discern any particular trends in clinical course, outcomes, or response to treatment for these cases. As well, the clinical courses for two of the heteroresistant cases could not be further analyzed due to lack of access to relevant records. Since some heteroresistant cases involved patients with extreme underlying illness, a true estimate of the extent or success of treatment or the clinical course associated with heteroresistant CoNS bacteremia could not be made due to palliative care decisions involving termination of treatment.

In conclusion, our study reaffirms the link between glycopeptide exposure and development of heteroresistance in NICU patients, though also confirms that the phenomenon is quite rare despite the ubiquity of such exposure. While heteroresistant CoNS is associated with greater morbidity and mortality in NICU patients, such an association remains unclear in adult patients, who are typically affected by CoNS species distinct from the more prevalent *Staphylococcus capitis* in neonates. However, our study does suggest that those patients who are at the extremes of age, who have longstanding comorbidities, and who require prolonged use of central vascular catheters should be considered for surveillance of glycopeptide heteroresistant CoNS.

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Tables*Table 1: Mean glycopeptide MIC for heteroresistant and non-heteroresistant CoNS isolates from NICU inpatients and adult inpatients*

	Mean MIC of heteroresistant isolates	Mean MIC of non-heteroresistant isolates	P value (by Mann-Whitney U Test)
<i>NICU isolates</i>			
Vancomycin MIC by microdilution Etest®	2.91 ± 0.54 µg/mL	2.00 µg/mL	0.001
Vancomycin MIC by macrodilution Etest®	11.27 ± 5.00 µg/mL	4.20 ± 1.32 µg/mL	0.000
Teicoplanin MIC by macrodilution Etest®	29.09 ± 13.03 µg/mL	4.60 ± 2.17 µg/mL	0.000
<i>Adult inpatient isolates</i>			
Vancomycin MIC by microdilution Etest®	3.50 ± 0.93 µg/mL	2.14 ± 0.87 µg/mL	0.003
Vancomycin MIC by macrodilution Etest®	4.75 ± 1.39 µg/mL	3.44 ± 1.17 µg/mL	0.022
Teicoplanin MIC by macrodilution Etest®	15.00 ± 7.01 µg/mL	3.62 ± 2.42 µg/mL	0.000

Table 2: Demographic and exposure factors for NICU cases

	Heteroresistant Cases	Non-heteroresistant Cases	P-value
Number of cases	14	10	N/A
Gender			
Males	9	5	0.678*
Females	5	5	
Mean length of stay (days)	82.5 ± 36.3	104.2 ± 61.8	0.302**
Mean central line days prior to first positive culture	15.0	11.6	0.962‡
Mean antibiotic exposure 1 month prior to first positive culture (days)	8.7	2.8	0.124**
Mean vancomycin exposure 1 month prior to first positive culture (days)	4.0	3.0	0.041**

*p-value obtained via Fisher's exact test

**p-value obtained via two-sample t test

‡p-value obtained via Mann-Whitney U test

Table 3: Demographic and exposure factors for adult inpatient cases

	Heteroresistant Cases	Non-heteroresistant Cases	P-value
Number of cases	6	33	N/A
Gender			
Males	5	19	0.376*
Females	1	14	
Age at admission (years)	66.0	58.3	0.324**
Mean length of stay (days)	46.7 ± 46.7	106.3 ± 140.1	0.317**
Mean central line days prior to first positive culture	30.0 ± 26.0	21.9 ± 26.3	0.565**
Mean antibiotic exposure 1 month prior to first positive culture (days)	7.8	9.2	1.00‡
Mean vancomycin exposure 1 month prior to first positive culture (days)	0.75 ± 1.5	2.1 ± 4.9	0.879‡

*p-value obtained via Fisher's exact test

**p-value obtained via two-sample t test

‡p-value obtained via Mann-Whitney U test