Pseudo-Outbreak of Vancomycin-Resistant-Enterococcus (VRE) Colonization in a Neonatal Intensive Care Unit Using Spectra VRE Surveillance Medium

Rita M. Gander, Dominick Cavuoti, Adnan Alatoom, Paul Southern Jr., Debra Grant, Kathleen Salinas, Donna Gaffney, Jennifer MacKenzie and Linda Byrd

Published Ahead of Print 19 December 2012.

Updated information and services can be found at:
http://jcm.asm.org/content/51/3/810

These include:

**REFERENCES**

This article cites 13 articles, 8 of which can be accessed free at:
http://jcm.asm.org/content/51/3/810#ref-list-1

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»
Vancomycin-resistant enterococci (VRE) colonization of the gastrointestinal tract appears to be rare in neonates (1, 2). Most colonized neonates that develop infections have been associated with clusters and outbreaks (1, 2). Risk factors for VRE colonization in neonates include low birth weight, prematurity, and long-term antimicrobial therapy (2). Contact isolation precautions are usually implemented if VRE is detected in surveillance culture results in a NNICU population that initiated an outbreak investigation and contact isolation of a group of neonates. Most colonized neonates that develop infections have been associated with false-positive cultures. The false-positive VRE surveillance cultures than those receiving other formulas ($P < 0.001$). Application of topical products was not associated with false-positive cultures. The false-positive $E. faecalis$ strains were typed by Diversilab Rep-PCR (bioMérieux, Marcy l’Etoile, France) and found to represent eight different groups of isolates. The utility of the Spectra VRE media appeared to be significantly impacted by the age of the patients screened.

**MATERIALS AND METHODS**

Spectra VRE surveillance cultures. A total of 989 rectal swabs were collected from 676 adult and neonatal patients at Parkland Memorial Hospital, a 672-bed tertiary care medical center, from 9 November 2011 through 27 March 2012. The NNICU is a 90-bed unit that houses babies from birth until discharge or transfer to another Parkland unit. Weekly surveillance for VRE was initiated when vancomycin-resistant $E. faecium$ was recovered from a neonate’s urine. In January, the surveillance frequency was decreased to biweekly. Eswabs with liquid Amies transport medium (Becton Dickinson, Sparks, MD) were used to collect rectal samples. The swabs were then plated onto Spectra VRE agar (Remel) and incubated aerobically in ambient air at 35°C for 24 ± 5 h. After incubation, the plates were evaluated for suspected vancomycin-resistant $E. faecium$ that appeared as navy blue or pink-purple colonies and vancomycin-resistant $E. faecalis$ that appeared as light blue to blue colonies. Suspect VRE colonies were tested for pyrrolidonyl arylamidase production by disks (Key Scientific Products, Stamford, TX), and positive colonies were subcultured to sheep blood agar plates and saved frozen for later definitive identification and susceptibility testing. For the second phase of the study, suspect VRE colonies were prospectively inoculated into MicroScan Pos Gram combo panels (Siemens Healthcare Diagnostics, Inc., West Sacramento, CA) for definitive identification and susceptibility testing.

Interfering substances challenge. Six products applied to neonates’ buttocks—Sensi Care Protective Barrier, Aloe Vesta Skin Protectant, Bou dreaux’s Butt Paste, zinc oxide cream, nystatin powder, and nystatin cream—were evaluated for each product’s ability to produce false-negative or false-positive results with Spectra VRE. Three vancomycin-resistant strains—one adult $E. faecium$, one adult $E. faecalis$, and one neonatal $E. faecium$—were tested. In addition, two vancomycin-susceptible strains, including a neonatal $E. faecalis$ detected as a false positive, and $E. faecalis$ ATCC 29212 strains were tested. Bacterial concentrations of $1.5 \times 10^2$ CFU/ml and $1.5 \times 10^3$ CFU/ml were prepared in 0.85% sterile saline from fresh subcultures; 0.1-ml portions of the diluted bacterial suspensions were added to the appropriate containers. The final concentrations in the Eswab transport media were approximately $1.5 \times 10^2$ CFU/ml and $1.5 \times 10^3$ CFU/ml. Each test was performed in duplicate. The products were...
False-positive Spectra VRE Results in Neonates

TABLE 1 Comparison of the performance of Spectra VRE medium for surveillance cultures of adult and neonatal fecal samples

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Total no. of tests</th>
<th>No. of results (%)</th>
<th>Positive</th>
<th>False positive&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>580</td>
<td></td>
<td>87 (15)</td>
<td>20 (3)</td>
<td>473 (82)</td>
</tr>
<tr>
<td>Neonates</td>
<td>409</td>
<td></td>
<td>1 (&lt;1)</td>
<td>55 (13)</td>
<td>353 (86)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Multiple lots of media were used during the study period.

<sup>b</sup> Determined using a comparison of proportions for the false-positive adult and neonatal results (<i>P</i> < 0.001).

RESULTS

Spectra VRE surveillance results. A total of 580 fecal swabs were collected from 404 adult patients as shown in Table 1. Vancomycin-resistant <i>E. faecium</i> was recovered from 87 (15%) of the samples plated on Spectra VRE agar. Negative culture results were observed for 473 (82%) of the fecal swabs. False-positive results with blue or purple colonies growing on the media were seen for 20 (3%) of the cultures. The 20 isolates included 14 <i>E. faecalis</i> isolates with vancomycin MICs ranging from 0.5 to 4.0 μg/ml (median 2.0 μg/ml), 3 <i>E. gallinarum</i> isolates with vancomycin MICs of >16.0 μg/ml, and three <i>E. durans</i> isolates with vancomycin MICs of >16 μg/ml.

A total of 409 swabs from 272 neonates were plated on Spectra VRE. A single isolate of vancomycin-resistant <i>E. faecium</i> was recovered. Negative results were seen for 353 (86%) of the cultures. False-positive results were seen for 55 (13%) of the cultures collected from 45 patients; all isolates were identified as <i>E. faecalis</i> with vancomycin MICs ranging from 1.0 to 4.0 μg/ml (median, 2.0 μg/ml). False positives were observed throughout the study period on seven different lots of media.

Effect of topical products on the detection of VRE on Spectra VRE media. Exposure of the vancomycin-susceptible strains to six topical products used on neonates did not produce false-positive results on the Spectra VRE media. However, low concentrations of vancomycin-resistant <i>E. faecium</i> and <i>E. faecalis</i> strains (1.5 × 10⁵ CFU/ml) were inhibited by all of the products tested, with the exception of the <i>E. faecium</i> neonatal strain; this isolate was able to grow on Spectra VRE medium after exposure to nystatin powder. At the higher bacterial concentrations (1.5 × 10⁶ CFU/ml), all of the VRE strains were inhibited by nystatin powder, and the adult <i>E. faecalis</i> strain was also inhibited by nystatin cream.

DNA fingerprinting of vancomycin-susceptible <i>E. faecalis</i> adult strains and neonatal <i>E. faecalis</i> strains demonstrating false-positive results on Spectra VRE. The typing results are shown in Fig. 1. For the 14 isolates recovered from neonates, 8 different groups were identified: groups 1 through 5 and groups 7 through 9 with two or more band differences and <95% similarity between groups. Within group 1 (G1), G3, G5, and G8, there were applied to a sterile gloved hand or other clean disposable surface to simulate the amount used on a patient. Eswab (Becton Dickinson) were lightly rolled across the topical products and placed in the seeded transport containers. Spectra VRE plates were inoculated with the swabs and incubated overnight.

Repetitive sequence-based PCR (Rep-PCR). Nineteen vancomycin-susceptible <i>E. faecalis</i> that demonstrated false-positive results on Spectra VRE agar, including 13 isolates from neonates and 6 from adults, were typed using a Diversilab Enterococcus DNA fingerprinting kit (bioMérieux). Briefly, DNA was extracted from colonies using an UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA). In accordance with the manufacturer’s instructions, extracted DNA was amplified using a thermocycler and then separated, detected, and analyzed using a microfluidics DNA chip (bioMérieux) with an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA).

The relatedness was determined by cluster analysis and guidelines provided by the manufacturer. Isolates were categorized as indistinguishable, similar or different. In general, “different” was defined as <95% similarity and ≥2 band differences, “similar” was defined as <97% similarity and 1 band difference, and “indistinguishable” was defined as >95% similarity and no band differences.

Neonatal patient demographics. Medical record review was performed to identify demographic characteristics, nutrition, and ointments in use at the time of testing. This study was exempt from the requirement for institutional review, and the privacy rule of the Health Insurance Portability and Accountability Act did not apply because the data were gathered as part of a hospital outbreak investigation.

Statistical analysis. Group characteristics were compared between groups using a median two-sample test for continuous measures or test of proportion or Pearson chi-square test for categorical variables where appropriate. All <i>P</i> values in the analyses presented are two-sided and considered significant when <i>P</i> was ≤0.05 unless otherwise stated. The data were analyzed using the SAS version 9.2 (SAS, Inc., Cary, NC) statistical package and the WINKS Statistical Data Analysis software version 7.0 software (TexasSoft, Cedar Hill, TX).

<FIG 1 Rep-PCR dendrogram and simulated electrophoresis results for neonatal false-positive <i>E. faecalis</i> isolates corresponding to keys 1, 2, 4 to 8, 11, 13 to 17, and 19 and adult false-positive <i>E. faecalis</i> strains corresponding to keys 3, 9, 10, 12, and 18.>
multiple indistinguishable strains. Within G4, there were two similar but not indistinguishable strains.

The five strains recovered from adults were assigned to groups 3, 6, 7, and 9. Two adult isolates placed in G7 were indistinguishable. Three adult strains were indistinguishable from neonatal isolates placed in the same groups, G3, G7, and G9.

**Comparison of demographics, nutrition, and topical ointments in neonatal patients with false-positive and negative surveillance cultures.** The demographic characteristics and nutrition at the time of testing were compared between neonates with negative and false-positive surveillance cultures as shown in Table 2. The median age at testing was statistically significant between the two groups, P < 0.001; the false-positive group demonstrated a higher median age. When examining the nutrition received during the period in which testing occurred, only comparisons of the Similac Expert Care products showed statistical significance with P < 0.001; babies with false-positive results were less likely to have received breast milk. Babies with false-positive results were also less likely to have received breast milk. The number of chart-documented uses of topical products was too low to allow statistical comparisons.

**DISCUSSION**

False-positive results for VRE fecal colonization were significantly higher in neonatal screening cultures than in adult cultures, 13% versus 3%, respectively, contributing to a pseudo-outbreak of colonized infants in the NICU. As a result, the neonates were unnecessarily placed in isolation, increasing the labor and hospital costs in caring for these neonates. In addition, a recent review of the possible impacts of contact precautions on patients identified adverse outcomes, including reduced contact with clinical staff and changes in systems of care that produced delays and increases in preventable, noninfectious adverse events (4). These results suggested that there are differences between adult and neonatal fecal samples that influenced the results on the Spectra VRE agar. The published evaluations and manufacturer’s published findings of the Spectra VRE agar did not segregate the results according to the ages of the patients tested (5, 6, 7, 8). This is the first report to describe differences in the performance of Spectra VRE based on the patient populations tested.

All of the isolates that produced false-positive results in neonates were vancomycin-susceptible *E. faecalis*. In Peterson’s evaluation of Spectra VRE agar testing 399 fecal specimens, two vancomycin-sensitive *E. faecalis* produced light blue colonies on the media and were interpreted as false-positive results (7). The neonatal strains of *E. faecalis* in our study were typed to determine whether the vancomycin-susceptible strains demonstrating false-positive results were clonal. Using Rep-PCR, the DNA fingerprinting results showed that the strains were not clonal with 8 unrelated groups among 14 strains. Therefore, the false-positive results did not appear to be caused by an aberrant strain of *E. faecalis*.

In adults, 6 of 20 bacterial strains producing false-positive results were vancomycin-resistant *E. gallinarum* and *E. durans*. *E. gallinarum* has an intrinsic intermediate level of vancomycin resistance mediated by the vanC gene located on the chromosome and does not pose a risk for the dissemination of vancomycin resistance to other bacteria. The manufacturer indicated in the package insert that additional antibiotics, in combination with vancomycin, are present in the Spectra VRE medium to suppress the growth of *E. gallinarum* (8), although we had breakthrough...

---

**TABLE 2** Comparison of neonatal patients with false-positive and negative surveillance cultures for VRE

<table>
<thead>
<tr>
<th>Demographic characteristic</th>
<th>False positive (n = 55)</th>
<th>Negative (n = 353)</th>
<th>P&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n or median</td>
<td>% or range</td>
<td>n or median</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>35</td>
<td>153</td>
</tr>
<tr>
<td>Age at testing</td>
<td>6 wks</td>
<td>4 days to 20 wks</td>
<td>3 wks</td>
</tr>
<tr>
<td>Birth wt (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1,000</td>
<td>5</td>
<td>9</td>
<td>47</td>
</tr>
<tr>
<td>1,000–2000</td>
<td>22</td>
<td>40</td>
<td>134</td>
</tr>
<tr>
<td>&gt;2,000</td>
<td>28</td>
<td>51</td>
<td>172</td>
</tr>
<tr>
<td>Preterm</td>
<td>41</td>
<td>75</td>
<td>259</td>
</tr>
<tr>
<td>Nutrition at testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk</td>
<td>41</td>
<td>75</td>
<td>305</td>
</tr>
<tr>
<td>Similac Special Care&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16</td>
<td>29</td>
<td>119</td>
</tr>
<tr>
<td>Similac Special Care 24 High Protein</td>
<td>1</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Similac Expert Care&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61</td>
<td>17</td>
<td>61</td>
</tr>
<tr>
<td>Similac Expert Care Neosure</td>
<td>16</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>Similac Advance Early</td>
<td>19</td>
<td>36</td>
<td>114</td>
</tr>
<tr>
<td>Neocate Infant with DHA + ARA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Isomil soy</td>
<td>1</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>Pregestimil LIPIL</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total parenteral nutrition + fat emulsion infusion</td>
<td>3</td>
<td>5</td>
<td>36</td>
</tr>
</tbody>
</table>

<sup>a</sup> Includes Similac Special Care 20-cal/oz, 24-cal/oz, or 30-cal/oz formulations.

<sup>b</sup> Includes Similac Expert Care 24 cal with iron or alimentum formulation.

<sup>c</sup> Neocate Infant with (DHA) decosohexanoic acid plus (ARA) arachadonic acid.

<sup>d</sup> *, P ≤ 0.05.
colonies in our surveillance cultures. A single vancomycin-resis-
tant *E. durans* strain was also recovered on Spectra VRE in the
Peterson study (7). The false-positive rate for Spectra VRE at 24 h
of incubation was <1% in three different studies (5, 6, 7). If the *E.
gallinarum* and *E. durans* isolates were excluded from our study,
the false-positive rate in adult cultures decreased to 2%, com-
parable to these studies.

Enterococci other than *E. faecalis* and *E. faecium* were also recov-
ered on other chromogenic agars. In evaluations of CHROMagar
VanRE, false-positive results were observed with *E. gallinarum*, *E.
casseliflavus*, and *E. raffinosus* (9, 10). In another study, 40% of
green colonies consistent with vancomycin-resistant *E. faecalis*
on the CHROMagar VanRE were later identified as vancomycin-sus-
ceptible *E. faecalis* (11). Grabsch et al. also detected a high number
of vancomycin-sensitive enterococci on another chromagar,
chromID VRE, although the medium was incubated for 48 h (12).

The possible influence of the babies’ ages at the time of testing
on the surveillance cultures was examined. A statistically signif-
ificant difference in the age at testing was noted between those with
false-positive and negative surveillance cultures. The older infants
with a median age of 6 weeks were more likely to have false-positi-
tive cultures than the younger neonates. Extended hospitalization
in NNICUs with prolonged antibiotic therapy, parenteral nutri-
tion, delayed oral feedings, and intubation seems to affect the
composition of the intestinal flora (13).

Nurtition at the time of testing, which might have altered the
composition of the feces, was also examined. Neonates receiving
Similac Expert Care formulations were less likely to be associated
with false-positive fecal cultures. The babies receiving these for-
mulations were the youngest group of neonates. The older group
of babies, with a median age of 6 weeks, who received other types
of formula were more likely to have false-positive surveillance
cultures. The Similac Expert Care and Advance products for older
neonates were more complex, containing additional nutrients
such as monoglycerides, carrageenan, galacto-oligosaccharides,
and lycopene. Galacto-oligosaccharides have been shown to pro-
mote an increase in beneficial bacteria such as bifidobacteria in the
gastrointestinal tracts of preterm infants (14) that could change
the composition of the feces.

We also investigated the possible effects of topical products
that were used on the neonates during collection of the fecal
swabs. None of the ointments appeared to contribute to the false-
positive culture results in the babies, although the number of doc-
umented applications was low. However, some products, such as
nystatin, had an inhibitory effect on the recovery of VRE. The
manufacturer evaluated miconazole as a possible interfering sub-
stance and indicated that the antifungal might reduce the recovery
of VRE (8).

Findings from this pseudo-outbreak of VRE colonization in
neonates suggested that verification studies from manufacturers
and hospitals should include specimens from all age groups in-
tended for inclusion in surveillance culturing to ensure that the
screening medium will accurately detect the colonizing organisms
in fecal specimens from each group. In addition, our findings
suggested that additional identification and susceptibility testing
should be performed to confirm that the colonized babies
were vancomycin-resistant *E. faecalis* and *E. faecium* before reporting the results.

ACKNOWLEDGMENTS
We are grateful to the staff of the microbiology laboratory at Parkland
Health and Hospital System, especially Susan Webb, for technical assis-
tance in this investigation. We thank Cari Brown and Cheryl Lair for
providing information concerning infant formulas. Finally, we thank Al-
lan Elliot for help in the statistical analysis of the data.

REFERENCES
vancomycin-resistant *Enterococcus faecium* outbreak caused by two geo-
32:82–86.
2. Sherer CR, Sprague BM, Campos JM, Nambiar S, Temple R, Short B,
Singh N. 2005. Characterizing vancomycin-resistant enterococci in neo-
3. Rank EL. 2012. Chromogenic agar media in the clinical, food, and envi-
outcomes associated with contact precautions: a review of the literature.
5. Jenkins SG, Raskoshina L, Schuetz AN. 2011. Comparison of perfor-
mance of the novel chromogenic Spectra VRE agar to that of bile esculin
azide and *Campylobacter* agars for detection of vancomycin-resistant en-
Comparison of medium, temperature, and length of incubation for detec-
2509.
7. Peterson JF, Doern CD, Kallstrom G, Riebe KM, Sander T, Dunne WM,
Jr, Ledeboer NA. 2010. Evaluation of Spectora VRE, a new chromogenic
agar medium designed to screen for vancomycin-resistant *Enterococcus
8. Remel Diagnostics. 2011. Spectora<sup>TM</sup> VRE package insert. Remel Diagnos-
tics, Lenexa, KS.
Haase G. 2009. Comparison of two chromogenic media for selective iso-
lization of vancomycin-resistant enterococci from stool specimens. J. Clin.
11. Stamper PD, Shulder S, Bekalo P, Manandhar D, Ross TL, Speser S,
Kimgery J, Carroll KC. 2010. Evaluation of BBL CHROMagar VanRE for
detection of vancomycin-resistant enterococci in rectal swab specimens.
study of selective chromogenic (chromID VRE) and bile esculin agars for
isolation and identification of *vanR*-containing vancomycin-resistant en-
A. 2002. Supplementation of a bovine milk formula with an oligosaccha-
ride mixture increases counts of fecal bifidobacteria in preterm infants.