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BRIEF REPORT

Vancomycin-Nonsusceptible Enterococci Mediated by *vanC* at a Large Children's Hospital: Prevalence, Susceptibility, and Impact on Care of Enterococcal Bacteremia

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Enterococcus gallinarum and *casseliflavus* have inherent vancomycin resistance and, though known as pathogens, have not been well characterized in pediatric patients. We identified a significant prevalence of these enterococcal species among immunocompromised patients at a large pediatric institution and describe the impact on patient care, antibiotic stewardship, and infection control.

Keywords. *Enterococcus*; *vanC*; vancomycin; infection control.

Vancomycin-resistant *Enterococcus faecium* and *faecalis* (VRE) have high-level vancomycin resistance mediated by the transposable *vanA* and *vanB* genes, which alter the terminal amino acids of peptidoglycan precursors, dramatically reducing vancomycin inhibition of cell wall synthesis [1, 2]. *Enterococcus gallinarum* and *casseliflavus* have intrinsic low-level vancomycin resistance mediated by a chromosomally integrated *vanC* gene, which also alters peptidoglycan precursors. Unlike VRE, these enterococcal species are usually ampicillin-susceptible, and may even still be vancomycin-susceptible per Clinical Laboratories Standards Institute (CLSI) breakpoints [3]. Though recognized as pathogens, particularly in immunocompromised patients [3–6], *vanC*-containing enterococci (VCE) are not well characterized in pediatric patients [7]. We describe the prevalence and susceptibility of *Enterococcus*-positive blood and stool cultures at a large children's hospital over a 6-year period and the impact of VCE on patient care.

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METHODS

Data Extraction

We obtained a list of blood cultures positive for *Enterococcus* spp. from the years 2013–2018 from the electronic medical record (EMR). Only first-time positive blood cultures, defined as at least 2 weeks after any previous *Enterococcus* blood culture, were included. For patients with VCE bacteremia, we reviewed the EMR for underlying medical conditions, presence of a central catheter, neutropenia, and days of bacteremia. We similarly obtained a list of stool cultures positive for vancomycin-nonsusceptible *Enterococcus* from the years 2013 to 2018, and samples from unique patients in a calendar year were included. Stool surveillance cultures are routinely collected on bone marrow transplant (BMT) patients but infrequently collected in other patients.

Blood Cultures

Our institution routinely draws at least 2 blood cultures before starting antibiotics. Blood cultures are monitored by BACTEC (BD, Franklin Lakes, NJ, USA). After detection of growth, samples are processed by multiplex polymerase chain reaction (PCR) panel (BCID, BioFire, Salt Lake City, UT, USA), which, among other targets, detects the genus *Enterococcus* and the *vanA*/*vanB* genes. Simultaneously, isolates are incubated on blood agar and identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF). Mean inhibitory concentrations (MICs) are obtained by Microscan (Beckman Coulter, Atlanta, GA, USA) and categorized per CLSI guidelines (for vancomycin: susceptible ≤4 μg/mL, intermediate 8–16 μg/mL, and resistant ≥32 μg/mL) [8]. Vancomycin-intermediate and vancomycin-resistant isolates were classified as vancomycin-nonsusceptible. Per laboratory protocol, not all repeat positive cultures are evaluated beyond gram stain. Therefore, for polymicrobial isolates, days of VCE bacteremia was inferred only if VCE was the sole identified organism that would demonstrate gram-positive cocci in chains on gram stain.

Stool Samples

Stool samples were cultured on bile esculin plates with and without vancomycin. Colonies were subcultured for identification, and susceptibilities were obtained by Microscan. Vancomycin-nonsusceptible isolates were included in the analysis.

Statistical Analysis

All comparisons were assessed by Fisher exact test. Significance was set at $P < .05$.

Institutional review board approval was obtained through the Colorado Multiple Institutional Review Board (19-0692). Informed consent was waived.

RESULTS

Enterococcus Bacteremia

During the 6-year study period, we identified 240 first-time positive *Enterococcus* blood cultures in 171 unique patients (Table 1). Seventeen of the 171 unique patients (9.9%), had at least 1 episode of VCE bacteremia. One patient accounted for 33 bacteremic events (14% of total). A second patient had 6 bacteremic events (3% of total), and 3 additional patients had 4 bacteremic events (2% of total each). No other patient accounted for more than 3 bacteremic events.

Enterococcus casseliflavus Bacteremia

Four patients accounted for 13 bacteremic events. All 13 initial cultures were polymicrobial. Twelve (92%) were associated with a central catheter. Of the 12 events for which days of bacteremia could be inferred, 1 patient (8%) had bacteremia for >1 day (2 days). Two of the 4 patients (50%) had underlying oncologic diagnoses (1 with acute neutropenia), 1 (25%) had complications from Ehlers-Danlos, and 1 (25%) was a febrile infant for whom *E. casseliflavus* was thought to be a contaminant by the clinical team.

Enterococcus gallinarum Bacteremia

Thirteen patients accounted for 16 bacteremic events. Six (38%) of the 16 initial cultures were polymicrobial. All events were associated with a central catheter. Of the 14 events for which days of bacteremia could be inferred, 9 patients (63%) were bacteremic for >1 day (median [range], 2 [1–6] days). Two patients were bacteremic for >3 days with vancomycin-nonsusceptible isolates. Though both patients were being treated with vancomycin on culture day 1, neither was on effective therapy until day 3. Nine of the 13 patients (69%) had an underlying oncologic diagnosis, 2 (15%) had aplastic anemia, 1 (8%) was undergoing chemotherapy for hemophagocytic lymphohistiocytosis, and 1 (8%) had complications of Ehlers-Danlos syndrome. Ten of these 16 events (62%) occurred in patients with an acute ANC ≤20.

Compared with *E. casseliflavus*, *E. gallinarum* was more often isolated in a mono-microbial culture, associated with >1 day of bacteremia, and identified in an acutely neutropenic patient ($P < .01$ for all comparisons).

Susceptibility

Of the 240 total *Enterococcus* isolates, 16 (7%) were ampicillin-susceptible and vancomycin-nonsusceptible, 15 (6%) were ampicillin-resistant and vancomycin-susceptible, and 7 (3%) were not susceptible to either antibiotic (Table 1). The hematology/oncology/BMT unit had the highest proportion of ampicillin resistance and VRE ($P < .01$) (Table 1). Of our 23 vancomycin-nonsusceptible isolates, 15 (65%) were VCE and 7 (30%) were *VRE*. Of 29 total VCE isolates, none

Table 1. Susceptibility of *Enterococcus* Blood Isolates to Vancomycin and Ampicillin Categorized by Species and Hospital Unit; Isolates Were Obtained From the Years 2013–2018

Enterococcal Species	Total No. (% of Total)	Vancomycin-Susceptible and Ampicillin-Susceptible (% of Species)	Vancomycin-Nonsusceptible and Ampicillin-Susceptible (% of Species)	Vancomycin-Susceptible and Ampicillin-Resistant (% of Species)	Vancomycin-Nonsusceptible and Ampicillin-Resistant (% of Species)
Enterococcus (all)	240	202 (84)	16 (7)	15 (6)	7 (3)
Enterococcus casseliflavus	13 (5.5)	10 (77)	3 (23)	0	0
Enterococcus gallinarum	16 (7)	4 (25)	12 (75)	0	0
Enterococcus faecalis	161 (67)	161 (100)	0	0	0
Enterococcus faecium	40 (17)	19 (48)	0	14 (35)	7 (18)
Enterococcus avium	1 (.5)	1 (100)	0	0	0
Enterococcus durans	3 (1)	2 (67)	1 (33)	0	0
Enterococcus hirae	2 (1)	2 (100)	0	0	0
Enterococcus raffinosus	1 (0.5)	0	0	1	0
Enterococcus spp. (unidentified)	3 (1)	3 (100)	0	0	0
Hospital unit					
All	240	202 (84)	15 (6)	16 (7)	7 (3)
CICU/CPCU	20 (8)	19 (95)	1 (5)	0	0
HEM/ONC/BMT	54 (23)	29 (54)	9 (16.5)[a]	10 (18.5)[a]	6 (11)[a]
NICU	49 (20)	49 (100)	0	0	0
PICU	26 (11)	22 (84.5)	1 (4)	2 (7.5)	1 (4)
MED (3rd, 6th, 8th, 9th floors)	91 (38)	83 (91)	4 (4.5)	4 (4.5)	0

Two hundred forty isolates were obtained from 171 unique patients.

Abbreviations: CICU, cardiac intensive care unit; CPCU, cardiac progressive care unit; HEM, hematology; ONC, oncology; BMT, bone marrow transplant; NICU, neonatal intensive care unit; PCIU, pediatric intensive care unit; MED, general medical floor (MED).

[a]Denotes statistical significance by Fisher's exact test: $P < .01$.

were ampicillin-resistant and 15 (52%) were vancomycin-nonsusceptible. All 14 vancomycin-susceptible VCE isolates had a vancomycin MIC of 2–4 μg/mL. *E. gallinarum* was more often vancomycin-nonsusceptible than *E. casseliflavus* ($P < .01$).

Stool Cultures

From 315 surveillance stool cultures, we identified 60 vancomycin-nonsusceptible isolates in 58 patient-samples. VCE and VRE accounted for 49 (82%) and 11 (18%) of these isolates, respectively. Two of the 47 patients (4%) with vancomycin-nonsusceptible VCE colonized stool had bacteremia with the same vancomycin-nonsusceptible VCE species compared with 3 of the 9 patients (33%) colonized with VRE ($P < .05$). Six patients with VCE bacteremia had stool cultures available. Two (33%) were colonized with the same species of VCE (both vancomycin-nonsusceptible); none were colonized with VRE. Four patients with VRE bacteremia had stool cultures available. Three (75%) were colonized with the same species of VRE; none were colonized with VCE.

DISCUSSION

In our study, 12.1% of enterococcal bacteremia was VCE, higher than previously reported [3, 5, 7]. The highest frequency of VCE and VRE was in the hematology/oncology/BMT unit. All except 1 episode of VCE bacteremia was associated with a central catheter, and most unique patients were immunocompromised with acute neutropenia. Compared with *E. casseliflavus*, *E. gallinarum* was more often identified as a single pathogen and associated with >1 day of bacteremia, suggesting increased pathogenicity.

Among BMT patients, vancomycin-nonsusceptible VCE were more frequent stool colonizers than VRE, described previously [9]. Patients with vancomycin-nonsusceptible VCE-colonized stool were less likely to develop bacteremia with the same enterococcal species than those colonized by VRE. Furthermore, in patients with *Enterococcus* bacteremia, stool cultures often predicted the blood isolate (75% for VRE vs 33% for vancomycin-nonsusceptible VCE). Though our sample size is small, these data suggest that stool surveillance cultures may have some predictive value for *Enterococcus* bacteremia in our BMT patient population, though more systematic study is needed to make firm conclusions.

A significant prevalence of VCE has impacted our management of enterococcal bacteremia. Our data suggest that empiric vancomycin monotherapy may not be appropriate for patients at risk for VCE, even if VRE is not detected by multiplex PCR. Instead, a regimen of daptomycin OR linezolid OR ampicillin PLUS vancomycin may be more appropriate while awaiting speciation. (We often recommend ampicillin in addition to vancomycin for patients at risk of VCE.) If speciation proves the isolate is VCE, immediate transition to ampicillin is recommended. These suggestions are tempered by a paucity of VCE treatment data, though vancomycin therapy even in vancomycin-susceptible VCE may be associated with treatment failure [10].

Infection control practices may also be affected by VCE. Unlike *vanA*/*vanB*, the *vanC* gene is not transferrable between organisms. Therefore, patients with VCE may not require the stringent isolation practices used for patients with VRE, supported by the fact that VCE has rarely been reported in outbreaks, though further studies are needed [11–13].

A primary limitation of our study is single patients with multiple bacteremic episodes. However, many repeat positive cultures with the same *Enterococcus* species demonstrated different susceptibility patterns. Therefore, we kept these repeat isolates in the analysis rather than discard repeat positive cultures, which we felt would result in a somewhat arbitrary loss of important susceptibility data. Notably, lengthening the definition of first-time positive cultures to 4 weeks results in the loss of only 2 isolates (neither VCE). Other limitations include low absolute numbers of VCE and VRE bacteremia, few bacteremic patients with available stool cultures, routine collection of stool cultures only on BMT patients, and difficulty generalizing VCE prevalence to other institutions.

Despite these limitations, our high prevalence of VCE has affected management of patients with *Enterococcus* bacteremia and would be important to recognize at other institutions. Future directions include large case–control or prospective studies to clarify risk factors and optimal treatment of VCE, systematic stool studies with molecular sequencing to evaluate the association of VCE stool colonization with bacteremia, and investigation into the epidemiology of VCE spread.

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Author contributions. All authors contributed to the study design and data collection. D.S.D. and M.C.W. performed the data analysis. D.S.D. was the primary writer of the manuscript. All authors contributed to the final manuscript.

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