Brief Report

Oral antimicrobial options for vancomycin-resistant *Enterococcus* isolates in urine culture

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Abstract

Objectives: The present study aimed to investigate the susceptibility profiles of vancomycin-resistant *Enterococcus* isolates in urine culture to create an antibiogram to guide selection of oral antimicrobials in British Columbia (BC), Canada. **Methods:** An audit was conducted on all urine cultures reported from January 1, 2021, to December 31, 2023, in LifeLabs BC microbiology laboratories. *Enterococcus* species in urine were routinely tested with ampicillin, ciprofloxacin, nitrofurantoin, tetracycline, and vancomycin. Linezolid and fosfomycin were tested in selected cases. **Results:** Three hundred and thirty-five vancomycin-resistant *Enterococcus faecium*, 47 vancomycin-resistant *Enterococcus faecalis*, 48 *Enterococcus gallinarum*, 25 *Enterococcus casseliflavus*, and no *Enterococcus flavescens* isolates were reported in urine culture. Vancomycin-resistant *E. faecium* isolates were >90% susceptible to linezolid, but <15% susceptible to ampicillin, ciprofloxacin, nitrofurantoin, and tetracycline. Vancomycin-resistant *E. faecalis* isolates were >90% susceptible to ampicillin, linezolid, and nitrofurantoin, but <10% susceptible to ciprofloxacin and tetracycline. *E. casseliflavus* isolates were >90% susceptible to ampicillin, nitrofurantoin, and tetracycline. *E. gallinarum* isolates were >90% susceptible to ampicillin and nitrofurantoin. In the seven and 263 selected cases of vancomycin-resistant *E. faecium* and *E. faecalis*, respectively, fosfomycin susceptibility rates were 57% and 86%, respectively. **Conclusions:** Ampicillin and nitrofurantoin may be considered for urinary tract infections secondary to vancomycin-resistant *E. faecalis*, *E. casseliflavus*, and *E. gallinarum*. Tetracycline may also be considered for *E. casseliflavus*. Linezolid remained to be the only reliable oral antimicrobial for vancomycin-resistant *E. faecium*.

Keywords: Ampicillin, Antimicrobial, Ciprofloxacin, *Enterococcus* species, Fosfomycin, Linezolid, Nitrofurantoin, Oral therapy, Tetracycline, Vancomycin-resistant *Enterococcus*

1. INTRODUCTION

Gram-negative bacilli, such as *Escherichia coli*, are generally the most frequent pathogenic causes of urinary tract infections (UTI) [1]. UTI secondary to *Enterococcus* species, a Gram-positive coccus, occasionally occur [1]. Vancomycin-resistant *Enterococcus* (VRE) infections are emerging [2], but there is a lack of guidance on their empirical antimicrobial choice. Published literatures generally recommend use of antimicrobial susceptibility testing (AST) to guide treatment selection [3,4]. However, there is turnaround time for this laboratory workup of AST but available antibiograms specifically for VRE are lacking. Intravenous daptomycin has been shown to be highly effective in achieving microbiological cure in patients with VRE bacteremia [5,6], but intravenous therapies may be impractical to treat uncomplicated UTI in patients who are stable for outpatient oral therapies. Although it can be argued that presence of *Enterococcus* species may suggest asymptomatic bacteriuria rather than true UTIs, patients who are pregnant or undergoing endourological procedures would still require treatment for asymptomatic bacteriuria [7].

The present study aimed to investigate the susceptibility profiles of all VRE isolates reported in urine culture in a 3-year period. The goal was to create an antibiogram to guide selection of empirical oral antimicrobials in British Columbia

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(BC), Canada, when VRE UTI is suspected but full AST results are pending. When diagnostic laboratories apply selective testing or cascading on antimicrobials for stewardship reasons, not all AST results are instantly available [8]. Clinicians may need to determine their prescribing practice based on the local epidemiology of antimicrobial resistance.

2. METHODS

2.1. Data collection and analysis

The current retrospective audit was conducted using Microbiology Electronic Worksheet System (MEWS; Version 5.00.267; LifeLabs, Toronto, ON, Canada), a software application that generated data of all reported urine cultures from January 1, 2021 to December 31, 2023 in LifeLabs BC microbiology regional laboratories, connected with 129 collection centers in community. Three years of data from patients of all age and gender were included to reduce bias secondary to seasonality, differences in clinical practices, and other potential confounders. As per Clinical and Laboratory Standard Institute (CLSI), a minimum sample size of 30 isolates is needed to create an antibiogram [9]. Therefore, 3 years of data were needed to generate a minimum sample size of 30 isolates for vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*, the two main microorganisms known to have acquired vancomycin resistance. *Enterococcus casseliflavus, Enterococcus flavescens* and *Enterococcus gallinarum*, known to have intrinsic resistance to vancomycin, were also included in the study [10]. GraphPad Prism (Version 6.0c; GraphPad Software Incorporation, Boston, MA, United States) was the software application used to perform statistical analysis when needed. Two-tailed Fisher's exact tests were used to determine categorial differences between groups. $P < 0.05$ was determined to be statistically significant.

2.2. Identification of microorganisms in urine culture

The workup of the urine specimens depended on the information provide by clinicians on the laboratory requisition forms. Urine labeled as midstream, catheter, pedi-bag, suprapubic catheter, Ileal conduit and bladder stoma specimens underwent routine urine culture workup, in which a 0.001 mL loop of each urine specimen was inoculated onto BD BBL™ CHROMagar™ plates (Becton, Dickinson and Company, Sparks, MD, United States), which had media for isolation, differentiation and enumeration of urinary tract pathogens. These inoculated plates were incubated in oxygen incubator and read after 18 h of incubation. Urine specimens labeled as suprapubic bladder aspiration, cystoscopy, pre- and post-prostatic massage, renal stent, and nephrostomy specimens underwent special urine culture workup, in which a 0.01 mL loop was inoculated on both BD BBL™ CHROMagar™ and Sheep Blood Agar Base with 5% Sheep Blood (Oxoid Company, Nepean, ON, Canada) plates. These inoculated plates were incubated in oxygen incubator and read twice, after 18 and 36 h of incubation, to improve the yield of positive results in these special cases. Urine specimens without any of the above specifications on the laboratory requisition forms underwent routine culture workup. Duplicate urine specimens from the same patient collected on the same day were not processed and thereby excluded in the current analysis.

After the incubation period, only microorganisms with a minimum of 10 colony counts (equivalent to about 10 million colony forming units per liter for routine culture or 1 million colony forming units per liter for special culture) were considered significant amount that warranted the identification stage. A mix of 3 or more different types of microorganisms would indicate contamination and therefore would not proceed to the identification stage. When microorganisms were identified, they were isolated and speciated using VITEK2 System (bioMerieux Incorporation, Durham, NC, United States) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics GmbH & Company KG, Bremen, Germany), as per the manufacturers' manual instructions. The final results of urine culture and its microorganism identification were recorded in our MEWS software.

2.3. AST

Suspension of microorganisms was put in the VITEK 2 System for the AST as per the manufacturer's manual instructions, which had been previously validated with the CLSI-approved methods [10]. Antimicrobials routinely included for testing with *Enterococcus* species in urine were ampicillin, ciprofloxacin, nitrofurantoin, tetracycline, and vancomycin. The clinical breakpoints for interpretation of the minimum inhibitory concentration (MIC) results were from the CLSI M100 Performance Standards for AST of the corresponding year that determined whether a bacterial isolate is susceptible, intermediate, or resistant toward an antimicrobial [10]. For fosfomycin, CLSI provided clinical breakpoints for *E. faecalis* only, which were extrapolated to other *Enterococcus* species in the current study. *Enterococcus* species were tested with fosfomycin only if it was requested or no other susceptible oral antimicrobials options were available. Only vancomycin-resistant *E. faecalis* and *E. faecium* isolates were tested with linezolid. *E. faecalis* and *E. faecium* isolates were determined to have acquired vancomycin resistance when their vancomycin MIC were suggestive of resistance. Their resistance was then further confirmed with vanA and vanB polymerase chain reaction (PCR) testing. *Enterococcus* species isolates were determined to have intrinsic vancomycin resistance when the microorganism identification methods determined these isolates to be *E. casseliflavus*. *E. flavescens*

and *E. gallinarum* followed with confirmation with vanC PCR testing [10]. The AST final results were recorded in our MEWS software.

3. RESULTS

In 2021–2023, 1,093,498 urine specimens were submitted to LifeLabs BC regional microbiology laboratories for culture, of which 23 8588 of them reported growth of microorganisms. A total of 335 vancomycin-resistant *E. faecium* and 47 vancomycin-resistant *E. faecalis* were reported in urine culture, with their susceptibility profiles presented in Table 1. In the same period, 48 *E. gallinarum*, 25 *E. casseliflavus*, and no *E. flavescens* isolates were reported in urine culture, with their susceptibility profiles presented in Table 2. In comparison, there were 23 306 and 943 vancomycin-susceptible *E. faecalis* and vancomycin-susceptible *E. faecium* isolates reported in urine culture, respectively. *E. coli* was the most common microorganism identified in urine culture during this period (n = 147 742).

4. DISCUSSION

The current 3-year audit of urine culture generated an antibiogram of VRE that might guide selection of empirical oral antimicrobials in BC, Canada, when VRE UTI is suspected but with full AST result pending. The data suggested that we should not always assume the need to upgrade to broad-spectrum antimicrobials for VRE. In the present study, vancomycin-resistant *E. faecalis, E. casseliflavus*, and *E. gallinarum* isolates were >90% susceptible to ampicillin and nitrofurantoin (Tables 1 and 2). Furthermore, *E. casseliflavus* isolates were >90% susceptible to tetracycline, which was significantly different from the susceptibility rate of *E. gallinarum* (Table 2). The antimicrobial options for vancomycin-*resistant E. faecium* remained a challenge, with linezolid being the only reliable option (100% susceptibility rate). We believe that this local epidemiology of antimicrobial resistance should be made readily available to prescribers to not only guide treatment selection, but also reduce use of antimicrobials which are likely to be ineffective and unnecessarily cause adverse drug reactions to patients.

In the selected cases of isolates being tested, fosfomycin was unable to achieve >90% susceptibility rate among the vancomycin-resistant *E. faecalis* and *E. faecium* isolates (Table 1), and, thus, might not be a good empirical therapy for VRE. Although linezolid was shown to achieve 100% susceptibility rate in the present study, it is important to consider the high drug cost and potential adverse effects of linezolid. Linezolid is known to cause cytopenia especially with prolonged use and, thus, requires careful monitoring [11]. However, UTI generally requires only 3–10 days of treatment and this short duration is unlikely to cause cytopenia. Another serious potential adverse effect of linezolid is serotonin toxicity due to concomitant administration of MAO inhibitors that increase serotonin concentrations [12].

As mentioned in the introduction, asymptomatic bacteriuria VRE in pregnancy would require antimicrobial treatment [7]. The choice of antimicrobials would be limited due to avoidance of drugs with high potential of teratogenicity. *Enterococcus*

Table 1. Antimicrobial susceptibility profiles of vancomycin-resistant *E. faecalis* and *E. faecium* reported in urine culture

Microorganisms	*n*	Antibiotic (% susceptible)					
		Ampicillin^	Ciprofloxacin^	Linezolid	Nitrofurantoin^	Tetracycline	Fosfomycin*
E. faecalis	47	96	6	100	96	4	
E. faecium	335	5	0	100	13	12	
E. faecalis	7						86
E. faecium	263						57

*The Clinical and Laboratory Standards Institute fosfomycin clinical breakpoints for *Enterococcus faecalis* were extrapolated for other *Enterococcus* species. *Enterococcus* species were tested with fosfomycin only if requested or had no other susceptible oral antimicrobials options available.

Note: ^P<0.05 between the two *Enterococcus* species. *E. faecalis*: *Enterococcus faecalis*; *E. faecium*: *Enterococcus faecium*.

Table 2. Antimicrobial susceptibility profiles of vancomycin-resistant *E. casseliflavus* and *E. gallinarum* reported in urine culture

Microorganisms	*n*	Antibiotic (% susceptible)					
		Ampicillin	Ciprofloxacin	Linezolid	Nitrofurantoin	Tetracycline^	Fosfomycin*
E. casseliflavus	25	100	84		100	96	
E. gallinarum	49	92	80		98	59	
E. casseliflavus	1						100
E. gallinarum	3						100

*The Clinical and Laboratory Standards Institute fosfomycin clinical breakpoints for *Enterococcus faecalis* were extrapolated for other *Enterococcus* species. *Enterococcus* species were tested with fosfomycin only if requested or had no other susceptible oral antimicrobials options available.

Note: ^P<0.05 between the two *Enterococcus* species. *E. casseliflavus*: *Enterococcus casseliflavus*; *E. gallinarum*: *Enterococcus gallinarum*.

species are intrinsically resistant to cephalosporins, aminoglycosides, and trimethoprim-sulfamethoxazole and, thereby, would not be considered [10], despite being some of the antimicrobials recommended for UTI in pregnancy by the American College of Obstetricians and Gynecologists' (ACOG) Committee on Clinical Consensus–Obstetrics [13]. Nitrofurantoin may be a feasible option as this was shown to have >90% susceptibility rates, except for vancomycin-resistant *E. faecium* isolates, in the present study and is deemed to be compatible for pregnancy by ACOG [14]. Fosfomycin could be an alternative provided that AST showed the patient's *Enterococcus* species isolate was susceptible. However, both nitrofurantoin and fosfomycin have limited penetration in the renal parenchyma and thus not recommended for upper UTI [13]. Tetracycline and ciprofloxacin are generally not recommended during pregnancy due to theoretical congenital risks [15,16]. The safety of linezolid during pregnancy remains to be a point of discussion. Linezolid has been classified by the United States Food and Drug Administration as category C for use in pregnancy, meaning use only if benefit outweigh risk to the fetus, but a review study found no teratogenic effect [17]. If AST shows no feasible oral antimicrobial options, intravenous daptomycin may be considered for testing. Daptomycin is classified as category B for use in pregnancy, because case reports demonstrated successful use during the second and third trimesters of pregnancy and animal reproductive studies have not shown any fetal harm [18].

The present study showed that vancomycin-resistant *E. faecalis* had a more favorable susceptibility profile compared to *E. faecium*, as significant different susceptibility rates were observed in ampicillin, ciprofloxacin, and nitrofurantoin (Table 1). These results are consistent with a study of 4 208 *Enterococcus* species collected from patients in 49 European hospitals in 27 countries, in which *E. faecalis* isolates appeared to be more susceptible to amoxicillin and ciprofloxacin compared to *E. faecium* [19]. However, this European study did not provide these AST results specific to VRE. This illustrated the strength of this study which was specific for VRE and included many commonly prescribed oral antimicrobials, such as ampicillin, ciprofloxacin, fosfomycin, nitrofurantoin, and tetracycline. Another strength of this study was the inclusion of a large volume of community urine culture data from 129 collection centers in community (n = 1,093,498). The data should be generalizable to the community practice in this province. The antibiogram generated in the present study followed the guidance given by CLSI, such as inclusion of a minimum of 30 isolates, a minimum of 1 year of data, inclusion of only diagnostic isolates, inclusion of only final results, inclusion of antimicrobials routinely tested, exclusion of duplicates, and exclusion of intermediate or susceptible-dose-dependent results [9].

One major limitation of the present study was the exclusion of hospital microorganism isolates. We hope that the present study would influence hospital laboratories to conduct a similar antibiogram specific for VRE in their sites. It is unknown whether the results would be generalizable to other countries as resistance patterns may differ until proven otherwise. Another limitation was that some antibiotic-microorganism combination in the present study failed to reach the minimum sample size of 30 and, therefore, require readers to interpret results with caution due to potential selection bias. One other limitation was that diagnostic laboratories generally would not report microorganisms in urine culture with insignificant amount or mixed with at least two other types of microorganisms suggestive of contamination. It also indicated that the present study focused on clinically significant urine specimens. Nevertheless, as mentioned in the *Introduction*, it is important to note that certain clinical situations, such as pregnancy and time before endourological procedures, would still require treatment for asymptomatic bacteriuria [7]. At last, the microbiology regional laboratories did not include patients' clinical data, progress of their illness, and follow-up testing results. However, management of UTI generally depends on patients' self-management of symptoms and do not always require follow-up testing [20].

5. CONCLUSIONS

This 3-year study demonstrated that ampicillin and nitrofurantoin may be considered for UTI secondary to vancomycin-resistant *E. faecalis*, *E. casseliflavus*, and *E. gallinarum* in BC, Canada. Tetracycline may also be considered for *E. casseliflavus*. However, linezolid remained the only reliable oral antimicrobial option for vancomycin-resistant *E. faecium* until the full AST result is available. More studies are needed to validate the findings in the present study.

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CONFLICT OF INTEREST

Eugene Y.H. Yeung is working as a microbiologist, physician, pharmacist, and clinical assistant professor. The views and opinions expressed are those of the authors and do not necessarily reflect the views or positions of their employers.

AUTHOR CONTRIBUTIONS

Conceptualization: Roxanna S. D. Mohammed
Investigation: Eugene Y. H. Yeung
Methodology: Eugene Y. H. Yeung
Writing – original draft: Eugene Y. H. Yeung
Writing – review & editing: Roxanna S. D. Mohammed

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

Data used in this work is available from the corresponding author upon reasonable request.

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