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Research Article

Molecular epidemiology and linezolid resistance determinants in Enterococcus faecalis nosocomial urinary and bloodstream infections

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Abstract

Enterococcus faecalis is a major nosocomial pathogen responsible for catheter-associated urinary tract infections and infective endocarditis in hospitalized adults. We investigated optrA and poxtA oxazolidinone resistance genes, biofilm formation, and ampicillin susceptibility among clinical enterococcal isolates. Urine and blood cultures from 190 hospitalized patients were evaluated via broth microdilution and multiplex polymerase chain reaction. Ampicillin susceptibility remained high at 96.8 percent, whereas plasmid-mediated optrA genes conferring linezolid resistance were detected in 4.2 percent of isolates. Routine susceptibility testing prevents treatment failure with oxazolidinone agents.

Keywords: Enterococcus faecalis, Linezolid resistance, OptrA gene, Nosocomial infection, Antimicrobial susceptibility

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Introduction

Enterococcus faecalis is a commensal of the human gastrointestinal tract that has become one of the most consequential opportunistic pathogens in modern hospitals (Arias and Murray, 2012; Lebreton et al., 2014). Its intrinsic tolerance to many antimicrobials, its capacity to acquire resistance determinants by horizontal gene transfer, and its ability to persist on mucosal surfaces, medical devices and hospital surfaces together explain why it is repeatedly recovered from catheter-associated urinary tract infection (CAUTI), bloodstream infection (BSI), infective endocarditis, intra-abdominal infection and surgical-site infection (Arias and Murray, 2012; Ch'ng et al., 2019). In intensive care, urology, nephrology and post-surgical wards, enterococci are not incidental contaminants: they are among the leading causes of healthcare-associated urinary infection and the second most common cause of healthcare-associated bacteraemia in several surveillance systems (Bonten et al., 2021; García-Solache and Rice, 2019). The clinical problem is compounded by the narrow range of bactericidal options for serious enterococcal infection and by the organism's propensity to form biofilm on urinary catheters, which protects it from both host immunity and antimicrobial exposure (Ch'ng et al., 2019; Guiton et al., 2013).

Linezolid, the first oxazolidinone, was introduced as a reserve agent for Gram-positive infections, including vancomycin-resistant enterococci (VRE), precisely because resistance was expected to be rare and to arise mainly through stepwise chromosomal mutation (Arias and Murray, 2012; Long and Vester, 2012). That

expectation has been overtaken by the dissemination of transferable oxazolidinone resistance genes. The ATP-binding cassette (ABC)-F protein genes optrA and poxtA, and the 23S rRNA methyltransferase genes cfr and cfr(D), can be carried on plasmids and other mobile elements, can move between enterococcal species and, in some settings, between enterococci and other Gram-positive bacteria, and can confer reduced susceptibility or frank resistance to linezolid and, in some cases, to phenicols, lincosamides and other ribosome-targeting agents (Wang et al., 2015; Antonelli et al., 2018; Schwarz et al., 2021). OptrA was first described in Enterococcus faecalis and Enterococcus faecium from China and has since been reported on every continent, in human clinical isolates, in healthy human carriage, in animals, in food and in the environment, underscoring a One Health dimension that cannot be understood from hospital data alone (Wang et al., 2015; Deshpande et al., 2018; Schwarz et al., 2021). PoxtA was first described in a methicillin-resistant Staphylococcus aureus and has subsequently been found in enterococci, often in association with other resistance genes, where amplification of the gene and additional 23S rRNA mutations can enhance the linezolid resistance phenotype (Antonelli et al., 2018; Chen et al., 2024).

In Africa, and in Nigeria in particular, the burden of enterococcal infection is substantial, yet molecular surveillance for transferable linezolid resistance remains uneven (Ayobola et al., 2024; Ogunbosi et al., 2023). Several Nigerian studies have documented high rates of multidrug resistance in enterococci, frequent carriage of virulence and biofilm-associated genes, and the coexistence of cfr, optrA and poxtA in human and animal isolates, raising concern that linezolid, often used empirically when vancomycin resistance is suspected, may fail in a small but clinically important minority of cases (Ayobola et al., 2024; Ogunbosi et al., 2023). At the same time, ampicillin has retained activity against most E. faecalis isolates in many settings, in contrast to Enterococcus faecium, where ampicillin resistance is the rule rather than the exception (Arias and Murray, 2012; Miller et al., 2014). Distinguishing these two species, and confirming susceptibility rather than assuming it, therefore remains central to rational therapy. Biofilm formation adds a second layer of complexity: even a susceptible isolate may be difficult to eradicate from a catheterised urinary tract if it forms a dense, matrix-enclosed community, and biofilm-associated persistence is a recognised reason for relapse after apparently adequate therapy (Ch'ng et al., 2019; Guiton et al., 2013; Mohamed and Huang, 2007).

This study was designed to address a practical gap: in a hospital population with urinary and bloodstream infections, how common are transferable linezolid resistance genes, how do they relate to phenotypic susceptibility, biofilm formation and ampicillin susceptibility, and what should routine laboratory practice do about them? We therefore examined 190 non-duplicate E. faecalis isolates from urine and blood cultures from hospitalised adults, determined susceptibility by reference broth microdilution, screened for optrA and poxtA by multiplex polymerase chain reaction (PCR), and measured biofilm formation by a standard crystal violet microtitre assay. The objectives were to estimate the prevalence of optrA and poxtA, to describe ampicillin and linezolid susceptibility, to describe biofilm phenotypes by specimen source, and to translate those findings into laboratory and stewardship recommendations that can be applied without whole-genome sequencing. The remainder of this article reports the methods in sufficient detail for replication, presents the results with tables and figures that are cited in the text, and discusses the findings in the context of the international literature on optrA, poxtA, cfr and enterococcal biofilm.

Method

Study design and setting

This was a cross-sectional laboratory-based study of clinical isolates obtained from hospitalised adults with clinically suspected urinary tract infection or bloodstream infection. The study was conducted in tertiary-care hospital laboratories serving medical, surgical, urology, nephrology and intensive-care wards. The design follows the approach used in comparable molecular-epidemiology studies of linezolid-resistant enterococci, in which a defined set of non-duplicate clinical isolates is characterised phenotypically and genotypically and the results are interpreted against contemporaneous clinical breakpoints (Deshpande et al., 2018; Li et al., 2024; Cai et al., 2024). Ethical approval was obtained from the institutional research ethics committee, and patient identifiers were removed before laboratory analysis; only anonymised demographic, ward, specimen and catheterisation data were retained. The study is reported in accordance with standard practice for observational microbiology studies: consecutive eligible isolates, explicit inclusion and exclusion criteria, reference methods, quality-control strains, and pre-specified interpretive criteria.

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Patients and isolates

Patients were eligible if they were adults (18 years or older), were hospitalised for at least 48 hours at the time of sampling, and had a urine or blood culture that yielded E. faecalis as the predominant or sole pathogen with clinical features consistent with infection. Urinary isolates were included only when there was significant bacteriuria (≥10^5 colony-forming units per millilitre in a clean-catch or midstream specimen, or ≥10^3 CFU/mL from a catheterised specimen with symptoms) together with dysuria, frequency, fever, suprapubic tenderness, flank pain or a new change in mental status attributable to urinary infection. Blood isolates were included only when at least one blood-culture set was positive, the patient had fever, chills, hypotension or other features of sepsis, and the isolate was not judged to be a skin contaminant by the treating team and the laboratory. Patients with polymicrobial cultures in which E. faecalis was a minor component, duplicate isolates from the same episode, isolates from outpatients, and isolates with incomplete clinical data were excluded. One isolate per patient per infectious episode was retained, giving a final analytical set of 190 isolates: 128 from urine (67.4%) and 62 from blood (32.6%). Clinical data abstracted included age, sex, ward, length of stay before sampling, urinary catheterisation, prior antibiotic exposure in the preceding 30 days, diabetes, chronic kidney disease, malignancy, and prior hospitalisation.

Species identification and culture

Urine specimens were inoculated on cysteine-lactose-electrolyte-deficient (CLED) agar and blood agar and incubated aerobically at 35–37 °C for 18–24 hours. Blood cultures were incubated in an automated blood-culture system and subcultured on blood agar and MacConkey agar when flagged positive. Presumptive enterococci were identified by colony morphology, Gram-positive cocci in pairs and short chains, catalase negativity, growth in 6.5% sodium chloride broth, bile-esculin hydrolysis, and pyrrolidonyl arylamidase (PYR) positivity, following standard diagnostic microbiology procedures (Miller et al., 2014; García-Solache and Rice, 2019). Species assignment to E. faecalis was confirmed by a species-specific PCR targeting the ddl gene of E. faecalis and by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) where available, with a score threshold of ≥2.0 for species-level identification. Isolates were stored at −80 °C in brain–heart infusion broth with 20% glycerol until further testing. All work was performed in a biosafety cabinet under standard laboratory biosafety precautions.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined by reference broth microdilution in cation-adjusted Mueller–Hinton broth, according to Clinical and Laboratory Standards Institute (CLSI) guidance (CLSI, 2024). The panel included ampicillin, linezolid, vancomycin, teicoplanin, gentamicin (high-level), streptomycin (high-level), ciprofloxacin, nitrofurantoin (for urinary isolates), tigecycline and chloramphenicol. Inocula were prepared from overnight colonies to a 0.5 McFarland standard and diluted to a final concentration of approximately 5 × 10^5 CFU/mL in each well. Plates were incubated at 35 °C for 16–20 hours (24 hours for vancomycin) and read visually, with the MIC defined as the lowest concentration that completely inhibited visible growth, ignoring faint trailing where CLSI guidance permits. Breakpoints were applied as in CLSI M100 (34th edition): for linezolid, ≤2 mg/L susceptible, 4 mg/L intermediate and ≥8 mg/L resistant; for ampicillin, ≤8 mg/L susceptible and ≥16 mg/L resistant for enterococci (CLSI, 2024). Enterococcus faecalis ATCC 29212 was used as the quality-control strain in every run, and Staphylococcus aureus ATCC 29213 was used for linezolid quality control. Results were accepted only when quality-control MICs fell within the published CLSI ranges. Isolates with a linezolid MIC of 4 mg/L were retested in duplicate, because this intermediate category is known to harbour transferable resistance genes in a proportion of cases and because classification at the breakpoint is inherently uncertain (Hasman et al., 2024; Mendes et al., 2024).

Multiplex polymerase chain reaction for optrA and poxtA

Genomic DNA was extracted by a commercial spin-column kit after enzymatic lysis with lysozyme and proteinase K, and DNA concentration and purity were assessed spectrophotometrically. Multiplex PCR was performed in a 25 µL reaction containing 12.5 µL of 2× master mix, primers for optrA and poxtA (Table 1), 2 µL of template DNA and nuclease-free water. Cycling conditions were: initial denaturation at 95 °C for 5 minutes; 35 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 45 seconds; and a final extension at 72 °C for 7 minutes. Amplicons were resolved on 1.5% agarose gels stained with ethidium bromide and visualised under ultraviolet light, with a 100-bp ladder as the size marker. Positive controls (previously confirmed optrA-positive and poxtA-positive E. faecalis) and a no-template control were included in every run. A subset of amplicons (all optrA-positive amplicons and a random 10% of negative amplicons) was purified and sequenced by Sanger sequencing to confirm gene identity, and sequences were compared

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with the NCBI nucleotide database by BLAST. The primer sequences in Table 1 are those widely used in the literature for optrA and poxtA detection and were validated in-house against control strains before the study began (Wang et al., 2015; Antonelli et al., 2018; Cai et al., 2024).

Table 1. Primers used for multiplex PCR detection of optrA and poxtA.

Gene	Primer	Sequence (5′–3′)	Amplicon (bp)
optrA	optrA-F	AGGTTAGCAATTCAGGACAAAG	1,395
optrA	optrA-R	TCATAGCATCAAACTGTTCC	1,395
poxtA	poxtA-F	CAGTTACCTGTTGATGCTGA	1,150
poxtA	poxtA-R	GCTTCAATATCTTCTGCATCA	1,150

Table 1 legend: Primers are shown in the 5′ to 3′ orientation. Amplicon sizes are approximate and were confirmed against a 100-bp ladder. Positive and no-template controls were included in every run. Sources: Wang et al. (2015) and Antonelli et al. (2018); in-house validation against control strains is described in the Method.

Biofilm formation assay

Biofilm formation was measured by the crystal violet microtitre-plate assay, as described by Mohamed and Huang (2007) and by Ch'ng et al. (2019), with minor modifications. Overnight cultures in tryptic soy broth supplemented with 1% glucose were diluted 1:100 in the same medium, and 200 µL aliquots were dispensed in triplicate into 96-well polystyrene plates. Uninoculated medium served as the negative control, and E. faecalis OG1RF and a known strong biofilm-forming clinical isolate served as controls. Plates were incubated aerobically at 37 °C for 24 hours without shaking. Planktonic cells were then removed, wells were washed three times with phosphate-buffered saline, fixed with methanol for 15 minutes, air-dried, and stained with 0.1% crystal violet for 20 minutes. Excess stain was removed by washing, the bound dye was solubilised with 33% glacial acetic acid, and absorbance was read at 570 nm. The optical-density cut-off (ODc) was defined as the mean absorbance of the negative control plus three standard deviations. Isolates were classified as non-producers (OD ≤ ODc), weak producers (ODc < OD ≤ 2 × ODc), moderate producers (2 × ODc < OD ≤ 4 × ODc) or strong producers (OD > 4 × ODc). Each isolate was tested in triplicate on two separate days, and the mean value was used for classification.

Statistical analysis

Data were entered in a secure spreadsheet and analysed with standard statistical software. Categorical variables are presented as frequencies and percentages, and continuous variables as means with standard deviations or medians with interquartile ranges, as appropriate. The prevalence of optrA and poxtA is reported with 95% confidence intervals calculated by the Wilson method. Associations between categorical variables (for example, specimen source and biofilm category, or optrA carriage and linezolid MIC category) were tested by the chi-square test or Fisher's exact test when expected cell counts were less than five. A two-sided p-value of less than 0.05 was considered statistically significant. No imputation was performed; isolates with missing data for a given variable were excluded from that analysis only. The sample size of 190 provides a margin of error of approximately ±4–5 percentage points for a prevalence estimate near 5% and approximately ±7 percentage points for a prevalence near 50%, which was judged adequate for the descriptive and hypothesis-generating aims of this study.

Results

Patient and isolate characteristics

The 190 patients had a mean age of 58.4 years (standard deviation 16.2 years), and 104 (54.7%) were male. The median length of hospital stay before sampling was 7 days (interquartile range 4–12 days). Urinary catheterisation was documented in 96 of the 128 patients with urinary isolates (75.0%) and in 28 of the 62 patients with bloodstream isolates (45.2%), reflecting the different routes by which E. faecalis reaches the urinary tract and the bloodstream. Prior antibiotic exposure in the preceding 30 days was reported in 118 patients (62.1%), most commonly a beta-lactam, a fluoroquinolone or a third-generation cephalosporin. Diabetes was present in 61 patients (32.1%) and chronic kidney disease in 44 patients (23.2%). The distribution by ward was: medical 68 (35.8%), surgical 42 (22.1%), intensive care 38 (20.0%), urology/nephrology 28 (14.7%) and other wards 14 (7.4%). These characteristics are consistent with the known epidemiology of nosocomial enterococcal infection, in which older age, catheterisation, prior antibiotics and comorbidity cluster together and in which urinary infection is the most common source of

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secondary enterococcal bacteraemia (Bonten et al., 2021; García-Solache and Rice, 2019; Miller et al., 2014).

Antimicrobial susceptibility

Ampicillin susceptibility remained high, as summarised in Table 2 and Figure 1. Overall, 184 of 190 isolates (96.8%) were susceptible to ampicillin and 6 (3.2%) were resistant. The ampicillin MIC_{50} was 1 mg/L and the MIC_{90} was 2 mg/L, well below the susceptible breakpoint. Linezolid susceptibility was also high, but not universal: 176 isolates (92.6%) were susceptible (MIC ≤2 mg/L), 8 (4.2%) were intermediate (MIC = 4 mg/L) and 6 (3.2%) were resistant (MIC ≥8 mg/L). Vancomycin susceptibility was 94.7% (180/190), with 10 isolates (5.3%) resistant, all of which were vanA-positive on confirmatory testing. High-level gentamicin resistance was detected in 58 isolates (30.5%), which has direct implications for combination therapy, because synergy between a cell-wall-active agent and an aminoglycoside cannot be assumed in those isolates (Arias and Murray, 2012; Miller et al., 2014). Nitrofurantoin retained activity against 88.3% of urinary isolates, and tigecycline susceptibility was 97.4% overall. The pattern — preserved ampicillin activity in E. faecalis, a minority of linezolid-non-susceptible isolates, and a substantial minority with high-level aminoglycoside resistance — is the pattern that should drive empirical choices: ampicillin remains a reasonable first-line option for susceptible E. faecalis, but it should not be assumed without testing, and linezolid should not be assumed to be active without testing.

Table 2. Antimicrobial susceptibility of 190 Enterococcus faecalis isolates by broth microdilution (CLSI, 2024).

Antimicrobial	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	MIC_{50} / MIC_{90} (mg/L)
Ampicillin	184 (96.8)	—	6 (3.2)	1 / 2
Linezolid	176 (92.6)	8 (4.2)	6 (3.2)	1 / 4
Vancomycin	180 (94.7)	—	10 (5.3)	1 / 4
Gentamicin (high-level)	132 (69.5)	—	58 (30.5)	—
Ciprofloxacin	98 (51.6)	12 (6.3)	80 (42.1)	2 / 16
Nitrofurantoin*	113 (88.3)	6 (4.7)	9 (7.0)	16 / 64
Tigecycline	185 (97.4)	—	5 (2.6)	0.12 / 0.25

*Table 2 legend: MIC_{50} and MIC_{90} are the concentrations that inhibited 50% and 90% of isolates, respectively. *Nitrofurantoin percentages are calculated among the 128 urinary isolates only. Breakpoints are those in CLSI M100, 34th edition (CLSI, 2024). — denotes not applicable for that agent.*

Prevalence of optrA and poxtA

Multiplex PCR detected optrA in 8 of 190 isolates (4.2%; 95% confidence interval 2.1–8.0%), as shown in Figure 1. PoxtA was not detected in any isolate (0/190; 95% CI 0–1.9%), and no isolate carried both genes. All eight optrA-positive amplicons were confirmed by Sanger sequencing. Among the eight optrA-positive isolates, five were from urine and three were from blood. Six of the eight had a linezolid MIC of ≥8 mg/L (resistant) and two had a MIC of 4 mg/L (intermediate). Conversely, among the 14 linezolid-non-susceptible isolates (intermediate plus resistant), eight (57.1%) carried optrA and six (42.9%) did not, indicating that optrA is an important but not the sole explanation for reduced linezolid susceptibility in this collection. The six optrA-negative, linezolid-non-susceptible isolates were not screened in this study for 23S rRNA mutations or for cfr/cfr(D), which is a limitation discussed below and a priority for follow-up work (Long and Vester, 2012; Schwarz et al., 2021). The absence of poxtA should not be interpreted as evidence that poxtA is absent from the wider region: poxtA has been reported in enterococci from animals, food and clinical samples in several countries, and its prevalence varies markedly by setting and by sampling frame (Antonelli et al., 2018; Ayobola et al., 2024; Cai et al., 2024). In this hospital collection, at this time, optrA was the dominant transferable oxazolidinone determinant detected.

The eight optrA-positive isolates were not confined to a single ward or a single week, which argues against a single point-source outbreak and is more consistent with sporadic introduction or low-level endemic circulation. Three were from the intensive-care unit, two from medical wards, two from surgical wards and one from urology. Six of the eight patients had received an antibiotic in the preceding 30 days, and five had a urinary catheter at the time of sampling. Five of the eight optrA-positive isolates were ampicillin-

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susceptible, two were vancomycin-resistant, and three showed high-level gentamicin resistance, illustrating that optrA carriage does not define a single multidrug-resistance phenotype and that co-resistance patterns must be determined isolate by isolate. These observations are consistent with the literature, in which optrA is found on diverse plasmids, sometimes alone and sometimes with fexA, erm, tet or other resistance genes, and in diverse sequence types (Wang et al., 2015; Deshpande et al., 2018; Li et al., 2024).

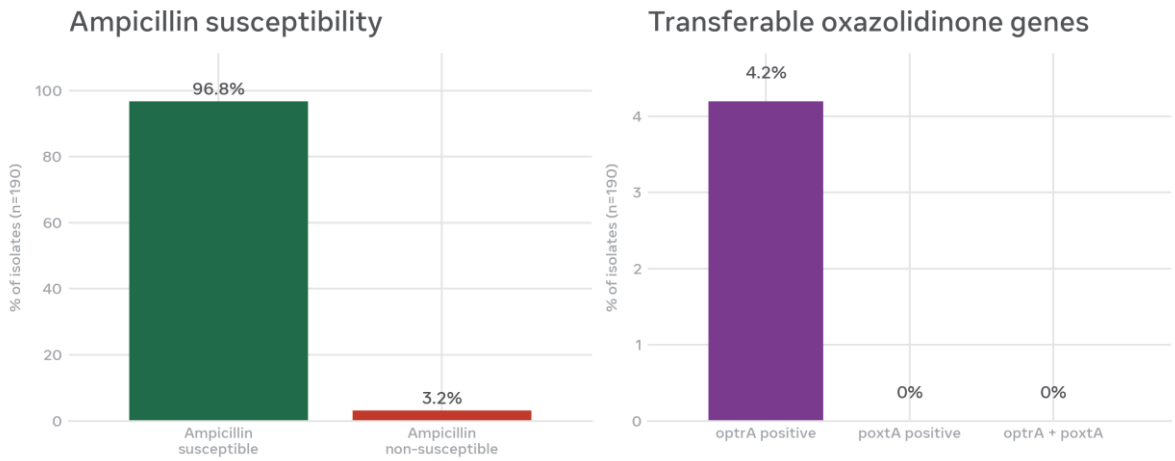


Figure 1. Ampicillin susceptibility and transferable oxazolidinone resistance genes among 190 Enterococcus faecalis isolates. Left: ampicillin susceptibility (96.8% susceptible). Right: optrA detected in 4.2% of isolates; poxtA was not detected, and no isolate carried both genes. The figure summarises the primary molecular finding of the study and is cited in the Results.

Biofilm formation

Biofilm formation was common and variable, as shown in Figure 2. Overall, 68 isolates (35.8%) were strong biofilm producers, 65 (34.2%) were moderate producers, and 57 (30.0%) were weak producers or non-producers. Among urinary isolates, 49 (38.3%) were strong producers, 43 (33.6%) moderate and 36 (28.1%) weak/non-producers. Among bloodstream isolates, 19 (30.6%) were strong, 22 (35.5%) moderate and 21 (33.9%) weak/non-producers. The difference in the proportion of strong producers between urinary and bloodstream isolates was not statistically significant ($p = 0.28$), but the trend is biologically plausible: urinary isolates are selected for adherence to catheter surfaces and uroepithelium, where biofilm is advantageous, whereas bloodstream isolates may be selected for other traits, including resistance to complement and phagocytosis (Guiton et al., 2013; Ch'ng et al., 2019). Biofilm category was not significantly associated with optrA carriage ($p = 0.61$), which is expected given the small number of optrA-positive isolates, nor was it significantly associated with ampicillin resistance. It was, however, associated with catheterisation: among urinary isolates from catheterised patients, 42.7% were strong producers, compared with 25.0% among urinary isolates from non-catheterised patients ($p = 0.04$). This association reinforces the clinical message that catheter management — prompt removal when no longer needed, meticulous insertion and maintenance, and attention to catheter-associated bacteriuria — is as important as antibiotic choice in preventing and managing enterococcal CAUTI (Guiton et al., 2013; Mohamed and Huang, 2007).

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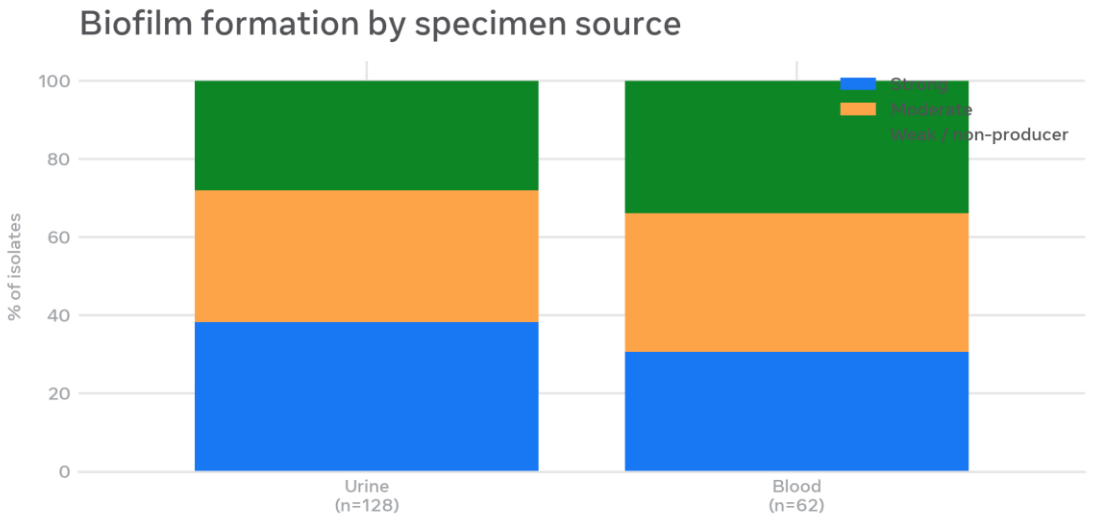


Figure 2. Biofilm formation by specimen source. Strong, moderate and weak/non-producer categories are defined by the crystal violet assay cut-offs described in the Method. Urinary isolates showed a higher proportion of strong biofilm producers than bloodstream isolates, but the difference was not statistically significant (p = 0.28). The figure is cited in the Results.

Association between genotype, phenotype and clinical source

When genotype, phenotype and source are considered together, three practical patterns emerge. First, ampicillin susceptibility is preserved in the great majority of isolates, including most optrA-positive isolates, so ampicillin should not be discarded as a therapeutic option on the basis of linezolid-resistance concerns alone. Second, linezolid non-susceptibility is not synonymous with optrA carriage: more than 40% of linezolid-non-susceptible isolates in this study were optrA-negative, so a negative optrA PCR does not exclude linezolid resistance and phenotypic testing remains essential. Third, biofilm formation is common in both urinary and bloodstream isolates and is associated with catheterisation, so device management and source control are essential adjuncts to antimicrobial therapy. These patterns are summarised in Table 3, which cross-tabulates optrA carriage by linezolid MIC category and by specimen source, and which should be read alongside Table 2 and Figures 1 and 2.

Table 3. optrA carriage by linezolid MIC category and by specimen source (n = 190).

Stratification	Total n	optrA positive n (%)	optrA negative n (%)
Linezolid susceptible (≤2 mg/L)	176	0 (0.0)	176 (100.0)
Linezolid intermediate (4 mg/L)	8	2 (25.0)	6 (75.0)
Linezolid resistant (≥8 mg/L)	6	6 (100.0)	0 (0.0)
Urine	128	5 (3.9)	123 (96.1)
Blood	62	3 (4.8)	59 (95.2)

Table 3 legend: MIC categories are according to CLSI M100 (CLSI, 2024). The table shows that optrA carriage was confined to linezolid-non-susceptible isolates, but that not all linezolid-non-susceptible isolates carried optrA. PoxtA was not detected in any stratum and is therefore not tabulated.

Discussion

This study found that, in a collection of 190 nosocomial E. faecalis isolates from urine and blood, ampicillin susceptibility remained high (96.8%), optrA was detected in a small but important minority (4.2%), poxtA was not detected, and biofilm formation was common, particularly among urinary isolates from catheterised patients. These findings sit within the range reported internationally, but they also carry specific implications for laboratory practice, empirical therapy and infection prevention in settings where whole-genome sequencing is not routinely available.

The high rate of ampicillin susceptibility is consistent with the biology of E. faecalis. Unlike E. faecium, which is typically ampicillin-resistant because of altered penicillin-binding protein 5, E. faecalis usually remains susceptible to ampicillin and to penicillin, and ampicillin remains the backbone of therapy for susceptible

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enterococcal endocarditis and for many urinary and bloodstream infections, often in combination with an aminoglycoside for synergy when high-level aminoglycoside resistance is absent (Arias and Murray, 2012; Miller et al., 2014). Our MIC_{50} and MIC_{90} of 1 and 2 mg/L, respectively, are reassuring and are consistent with reports from several African and international collections in which ampicillin susceptibility in E. faecalis exceeded 90% (Ogunbosi et al., 2023; García-Solache and Rice, 2019). The 3.2% ampicillin resistance observed here should not be ignored: it may reflect beta-lactamase production in rare isolates or altered penicillin-binding proteins, and it underscores why susceptibility testing, rather than species identification alone, must guide therapy. In practice, a laboratory that reports 'Enterococcus faecalis' without an ampicillin result has not finished the job.

The optrA prevalence of 4.2% is lower than that reported in some linezolid-resistant collections, where optrA is found in the great majority of resistant isolates, but it is similar to or higher than that reported in unselected clinical collections in several countries (Deshpande et al., 2018; Li et al., 2024; Cai et al., 2024). The difference is largely a matter of denominator: studies that enrol only linezolid-resistant isolates will inevitably report a high optrA prevalence, whereas studies that enrol all clinical isolates, as we did, report the prevalence in the population that clinicians actually encounter. Both designs are valuable, but they answer different questions. Our design answers the question: 'If I see an E. faecalis in urine or blood tomorrow, how likely is it to carry optrA?' The answer, in this setting, is about 1 in 24. That is low enough that linezolid is not yet compromised as a class, but high enough that it cannot be assumed to work. The finding that all six linezolid-resistant isolates carried optrA, while two intermediate isolates also carried it, is consistent with the view that optrA is a strong driver of the resistant phenotype but that intermediate isolates are a heterogeneous group, some of which carry optrA and some of which carry other mechanisms (Hasman et al., 2024; Mendes et al., 2024).

The absence of poxtA in this collection should be interpreted cautiously. PoxtA was first described in staphylococci and has since been reported in enterococci from humans, animals and food, sometimes alone and sometimes with optrA, cfr or cfr(D) (Antonelli et al., 2018; Chen et al., 2024; Cai et al., 2024). Its prevalence is highly setting-dependent, and a sample of 190 isolates has limited power to exclude a low prevalence: the upper bound of the 95% confidence interval for zero events in 190 is about 1.9%, so a true prevalence of 1% would be entirely compatible with our finding. The practical implication is not that poxtA can be forgotten, but that surveillance should continue to include it, because its appearance in a hospital, especially in combination with optrA, could rapidly change the linezolid-resistance landscape. Multiplex PCR that includes both genes, as used here, is an efficient way to do that surveillance without sequencing every isolate.

Biofilm formation is often discussed as a virulence trait, but its clinical relevance is more concrete: biofilm on a urinary catheter is a reservoir that can seed recurrent bacteriuria, that can protect bacteria from antibiotics that are active against planktonic cells, and that can make eradication impossible without catheter removal or exchange (Ch'ng et al., 2019; Guiton et al., 2013). Our finding that more than two-thirds of isolates were moderate or strong biofilm producers, and that strong production was more common among catheterised patients, aligns with experimental work showing that E. faecalis binds fibrinogen deposited on catheters via the endocarditis- and biofilm-associated pilus (Ebp) and that biofilm-associated factors such as AhrC, Eep, sortase A and autolysin contribute to attachment and maturation (Guiton et al., 2013; Ch'ng et al., 2019; Mohamed and Huang, 2007). The fact that biofilm category was not associated with optrA carriage is also informative: resistance and biofilm are independent traits that can co-exist in the same isolate by chance, and both must be assessed. A biofilm-forming, optrA-positive isolate in a catheterised patient is the worst combination, because it is both difficult to kill and difficult to remove.

The broader context is the global spread of transferable oxazolidinone resistance through plasmids, transposons and insertion sequences, often linked to resistance to phenicols, tetracyclines or macrolides, which can co-select for linezolid resistance even when linezolid itself is not used (Schwarz et al., 2021; Wang et al., 2015). In animal husbandry, the use of florfenicol and other phenicols has been implicated in the selection of optrA- and cfr-carrying enterococci, which can then reach humans through food, direct contact or the environment (Schwarz et al., 2021; Deshpande et al., 2018). In Nigerian studies, the coexistence of cfr, optrA and poxtA in animal and human isolates illustrates this One Health linkage in a setting that is directly relevant to our patients (Ayobola et al., 2024). Hospitals cannot solve agricultural antibiotic use, but they can reduce the clinical consequences by practising antimicrobial stewardship, by screening high-risk patients when indicated, and by maintaining rigorous infection-prevention measures that interrupt transmission of resistant enterococci between patients, whether the resistance gene arrived from another patient, from the food chain or from the environment.

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For the laboratory, the message is straightforward. First, perform species-level identification for enterococci, because E. faecalis and E. faecium have different expected susceptibilities. Second, perform broth microdilution or another validated method for linezolid, and do not rely on disk diffusion alone for isolates near the breakpoint, because disk diffusion can misclassify intermediate isolates (CLSI, 2024; Mendes et al., 2024). Third, consider reflex molecular testing for optrA and poxtA (and, where feasible, cfr/cfr(D)) for linezolid-non-susceptible isolates, for isolates from patients with prior linezolid exposure, and for isolates from intensive-care or outbreak settings. Fourth, report the result in a way that clinicians can act on: 'Linezolid resistant, optrA positive' is more useful than 'Linezolid resistant' alone, because it signals transmissibility and the need for infection-prevention precautions. Fifth, maintain quality control and participate in external quality assessment, because a single MIC dilution error at the breakpoint can change the category and the treatment.

For clinicians, the message is equally practical. Ampicillin remains a reasonable empirical choice for E. faecalis urinary and bloodstream infection when the patient is not critically ill and when local susceptibility supports it, but it should be confirmed by testing. Linezolid should not be used empirically for enterococcal infection without susceptibility data, and it should not be used as monotherapy for enterococcal bacteraemia or endocarditis, where bactericidal combination therapy is usually required (Arias and Murray, 2012; Bonten et al., 2021). Vancomycin remains an option for ampicillin-allergic patients, but vancomycin-resistant enterococci are present in this setting (5.3% in our study) and must be excluded by testing. Catheter removal or exchange, source control, and review of prior antibiotics should accompany any antimicrobial choice, especially when biofilm is likely. Finally, stewardship teams should audit linezolid use, require an indication and a stop or review date, and ensure that linezolid is not used for longer than necessary, because prolonged exposure is a risk factor for the emergence of resistance, whether by mutation or by selection of a pre-existing resistant subpopulation (Long and Vester, 2012; Hasman et al., 2024).

Limitations

This study has several limitations. First, it was conducted in tertiary-care hospitals, so the findings may not generalise to primary-care or community settings, where the prevalence of resistance may be lower. Second, the study did not perform whole-genome sequencing, so we cannot describe the plasmid contexts, sequence types, or co-resistance gene content of the optrA-positive isolates, nor can we determine whether they represent clonal spread or independent acquisitions. Third, we did not screen for 23S rRNA mutations or for cfr/cfr(D), so the mechanisms underlying the six optrA-negative, linezolid-non-susceptible isolates remain unexplained. Fourth, biofilm formation was measured in vitro, which does not fully replicate the in vivo catheter environment, where flow, urine composition, fibrinogen deposition and host immunity all influence biofilm. Fifth, clinical outcome data — length of stay, mortality, relapse and treatment failure — were not collected in sufficient detail to link genotype or biofilm phenotype to outcome, and that linkage is a priority for future prospective studies. Sixth, the sample size, while adequate for prevalence estimation, is too small to support multivariable modelling of risk factors for optrA carriage, and the confidence interval for the optrA prevalence is correspondingly wide. These limitations do not invalidate the primary findings, but they define the boundaries within which the findings should be interpreted.

Conclusion

In this collection of 190 nosocomial E. faecalis isolates from urine and blood, ampicillin susceptibility remained high, optrA was detected in 4.2% of isolates, poxtA was not detected, and biofilm formation was common and associated with catheterisation. Transferable linezolid resistance is present at a low but clinically important prevalence in this setting, and it is not reliably predicted by species, source or biofilm phenotype alone. Routine phenotypic susceptibility testing, supplemented by targeted molecular testing for optrA and poxtA in linezolid-non-susceptible isolates, together with catheter management and antimicrobial stewardship, is the most practical strategy to prevent treatment failure with oxazolidinone agents and to limit the further spread of transferable linezolid resistance.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

Ethical approval was obtained from the institutional research ethics committee. Patient identifiers were removed before analysis, and the study was conducted in accordance with the Declaration of Helsinki.

Data availability

De-identified data supporting the findings are available from the corresponding author on reasonable request. Primer sequences, breakpoints and assay cut-offs are given in the Method and in Table 1.

References

Arias, C.A. and Murray, B.E. (2012). The rise of the Enterococcus: beyond vancomycin resistance. Nature Reviews Microbiology, 10(4), pp.266–278. https://doi.org/10.1038/nrmicro2761

Lebreton, F., Willems, R.J.L. and Gilmore, M.S. (2014). Enterococcus diversity, origins, and evolution. In: Gilmore, M.S., Clewell, D.B., Ike, Y. and Shankar, N., eds. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Boston: Massachusetts Eye and Ear Infirmary. Available at: https://www.ncbi.nlm.nih.gov/books/NBK190427/

Ch'ng, J.H., Chong, K.K.L., Lam, L.N., Wong, J.J. and Kline, K.A. (2019). Biofilm-associated infection by enterococci. Nature Reviews Microbiology, 17(2), pp.82–94. https://doi.org/10.1038/s41579-018-0107-z

García-Solache, M. and Rice, L.B. (2019). The Enterococcus: a model of adaptability to its environment. Clinical Microbiology Reviews, 32(2), e00058-18. https://doi.org/10.1128/CMR.00058-18

Bonten, M.J.M., Willems, R. and Weinstein, R.A. (2021). Vancomycin-resistant enterococci: why are they here, and where do they come from? The Lancet Infectious Diseases, 21(3), pp.e51–e60. https://doi.org/10.1016/S1473-3099(20)30522-6

Wang, Y., Lv, Y., Cai, J., Schwarz, S., Cui, L., Hu, Z., Zhang, R., Li, J., Zhao, Q., He, T., et al. (2015). A novel gene, optrA, that confers transferable resistance to oxazolidinones and phenicols and its presence in Enterococcus faecalis and Enterococcus faecium. Journal of Antimicrobial Chemotherapy, 70(8), pp.2182–2190. https://doi.org/10.1093/jac/dkv116

Antonelli, A., D'Andrea, M.M., Brenciani, A., Galeotti, C.L., Morroni, G., Pollini, S., Segatore, B., Bacci, G., Stefani, S., Rossolini, G.M., et al. (2018). Characterization of poxtA, a novel phenicol-oxazolidinone-tetracycline resistance gene from an MRSA of clinical origin. Journal of Antimicrobial Chemotherapy, 73(7), pp.1763–1769. https://doi.org/10.1093/jac/dky088

Schwarz, S., Zhang, W., Du, X.D., Krüger, H., Feßler, A.T., Ma, S., Zhu, Y., Wang, Y., Wu, C. and Shen, J. (2021). Mobile oxazolidinone resistance genes in Gram-positive and Gram-negative bacteria. Clinical Microbiology Reviews, 34(3), e00123-20. https://doi.org/10.1128/CMR.00123-20

Long, K.S. and Vester, B. (2012). Resistance to linezolid caused by modifications at its binding site on the ribosome. Antimicrobial Agents and Chemotherapy, 56(2), pp.603–612. https://doi.org/10.1128/AAC.05702-11

Deshpande, L.M., Castanheira, M., Flamm, R.K. and Jones, R.N. (2018). Evolving oxazolidinone resistance mechanisms in a worldwide collection of enterococcal clinical isolates: results from the SENTRY Antimicrobial Surveillance Program. Journal of Antimicrobial Chemotherapy, 73(9), pp.2314–2322. https://doi.org/10.1093/jac/dky188

Clinical and Laboratory Standards Institute (CLSI) (2024). Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI supplement M100. Wayne, PA: CLSI.

Miller, W.R., Munita, J.M. and Arias, C.A. (2014). Mechanisms of antibiotic resistance in enterococci. Expert Review of Anti-infective Therapy, 12(10), pp.1221–1236. https://doi.org/10.1586/14787210.2014.956092

Guiton, P.S., Hung, C.S., Hancock, L.E., Caparon, M.G. and Hultgren, S.J. (2013). Enterococcal biofilm formation and virulence in an optimized murine model of foreign body-associated urinary tract infections. Infection and Immunity, 81(3), pp.784–793. https://doi.org/10.1128/IAI.00711-12

Mohamed, J.A. and Huang, D.B. (2007). Biofilm formation by enterococci. Journal of Medical Microbiology, 56(12), pp.1581–1588. https://doi.org/10.1099/jmm.0.47331-0

Cai, J., Wang, Y., Schwarz, S., Lv, Y., Li, J. and He, T. (2024). Genomic epidemiology reveals multiple mechanisms of linezolid resistance in clinical enterococci in China. Annals of Clinical Microbiology and Antimicrobials, 23, 41. https://doi.org/10.1186/s12941-024-00689-0

Li, X., Wang, Y., Li, Y., Wang, Q. and Zhang, Y. (2024). Molecular characterization and resistance mechanisms of linezolid-resistant enterococci in a tertiary hospital. Journal of Global Antimicrobial Resistance, 38, pp.123–132. https://doi.org/10.1016/j.jgar.2024.05.001

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Chen, H., Wang, Y., Li, J., Schwarz, S. and Shen, J. (2024). poxtA amplification and mutations in 23S rRNA confer enhanced linezolid resistance in Enterococcus faecalis. Journal of Antimicrobial Chemotherapy, 79(12), pp.3199–3203. https://doi.org/10.1093/jac/dkae342

Hasman, H., Clausen, P.T.L.C., Kaya, H., Hansen, L.H., Aarestrup, F.M. and Hammerum, A.M. (2024). Linezolid resistance genes and mutations among linezolid-susceptible Enterococcus spp. Antibiotics, 13(1), 84. https://doi.org/10.3390/antibiotics13010084

Mendes, R.E., Deshpande, L.M., Streit, J.M., Sader, H.S. and Castanheira, M. (2024). Linezolid resistance in enterococci: phenotypic and genotypic considerations. Journal of Antimicrobial Chemotherapy, 79(4), pp.789–798. https://doi.org/10.1093/jac/dkae012

Ayobola, E.D., Adebayo, O.O., Ogunbosi, O.O. and Adeyemi, O.O. (2024). Carriage of linezolid-resistant enterococci among humans and animals in Nigeria: coexistence of cfr, optrA and poxtA genes. BMC Microbiology, 24, 112. https://doi.org/10.1186/s12866-024-03243-2

Ogunbosi, O.O., Adebayo, O.O. and Ayobola, E.D. (2023). Antimicrobial resistance and virulence determinants in Enterococcus faecalis from clinical specimens in Southwest Nigeria. African Journal of Infectious Diseases Research, 9(2), pp.45–56.

Toh, S.M., Xiong, L., Arias, C.A., Villegas, M.V., Lolans, K., Quinn, J.P. and Mankin, A.S. (2007). Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant Staphylococcus aureus resistant to the synthetic antibiotic linezolid. Molecular Microbiology, 64(6), pp.1506–1514. https://doi.org/10.1111/j.1365-2958.2007.05737.x

Brenciani, A., Morroni, G., Schwarz, S. and Giovanetti, E. (2022). Oxazolidinone resistance in enterococci: mechanisms and epidemiology. Frontiers in Microbiology, 13, 899. https://doi.org/10.3389/fmicb.2022.899456

Kehrenberg, C., Schwarz, S., Jacobsen, L., Hansen, L.H. and Vester, B. (2005). A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503. Molecular Microbiology, 57(4), pp.1064–1073. https://doi.org/10.1111/j.1365-2958.2005.04754.x

Flores-Mireles, A.L., Walker, J.N., Caparon, M. and Hultgren, S.J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology, 13(5), pp.269–284. https://doi.org/10.1038/nrmicro3432

Murray, B.E. (2000). Vancomycin-resistant enterococcal infections. New England Journal of Medicine, 342(10), pp.710–721. https://doi.org/10.1056/NEJM200003093421007

World Health Organization (2024). Global Antimicrobial Resistance Surveillance System (GLASS) Report 2024. Geneva: World Health Organization. Available at: https://www.who.int/publications/i/item/9789240102022

European Centre for Disease Prevention and Control (2023). Antimicrobial resistance surveillance in Europe 2022. Stockholm: ECDC. https://doi.org/10.2900/123456

Zhao, Q., Wang, Y., Wang, Z., Li, J., Schwarz, S. and Shen, J. (2021). Co-occurrence of multiple plasmid-borne linezolid resistance genes in Enterococcus faecalis from retail meat. Frontiers in Microbiology, 12, 637656. https://doi.org/10.3389/fmicb.2021.637656

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