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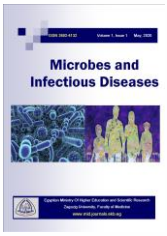

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Original article

Linezolid-resistant *Enterococcus* isolates in Menoufia University Hospitals and community-acquired infections: Genetic determinants and risk factors

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ABSTRACT

Background: *E. faecalis* and *E. faecium* are significant culprits in both hospital (HAIs) and community-acquired infections (CAIs), displaying inherent and acquired resistance to diverse antibiotic classes. Linezolid serves as a last-resort antibiotic for enterococcal infections. This study aimed to detect the prevalence of enterococcal infections in hospital and community contexts, assess the antibiotic susceptibility of *E. faecalis* and *E. faecium* and identify the underlying linezolid resistance mechanisms. **Methods:** Vitek-2 Compact System identified and determined the antibiotic susceptibility patterns for *E. faecalis* and *E. faecium* isolates. Conventional PCR assay was utilized to explore the acquired linezolid resistance genes *(PoxtA*, *OptrA*, and *Cfr*). The identification of 23S rRNA mutations G2505A was accomplished through PCR-based sequencing. **Results:** Among hundred Enterococcus isolates (56% *E. faecalis* and 44% *E. faecium*), genetic determinants of linezolid resistance were identified in 39 isolates. However, only 12 isolates were confirmed as being resistant to linezolid. Hospital-acquired infections had significantly more linezolid resistance determinants (79.5%) than those of community origin (20.5%). The most common resistance mechanisms among linezolid-resistant enterococci (LRE) were concurrent presence of *OptrA* and *PoxtA* (33.3%), 23SrRNA G2505A mutation (25%) and singular *OptrA* (25%) or *PoxtA* (8.3%). Notably, one hospital-acquired isolate (8.3%) showed 23S rRNA mutation alongside *OptrA* and *PoxtA* genes. Prior hospitalization, invasive devices, malignancy and immunosuppressives were risk factors for emergence of LRE. **Conclusion:** The distribution of *Enterococcus* species, antibiotic resistance and increasing linezolid resistance genes in hospital underscore the complexity of HAIs. This emphasizes the urgent importance of research and targeted interventions for effective management.

Introduction

Enterococcus species play a crucial role as inhabitants of the human intestinal tract. However, they also pose a significant risk as they are prominent contributors to various nosocomial infections. Their adaptability to diverse environmental conditions and resilience makes them implicated in community-acquired infections (CAIs) as well. Notably, *Enterococcus* spp. exhibit intrinsic resistance to several antibiotic classes,

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including cephalosporins and aminoglycosides, along with a notable genomic plasticity that enables the acquisition of resistance genes *via* mobile genetic elements [1].

Linezolid, a groundbreaking oxazolidinone approved for the management of severe infections stemming from Gram-positive microorganisms, notably vancomycin-resistant enterococci, functions as a bacteriostatic antibiotic. Its mode of action centres on the inhibition of protein synthesis, accomplished by binding to the V domain of the 23S rRNA component situated in the 50S ribosomal subunit. This targeted interference disrupts the crucial processes necessary for bacterial protein synthesis, ultimately impeding the proliferation and growth of the infectious agents [2].

Linezolid-resistant enterococci (LRE) may emerge through genetic alterations, including mutations in the 23s rRNA, such as G2576T or G2505A, or mutations affecting ribosomal proteins L3 and L4. Additionally, the acquisition of specific resistance genes significantly augments the development of linezolid resistance in these bacteria. Among *Enterococcus* spp., a spectrum of up to five identified acquired linezolid resistance genes includes *Cfr*, *Cfr(B)*, *Cfr(C)*, *Cfr(D)*, *OptrA*, and *PoxtA*. These genes play a crucial role in conferring resistance to linezolid, contributing to the challenges in effectively combating infections caused by *Enterococcus* strains [3].

PoxtA gene confers decreased susceptibility to oxazolidinones, phenicols, and tetracycline. *OptrA* gene codifies an ATP-binding cassette F (ABC-F) protein targeting the ribosome of Gram-positive bacteria and mediates resistance to both phenicols and oxazolidinones through ribosomal protection. *Cfr* is usually plasmid-located and confers cross-resistance to phenicol, lincosamide, oxazolidinone, pleuromutilin and streptogramin A (known as the PhLOPSA phenotype) [4].

This study targeted the assessment of the prevalence of *Enterococcus* spp. in both community- and hospital-acquired infections, analysis of their antibiotic susceptibility patterns, and shedding light on the genetic mechanisms behind linezolid resistance.

Patients and Methods

Study design:

The Department of Medical Microbiology and Immunology at Menoufia University, Faculty of Medicine, conducted this cross-sectional study during the period from October 2021 to April 2023. Patients' history was examined, focusing on factors such as hospitalization, prior antibiotic use or exposure to invasive procedures, and existing medical conditions. These data were gathered from patients admitted to various hospital departments and Intensive Care Units (ICUs) with different infection types that either emerged after 48 hours of admission or in patients visiting Outpatient Clinics at Menoufia University Hospitals (MUHs) and the National Liver Institute (NLI). Additionally, information was collected from individuals showing signs of infection before the initial 48 hours of hospital admission.

Ethical considerations:

This study was conducted according to the Helsinki Declaration. Prior to their participation in the study, informed consent was obtained from each patient. The study protocol was approved by the local Ethics Committee of the Faculty of Medicine, Menoufia University (IRB number 3/21 CARD 46).

Collection of clinical samples & identification of Enterococcus spp.:

A thorough collection of 660 samples comprising 300 from patients suffering from CAIs and 360 from patients with hospital-acquired infections (HAIs) was meticulously gathered. Samples of urine, blood, sputum, wound discharge, surgical drain discharge, burn wound swabs, and ascetic fluids were carefully collected. The procedure for processing specimens included culturing them on standard bacteriological media (Oxoid, England), and identifying *Enterococcus* species using the Vitek-2 Compact System identification cards (GP-REF 21342). To preserve the isolates for future analysis, they were stored in a solution consisting of 30% glycerol broth at -80°C [5]. Subsequently, obtained *Enterococcus* species underwent further evaluation as follows:

Antimicrobial susceptibility testing:

The susceptibility of *E. faecalis* and *E. faecium* isolates to ten antibiotics, (ampicillin, gentamicin high-level, streptomycin high-level, ciprofloxacin, erythromycin, vancomycin, linezolid, teicoplanin, tetracycline and tigecycline) was evaluated using the Vitek-2 Compact System with AST-P592 cards. The minimum inhibitory concentrations (MICs) were assessed and interpreted based on the guidelines outlined in the

Clinical and Laboratory Standards Institute (CLSI), 2023 recommendations [6].

Molecular characterization of linezolid resistance genes:

Detection of transferable genes (*PoxtA, OptrA* & *Cfr*) *via* conventional monoplex PCR involved the following procedure:

Genomic DNA extraction from *E. faecalis* and *E. faecium* isolates was performed using the Thermo Scientific GeneJET™ Genomic DNA Purification Kit, following the manufacturer's instructions. Primer sequences and the corresponding amplicon sizes are provided in the table below, utilizing primers sourced from Invitrogen, Thermo Fisher, UK.

The PCR protocol for amplifying *PoxtA, OptrA & Cfr* consisted of an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds. Primer annealing temperatures were set at 50°C for *cfr* [8], 55°C for *PoxtA* [3], and 58°C for *OptrA* [9], each for 30 seconds, followed by an extension step at 72°C for 30 seconds. A final extension step was performed at 72°C for 5 minutes. Subsequently, the resulting products were visualized via electrophoresis on a 1.5% agarose gel stained with ethidium bromide (Sigma, USA).

Identification of 23S rRNA G2505A mutations by PCR-based sequencing

PCR-based sequencing was applied only for linezolid-resistant enterococci (n=12) that displayed elevated linezolid MIC values by Vitek-2 Compact System. the PCR protocol for amplifying the 23S rRNA gene, followed by purification of the amplified product using the Invitrogen™ PureLink™ PCR Purification Kit from Thermo Fisher Scientific, USA was conducted according to the details provided in [7]. The amplified product of the 23S rRNA gene was purified using the Invitrogen™ PureLink™ PCR Purification Kit, sourced from Thermo Fisher Scientific, USA [7].

Sequencing procedures were carried out using the BigDye Terminator v3.1 cycle sequencing kit, paired with an ABI PRISM 310 Genetic Analyzer from Applied Biosystems, Foster City, CA, USA. The analysis of sequence results was meticulously undertaken through the ChromasLite and Mega 11 programs, facilitating the identification and characterization of mutations in the 23S rRNA gene, including the precise location and type of the mutations [10].

Statistical analysis

The data collected in this study were tabulated and statistically analyzed using SPSS (statistical package for the social science software) statistical package version 26. Pearson Chi-squared test (χ^2): the test of significance used to study association between qualitative variables. Fisher exact test (FE): is the test of significance used to study association between two qualitative variables if any of expected cells less than five. Z test is the test of significance used to compare two proportions. *p*-value <0.05 was considered to be statistically significant.

Results

Prevalence of Enterococcus spp. and specimen distribution

Enterococcus spp. represented 15.7% of the totally recovered isolates with *E. faecalis* being the most prevalent (54.9%) followed by *E. faecium* (43.1%), *E. gallinarum* and *E. raffinosus* (1% each). Considering *E. faecalis* and *E. faecium* in the community-acquired cases(n=40), *E. faecalis* accounted for 67.55% and *E. faecium* for 32.5% of the whole isolates. Meanwhile, both species comprised nearly equal percentages (48.3% and 51.7%, respectively) across HAIs (n=60), as shown in **figure (S1).**

Urine specimens exhibited the highest prevalence of *E. faecalis* and *E. faecium* (52%) and blood samples provided 30% of the total isolates. In contrast, specimens like pus and wound swabs showed minimal to no presence of *E. faecium*. *Enterococcus* species were detected alongside other organisms in various specimens including *E. coli, S. aureus, Klebsiella* spp., and coagulase-negative staphylococci.

Antimicrobial susceptibility testing

By using the Vitek-2 Compact System, no resistance to tigecycline was observed. Analyzing resistance patterns in both community and hospital settings, revealed significant differences in resistance rates between the two species. Specifically, in CAIs, *E. faecalis* exhibited a low resistance rate to ampicillin (7.4%), contrasting sharply with *E. faecium* (92.3%). Variations in susceptibility were also noted for gentamicin high level, ciprofloxacin, and tetracycline between the two species. Even though, *E. faecium* of community origin displayed no resistance to vancomycin or teicoplanin. It's interesting to note that, both species did not exhibit linezolid resistance in any of the

community-acquired infections as demonstrated in **figure (1).**

Regarding HAIs, it was remarkable that all *E. faecium* strains exhibited resistance to ampicillin, in stark contrast to 6.9% resistance among *E. faecalis* strains. The resistance to both gentamycin high level and ciprofloxacin showed significant difference between both species. Regarding streptomycin high level, erythromycin, vancomycin, linezolid, teicoplanin and tetracycline, the resistance trends in these antibiotics are relatively uniform between *E. faecalis* and *E. faecium* as shown in **figure (1).**

It is noteworthy that, all isolates (n=12) exhibiting phenotypic resistance to linezolid (12%) were sourced from patients with HAIs, primarily urinary tract infections (58.3%), followed by bloodstream (25%) and chest infections (16.7%). Among these isolates, 66.7% (8/12) were identified as *E. faecium*, while 33.3% (4/12) were *E. faecalis.*

Risk factors associated with linezolid resistance:

The usage of immunosuppressive medications, prior hospitalization, malignant tumors, and devices like urinary catheters and central venous catheters (CVCs), appeared to be linked to linezolid resistance. Consumption of antibiotics was observed in all patients infected with linezolid-resistant enterococci as shown in **table (2)**.

Antibiotic susceptibility profiles of the linezolid-resistant isolates:

In **table (3)**, it was evident that ampicillin, high-level gentamycin, high-level streptomycin, erythromycin, ciprofloxacin and tetracycline exhibited significantly reduced efficacy, with resistance rates ranging from 58.3% to 83.3% among LRE. Importantly, vancomycin displayed a resistance rate of 50%, and teicoplanin resistance also reached the same level. Conversely, tigecycline showed complete sensitivity at 100%.

Incidence and dispersion of linezolid resistance genetic markers:

Utilizing conventional PCR to screen for linezolid resistance genes (*PoxtA, OptrA & Cfr*) **(Figure 2)**, along with PCR-based sequencing targeting the 23SrRNA genetic mutation **(Figure 3):**

The applied PCR experiment revealed that, 39% of all isolates harboured linezolid resistance genetic determinants of which 38.5% (15/39) were *E. faecalis* and 61.5% (24/39) were *E. faecium* (**Table S1**). About 12.5% of *E. faecalis* and 18.2% of *E. faecium* isolates carried *PoxtA* gene. Additionally, 1.8% and 11.4% of *E. faecalis* and *E. faecium* isolates, revealed co-occurrence of *PoxtA* and *OptrA* genes, respectively (**Table S2**). *Cfr* gene wasn't detected in our study.

The presence of 23S rRNA mutations was examined among the 12 LRE including 4 *E. faecalis* and 8 *E. faecium* isolates, revealing that one *E. faecalis* isolate (25%; 1/4) and three *E. faecium* isolates (37.5%; 3/8) contained the G2505A mutation, as confirmed by PCR-based sequencing assay (**Table S2**).

Importantly, 31/39 (79.5%) and 8/39 (20.5%) of isolates carrying linezolid resistance genetic determinants were obtained from HAIs and CAIs, respectively with a highly significant statistical difference as shown in **table (4)**. Moreover, the co-existence of both the *OptrA* and *PoxtA* genes along with the 23S rRNA mutation, was only observed among hospital-acquired isolates, showing a significant statistical difference regarding the coexistence of both genes (p <0.05).

Out of the 39 enterococcal isolates carrying linezolid resistance genetic markers, 30.8% (n=12) exhibited resistance to the drug, while 69.2% (n=27) remained susceptible. Among LRE, genotypic analysis identified a combination of *OptrA* and *PoxtA* genes in 33.3% (4/12), and a 23S rRNA mutation (G2505A) in 25% (3/12). Conversely, linezolid-susceptible isolates showed no co-existence of resistance genes or 23S rRNA mutations. Transferable linezolid resistance genes were found as distinct entities in these isolates, as detailed in **table (4).**

Isolates bearing linezolid resistance genetic determinants were predominantly sourced from urine specimens. Among these isolates, 75% (3/4) carried the 23S rRNA mutation, 56.25% (9/16) contained the *OptrA* gene, 40% (6/15) harbored the *PoxtA* gene, and 60% (3/5) possessed both genes, as illustrated in **figure (4)**.

Isolates carrying the 23S rRNA mutation were primarily obtained from patients admitted to the Oncology department (50%; 2/4). Those harboring the *PoxtA* gene were frequently encountered in ICUs (33.3%; 5/15), while the highest prevalence of isolates with both *OptrA* and *PoxtA* genes was among ICU patients (80%; 4/5). The highest occurrence of isolates containing the *OptrA* gene was observed in the Internal Medicine Department (25%; 4/16), as depicted in **figure (4).**

Table 1. Primer sequence of target genes.

Primer name	Sequence 5^{-} -3^{-}	Product size (bp)	Reference
23S rRNA	F: GTAACGATTTGGGCACTGTCG R: CGATTAGTATTGGTCCGCTC	650 bp	7
Cfr	F:TGAAGTATAAAGCAGGTTGGGAGTCA R: ACCATATAATTGACCACAAGCAGC	700 bp	8
PoxtA	F: AAAGCTACCCATAAAATATC R: TCATCAAGCTGTTCGAGTTC	500bp	3
OptrA	F: TACTTGATGAACCTACTAACCA R: CCTTGAACTACTGATTCTCGG	400 bp	9

Table 2. Correlation between risk factors and linezolid resistance in the examined *Enterococcus* species

Risk factors	Linezolid-resistant cases (n=12)		Linezolid-sensitive cases (n=88)		Test of significance	*p*-value
	No.	%	No.	%		
Hospitalization*	12	100	48	54.5	FE*	<0.05
Previous surgery	4	333	25	28.4	FE	>0.05
Medical devices	10	83.3	40	45.5	$\chi 2$**=6.061	<0.05
Chronic diseases	9	75	59	67	FE	>0.05
Antibiotic use	12	100	75	85.2	FE	>0.05
Malignancy	4	33.3	7	8	FE	<0.05
Immunosuppressive drugs	5	41.7	4	4.5	FE	0.001

* FE: Fisher exact test, $\chi 2$**: Chi-squared test.

N.B: *p* value >0.05 ; statistically non-significant. *p*-value <0.05; statistically significant

Table 3. Antibiotic susceptibility pattern of linezolid-resistant enterococci.

Antibiotics	Linezolid-resistant enterococci (n=12)					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ampicillin	3	25	0	0	9	75
Gentamycin high level	2	16.7	0	0	10	83.3
Streptomycin high level	2	16.7	0	0	10	83.3
Erythromycin	2	16.7	1	8.3	9	75
Ciprofloxacin	5	41.7	0	0	7	58.3
Vancomycin	5	41.7	1	8.3	6	50
Teicoplanin	6	50	0	0	6	50
Tetracycline	2	16.7	0	0	10	83.3
Tigecycline	12	100	0	0	0	0

Table 4. Relationship between enterococcal infection source and linezolid resistance genotypic profile (n=100).

Variables	Community- acquired (n=40)		Hospital-acquired (n=60)		Total (n=100)		Test of significance	*p*-value
	No.	%	No.	%	No.	%		
PoxtA	3	7.5	12	20	15	15	2.94	>0.05
OptrA	5	12.5	10	16.6	15	15	0.33	>0.05
Co-existence of *OptrA* +*PoxtA*	0	0	6	10	6	6	FE	<0.05
23S rRNA mutation*	0	0	4	6.7	4	4	FE	>0.05
Isolates with linezolid resistance genetic determinants	8	20	31**	51.7	39	39	10.12	0.001

*23S rRNA sequencing was done only for the 12 linezolid-resistant isolates.
**One hospital-acquired isolate contained both *OptrA* and *PoxtA* genes plus 23S r RNA mutation.
N.B: *p* value >0.05; statistically non-significant. *p*-value <0.05; statistically significant.

Figure 1. Comparative analysis of antibiotic resistance in community and hospital-acquired *E. faecalis* and *E. faecium* isolates

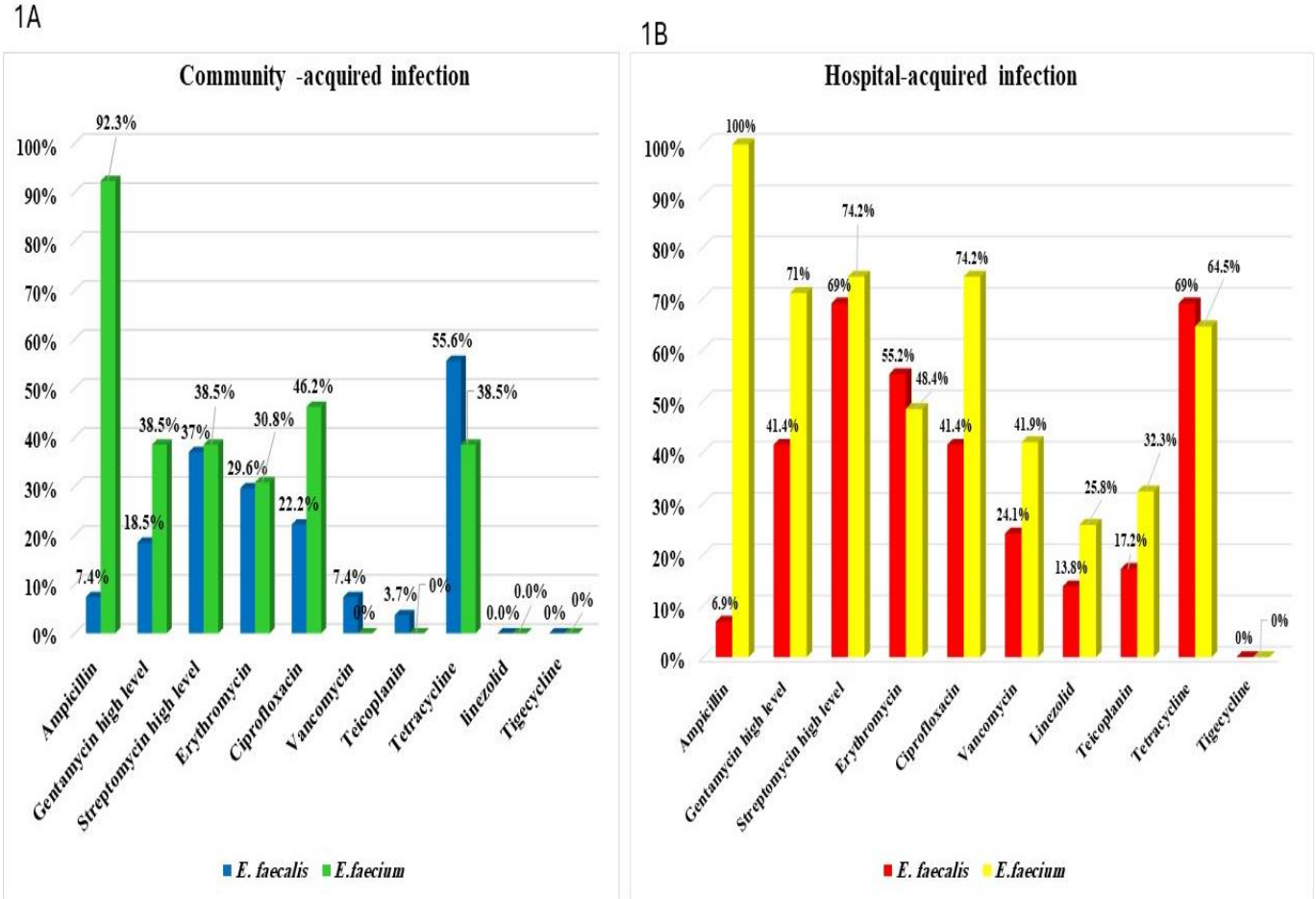
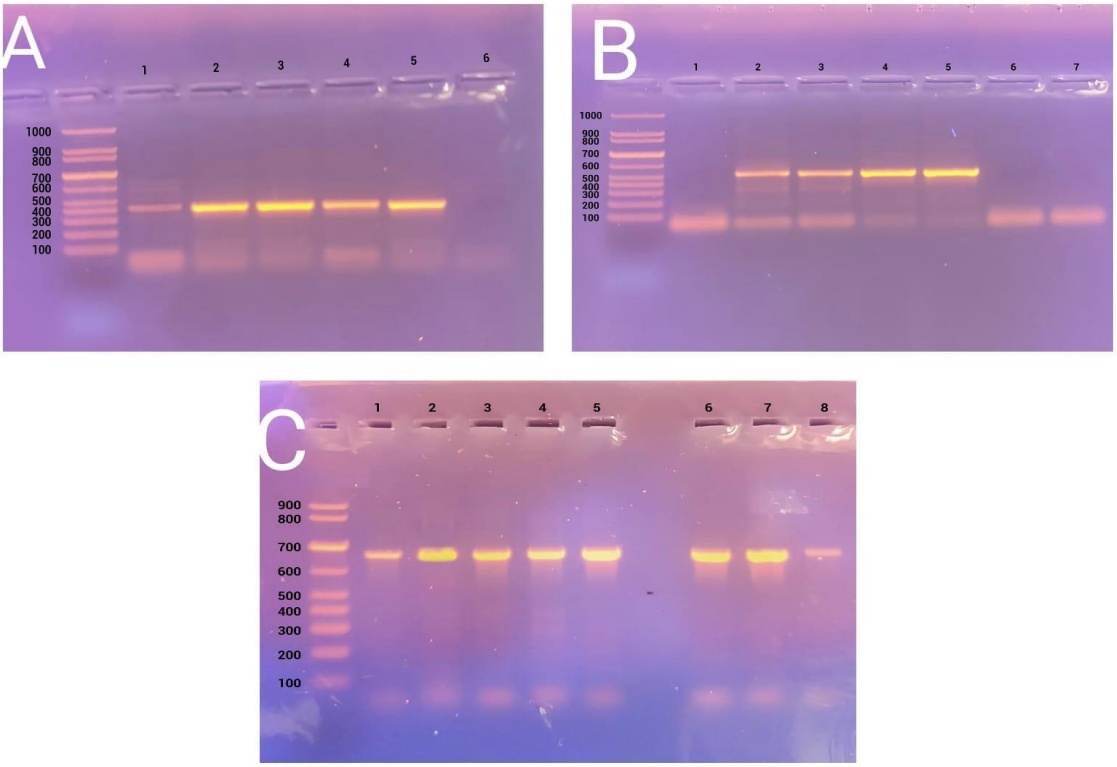


Figure 2. PCR gel electrophoresis of the amplified product of *PoxtA*, *optrA* and 23S rRNA gene. DNA ladder (100-1000bp)



A: lane 1, 2, 3, 4, 5 were positive for *optrA* gene at 400 bp.
B: lane 2, 3, 4, 5 were positive for *poxtA* gene at 500 bp.
C: all lanes were positive for 23sr RNA gene at 650bp.

Figure 3. Sequence alignment by MEGA 11 program which revealed the presence of G2505A mutation which is present to the left of the yellow highlighted sequence (red rectangle) and Electropherogram of PCR products of 23Sr RNA viewed by chromaslite program (red rectangle).

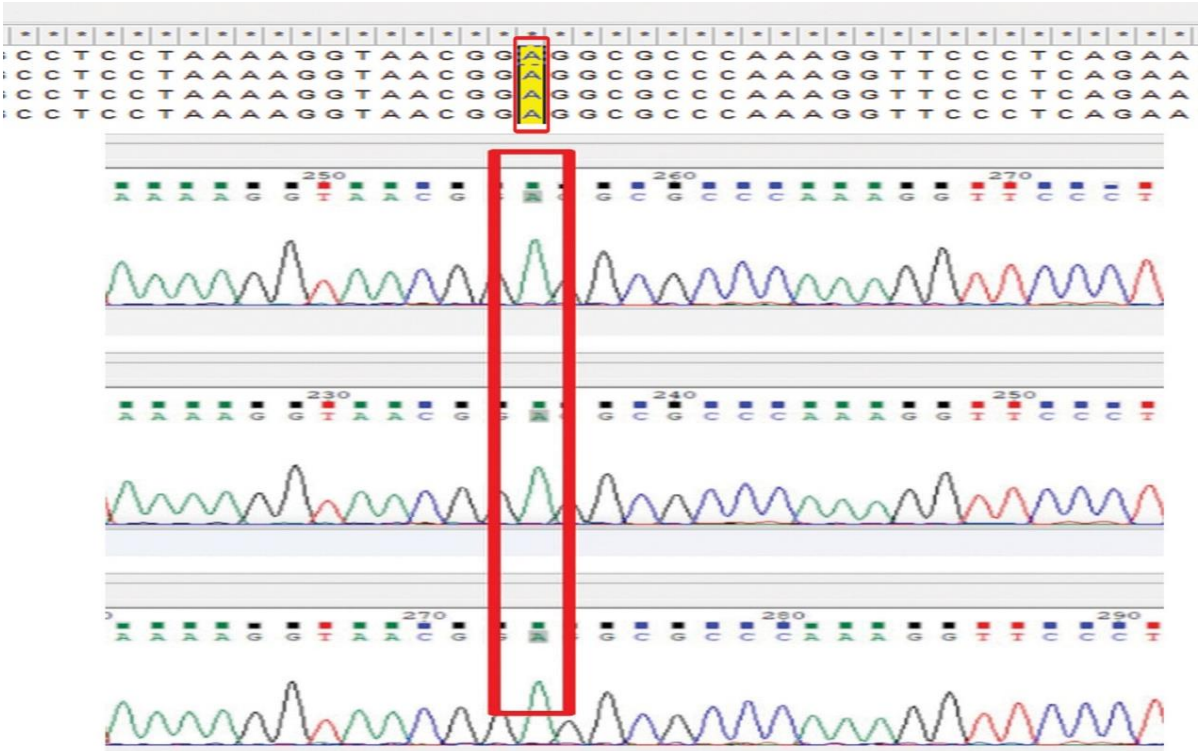
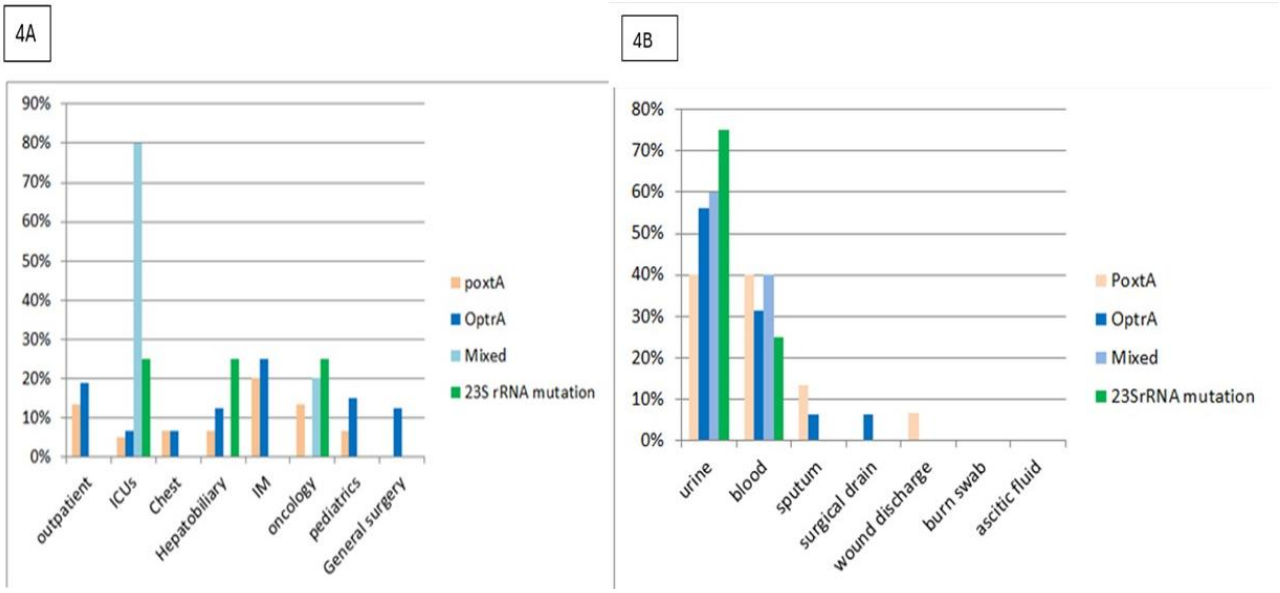


Figure 4. Distribution of the studied *Enterococcus* spp. isolates carrying linezolid resistance genetic determinants regarding departments (4A) and specimens (4B).



Discussion

Enterococcus species, particularly *E. faecalis* and *E. faecium*, are major concerns in hospitals due to their rapid dissemination, endangering both healthcare workers and patients. They rank among the top nosocomial pathogens and also cause community-acquired infections [11]. Their intrinsic resistance to antibiotics severely limits treatment options, emphasizing the importance of last-resort antibiotics like linezolid, especially for combating vancomycin-resistant enterococci (VRE) [12].

In this study, *E. faecalis* and *E. faecium* were selected for further analysis (100 isolates), consisting of 56% *E. faecalis* and 44% *E. faecium* obtained from individuals with either CAIs (40%) or HAIs (60%). Notably, *E. faecalis* was more prevalent (67.5%) in CAIs, while *E. faecium* was more common (51.7%) in HAIs. These findings closely resembled the prevalence rates reported by **Azzam et al.** [13] in Egypt, **Li et al.** [14] in China, and **Karna et al.** [15] in Nepal. According to current results, the primary sources of isolation of enterococci were urine specimens (52%), followed by blood (30%), with smaller proportions obtained from discharge from surgical drains, sputum, and ascetic fluid. This distribution closely matched the outcomes reported in a study conducted in Egypt by **El-Kazzaz and Abou El-Khier** [16].

Based on current data, *E. faecium* isolates displayed superior antimicrobial resistance than *E. faecalis* in both community and hospital settings. In CAIs, 92.3% of *E. faecium* isolates were resistant to ampicillin, compared to 7.4% in *E. faecalis*. In HAIs, 100% of *E. faecium* isolates were resistant to ampicillin, while *E. faecalis* had a minimal of 6.9% resistance rate. The resistance patterns aligned with study in Japan [17] Poland [18]. Additionally, *E. faecium* isolates from HAIs showed high resistance to gentamicin, streptomycin high level, ciprofloxacin, and tetracycline, consistent with findings in China [19] and higher than those in Egypt [20]. These variations highlight the complexity of antimicrobial resistance in different settings and regions.

The incidence of vancomycin resistance among isolates from CAIs was found to be lower than that in HAIs. Merely, 5% of CAI isolates exhibited vancomycin resistance, all of which were identified as *E. faecalis*. In contrast, the prevalence of vancomycin resistance in a hospital environment reached 33.3%, with higher percentage (41.9%) observed in *E. faecium* isolates. This discrepancy can be attributed to factors such as the presence of different bacterial species (*E. faecalis* in CAIs vs. *E. faecium* in HAIs), antibiotic selective pressure, and the conductive environment for resistance development in hospitals, where close patient contact and medical interventions contribute to

increased transmission of resistant strains [13]. These results demonstrated how quickly vancomycin resistance is spreading. Vancomycin is categorized as belonging to the Watch group in the WHO AWaRe classification, which includes most of the "highest-priority critically important antimicrobials". Antibiotics from this class ought to be reserved for certain, restricted uses. Vancomycin must be used rationally and in accordance with a stewardship program that takes into account the kind of infection, culture and sensitivity results, resistance, or antibiotic contraindications [13].

Linezolid stands as a critical antibiotic of last resort in addressing VRE infections. Its utilization is recommended exclusively for confirmed or suspected cases involving multi-drug-resistant organisms, and efforts should be made to de-escalate its use whenever feasible [21]. This study revealed a linezolid resistance rate of 12%, with 66.7% (8/12) attributed to *E. faecium* and 33.3% (4/12) to *E. faecalis*. Notably, these strains were isolated from patients experiencing HAIs, primarily within ICU settings (58.3%), followed by the Oncology department (25%) and Hepatobiliary and Chest departments (8.3% each). The observed resistance rate closely aligned with findings by **Kisk et al.** [22] in Egypt (10%) and **Mališová et al.** [7] in the Czech Republic (8%) representing a notable contrast to the lower rates reported by **Al-Mahdy et al.** [23] in Egypt (2.8%) **Zou and Xia,** [24] in China (4.5%).

Our main goal in this study was to better understand the phenomenon of linezolid resistance and to identify risk factors that are linked to it, such as hospital admissions, particularly in ICUs, the use of urinary catheters and/or central venous catheters (CVCs), the presence of malignant diseases, and the use of immunosuppressive medications. Notably, these parameters were found to be substantially similar to those found earlier by **Liu et al.** [25] in China and **Rodríguez-Noriega et al.** [26] in Mexico.

All LRE were isolated from patients who contracted HAIs in our investigation, which is parallel to the findings of **Kerschner et al.** [27]. This is consistent with research by **Olearo et al.** [28] which showed that 86% of LRE patients experienced long-term health problems. Furthermore, all LRE-infected patients received antibiotics at the time of the trial, precisely as reported by **Kerschner et al.** [27].

Of the patients with LRE, about 41.7% were prescribed immunosuppressive medications, and about 33.3% had malignant diseases. According to **Olearo et al.** 68% of LRE-infected individuals had malignant conditions, and 61% of them were on immunosuppressive medication [28]. Accordingly, 69% of patients in **Kerschner et al.'s** study had cancer, and 86% had impaired immune systems [27].

Herein, enterococci displaying resistance to linezolid exhibited notable resistance rates across various antibiotic classes. Particularly, they demonstrated elevated resistance against aminoglycosides (specifically high levels of gentamicin and streptomycin) and tetracycline (83.3%), mirroring the findings of **Chen et al.** [10] in China.

According to current findings, the predominant mechanism for LRE was co-existence of *OptrA* and *PoxtA* genes. Meanwhile, the second was point mutation in the V domain of 23S rRNA, specifically designated as G2505A. This particular genetic alteration was identified in 33.3% of the cases analyzed in this study, distributed across 3 of *E. faecium* and 1 of *E. faecalis* within the total sample size of 12 cases. Significantly, these findings remarkably aligned with those reported by **Hasman et al.** [29] in Denmark, reinforcing the prominence of the G2505A mutation in *E. faecium* as a major contributor to linezolid resistance. Furthermore, investigations by **Mališová et al.** [7] in the Czech Republic and **Gawryszewska et al.** [18] in Poland correlated with our results, indicating that mutations in the 23S rRNA were the predominant mechanism for linezolid resistance in *E. faecium*. Notably, these studies identified G2576T as the mutation, contrasting with our G2505A finding, highlighting potential regional variations in the prevalent mutations associated with linezolid resistance.

Interestingly, within our study, one hospital-acquired *E. faecalis* isolate exhibited a mutated 23S rRNA gene and concurrently carried both *PoxtA* and *OptrA* genes. This discovery implies the potential coexistence of multiple resistance mechanisms within a single strain. This observation finds support in the research conducted by **Misiakou et al.** [30] in Germany and **Egan et al.** [31] in Ireland, where they also identified combinations of 23S rRNA mutations and transferable linezolid resistance genes in hospital-acquired isolates. This may be explained by the fact that the hospital environment especially with lack of

infection control measures and presence of MDR organisms facilitate the spread of transferable resistance genes. Also, 23SrRNA mutations can occur via a homologous recombination process. Linezolid treatment of patients harbouring Enterococcus isolates with only few mutated 23S might result in higher linezolid resistance levels by facilitating an increase of mutated 23S rRNA copies [30].

Accordingly, *OptrA* gene was detected as a separate genetic marker in 25% (3/12) of LRE (2 *E. faecalis* & *1 E. faecium*). This was in line with the findings by ***Ruiz-Ripa et al.*** [32] in Spain and **Chen et al.** [10] in China which concluded that *OptrA* gene was the primary mechanism behind linezolid resistance among *E. faecalis* isolates. Additionally, *PoxtA* was discovered a distinct determinant in one of the LRE isolates (8.3%), which is less than the rate of 42.8% discovered by **Egan et al**. [31] in Ireland and comparable to the percentage reported by **Dejoies et al.** [33] in France (10.1%).

Among linezolid-sensitive Enterococcus isolates, *OptrA* gene was detected alone in about 13 isolates (5 from CAIs + 8 from HAIs). Similarly, *PoxtA* gene was detected alone in 14 isolates (3 from CAIs + 11 from HAIs). These genes might not be expressed or translated, or other mechanism may counteract their function, leading to a loss of drug resistance. However, their presence on conjugative plasmids or mobile genetic element facilitates their spread in hospitals lacking robust infection control and screening programs [34].

Conclusion

There is a dramatic increase in *OptrA* and *PoxtA* carriage and spread among *Enterococcus* spp. of community and hospital settings, which has a substantial effect on effectiveness of linezolid in treating enterococcal infections. Screening for linezolid resistance genes in susceptible isolates is imperative to curb the propagation of these genes, particularly within hospital premises. The identification of an isolate harbouring both *PoxtA* and *OptrA* genes alongside the G2505A 23SrRNA mutation is alarming due to heightened risk for hospitalized patients.

Limitations

Inability to perform sequencing of ribosomal protein to detect potential mutation due to financial constraints.

Abbreviations

HAIs: Hospital-acquired infections

CAIs: Community-acquired infections

UTI: Urinary tract infections

CVC: Central venous catheter

VRE: Vancomycin-resistant enterococci

LRE: linezolid-resistant enterococci

Data availability

The data used to support the findings of this study are available from the corresponding author on request.

Consent

All the studied cases signed a written informed consent prior to their participation in the study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Authors' contributions

Amal F. Makled contributed to the conceptualization (lead), formal analysis (equal), supervision (lead), visualization (equal), and writing of the original draft (equal). Sahar A. M. Ali and Asmaa M. Elbrolosy contributed to the conceptualization (equal), formal analysis (equal), supervision (equal), and review and editing (equal). Enas M. Ghoneim contributed to the conceptualization (equal). Esraa E. Elmahdy contributed to the methodology (equal), supervision (equal), and review and editing (equal). Shymaa S. Alkady contributed to the practical methodology (equal) and writing (equal).

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