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Prevalence and antimicrobial resistance of gram-positive pathogens in Lebanon: The need for surveillance and stewardship

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ABSTRACT

Background: Resistance in Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), poses a significant healthcare challenge globally. However, data on these organisms in Lebanon remain limited. This retrospective study aimed to assess the prevalence and antimicrobial resistance patterns of *Staphylococcus aureus* (*S. aureus*), coagulase-negative *Staphylococci* (CoNS), and *Enterococcus* spp. in clinical infections at the Lebanese Hospital Geitaoui – UMC from 2017 to 2023.
Methods: A total of 2676 isolates were collected from urine, blood, respiratory specimens, and other infection sites. Bacterial identification was performed following WHO clinical bacteriology procedures, utilizing gram staining, catalase and coagulase tests, and biochemical assays. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC) analysis, interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Statistical analyses were performed using SPSS® version 24, with significance set at $p < 0.05$.
Results: CoNS were the most prevalent (42.83 %), followed by *Enterococcus* spp. (28.81 %) and *S. aureus* (28.36 %). Blood cultures had the highest isolation rates (29.04 %), predominantly CoNS (76.45 %). *Enterococcus* spp. dominated urinary tract infections (85.01 %), while *S. aureus* was prevalent in wound/surgical site infections (59.23 %). Gender-specific trends showed CoNS and *S. aureus* more in males, while *Enterococcus* spp. infections were more common in females.
Conclusion: This study provides valuable insights into the prevalence and resistance patterns of Gram-positive pathogens in a Lebanese hospital setting. The findings highlight the need for continuous surveillance and stringent antibiotic stewardship to combat antimicrobial resistance effectively.

1. Introduction

Hospital settings are critical environments for gram-positive bacteria, particularly gram-positive cocci such as *Enterococcus* species, *Staphylococcus aureus (S. aureus)*, and coagulase-negative *Staphylococci* (CoNS) [1]. The Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) project, which monitors bloodstream infections of clinical significance among hospitalized patients in the United States, provided the information that, during a three-year period from April 1995 to April 1998, gram-positive bacteria accounted for 60 % of nosocomial bloodstream infections [2] (see Fig. 1).

CoNS have become significant pathogens in hospital environments [3]. The increase in CoNS-related nosocomial infections, with *S. epidermidis* and *S. haemolyticus* being the most significant species, has led researchers and physicians to reevaluate the importance of these bacteria [4].

MDR (Multidrug Resistance) generally refers to bacterial resistance against multiple antimicrobial agents, but its definition varies across studies. The most commonly accepted definition is resistance to three or more antimicrobial classes. XDR (Extensively Drug-Resistant) bacteria exhibit resistance to most available antibiotics, significantly limiting treatment options. Originally defined for *Mycobacterium tuberculosis*, XDR now applies to various bacteria following similar principles. PDR (Pandrug-Resistant) bacteria are resistant to all clinically relevant

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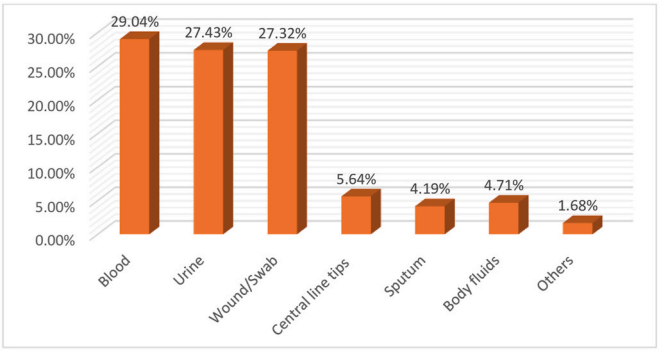

Fig. 1. Distribution of clinical specimens.

antimicrobial agents, but its definition also varies across studies, sometimes leading to inconsistent interpretations [5,6].

S. aureus, a major human pathogen, exhibits a variety of virulence characteristics as well as a tendency to develop resistance to almost all antibiotics 7–9. *S. aureus* produces various virulence factors such as adhesins, toxins, and enzymes that facilitate tissue invasion, immune evasion, and promote severe infections, including skin infections, pneumonia, and sepsis [10]. *S. aureus* produces surface adhesins (e.g., *clfA*, *fnbA/B*) that facilitate host tissue attachment, immune-evasive factors like protein A (*spa*), and exotoxins such as Panton-Valentine leukocidin (*lukS-PV*, *lukF-PV*) that cause cytotoxicity and tissue damage [11]. The emergence of methicillin-resistant *S. aureus* (MRSA) has been directly linked to the clinical use of methicillin [12]. When penicillin G was originally developed in the early 1940s, almost all strains of *S. aureus* were susceptible to it. Nowadays, almost all *S. aureus* strains are resistant to aminopenicillins, natural penicillins, and antipseudomonal penicillins [6,8]. Resistance to these drugs arises from the acquisition of genes encoding the enzymes, now known as β-lactamases, that render the antibiotics inactive. These enzymes were initially known as penicillinases.

The United States had its first reports of MRSA in 1968 [13]. Periodically occurring MRSA outbreaks were reported in several nations during the 1970s; these were frequently associated with high levels of methicillin use in intensive care units (ICUs). However, MRSA did not really start to pose a problem in American hospitals until the 1980s [14]. During this time, it first affected hospitals with large bed capacities and then started to afflict community hospitals [15]. Vancomycin was the only effective treatment for serious MRSA infections for a long time. However, four new drugs (quinupristin-dalfopristin, linezolid, daptomycin, and tigecycline) with action against MRSA have been launched in the recent four years. These new treatments are particularly welcome because over the past decade, there has been an increase in vancomycin resistance in *S. aureus* [16].

Vancomycin-resistant *Enterococci* (VRE) were not discovered until the mid-1980s, despite the fact that vancomycin has been used clinically since the late 1950s [17]. In the 1990s, VRE increased significantly and rapidly; it was first seen in ICUs and then expanded. What's particularly striking about this outbreak is that the vast majority of VRE cases were caused by *Enterococcus faecium* [18]. Two similar types of gene clusters, one including *vanA* and the other containing *vanB*, facilitate vancomycin resistance. Both classes achieve resistance by changing the vancomycin target site from D-alanine-D-alanine to D-alanine-D-lactate [19]. VRE exhibit resistance to vancomycin and other antibiotics, which, combined with factors like biofilm formation and surface protein production, makes them potent pathogens in hospital settings, causing infections such as urinary tract infections, bacteremia, and endocarditis [20]. VRE, particularly *Enterococcus faecium* and *Enterococcus faecalis*, employs aggregation substances (*asa1*), cytolysin (*cylA*), and biofilm-associated genes (*esp*) to evade host defenses and enhance colonization. Moreover, the vancomycin resistance mechanism in VRE is mediated by the *vanA* and *vanB* gene clusters, which alter peptidoglycan precursors to prevent vancomycin binding [21,22].

The rise in multidrug resistance worldwide is considered currently a significant public health threat. Reports on the emergence of virulent, multidrug-resistant bacterial pathogens from various sources increase the necessity of proper use of antibiotics, as well as the routine application of antimicrobial susceptibility testing to detect the antibiotic of choice and to screen for emerging MDR strains.

The rising resistance among Gram-positive organisms, particularly methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*, poses a major healthcare challenge [23]. However, data on this issue remain limited in Lebanon. This study aims to assess the prevalence of Gram-positive bacteria, including *Staphylococcus aureus*, coagulase-negative *Staphylococci*, and *Enterococcus* spp., across various infection sites. Additionally, it examines their antimicrobial resistance patterns through a retrospective analysis of samples collected at Lebanese Hospital Geitaoui – UMC from 2017 to 2023.

2. Methodology

2.1. Study design and setting

This retrospective study took place at the Lebanese Hospital Geitaoui – UMC in Beirut, Lebanon and encompassed all isolates detected from urine, blood, respiratory specimens, and other sites of infection, sent to the laboratory department between January 2017 and December 2023.

2.2. Data collection

An electronic search was conducted within the laboratory information system to identify all patients with positive culture specimens. The search was restricted to isolates of *Staphylococcus aureus*, *Enterococcus* spp., and coagulase-negative *Staphylococci*. Throughout this procedure, the patients' genders were noted. The ADAGIO automated zone size reader (Bio-Rad Laboratories, Hercules, CA, USA) [24] was used to gather antimicrobial susceptibility data from antimicrobial disk susceptibility testing. The analysis excluded duplicate cultures from the same patients.

2.3. Sample size

The analysis included all cultures that tested positive for at least one of the three specified bacterial isolates from the collection sites mentioned earlier, amounting to a total of 2676 isolates.

2.4. Ethical consideration

Ethical approval for this study was granted by the Institutional Review Board (IRB) of the Lebanese Hospital Geitaoui – UMC under code 2023-IRB-017.

2.5. Bacterial identification

Bacterial classification followed WHO basic laboratory procedures in clinical bacteriology [25]. Identification of *S. aureus* involved several diagnostic steps: initially, gram staining revealed characteristic violet cocci [26]. Further confirmation was achieved through a catalase test using the **Calbiochem® Catalase Assay Kit** [27], where the formation of bubbles indicated a positive result [28], followed by a positive coagulase test. For *Enterococcus* spp., identification procedures included gram staining, assessment of hemolysis patterns, and esculin hydrolysis on bile-esculin agar (Bio-Rad Laboratories, CA, USA), where *Enterococcus* species turned the medium black due to esculin hydrolysis [29]. Additional tests such as catalase activity and salt tolerance (6.5 % NaCl broth) were also employed. Similarly, identification of CoNS involved evaluation of colony characteristics, gram staining, and conducting

catalase and coagulase tests as part of the comprehensive identification process [30].

2.6. Antimicrobial susceptibility testing

Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method and the minimum inhibitory concentrations (MIC) in compliance with Clinical and Laboratory Standards Institute (CLSI) guidelines. For *Staphylococci*, several antibiotics were used including Cefoxitin (30 µg), Oxacillin (1 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Erythromycin (15 µg), Gentamicin (10 µg), Linezolid (30 µg), Rifampin (5 µg), Teicoplanin (30 µg), Vancomycin (30 µg), Tetracycline (30 µg), Trimethoprim/Sulfamethoxazole (25 µg). Moreover, the following antibiotics were used for *Enterococci*: Ampicillin (10 µg), Fosfomycin (200 µg), Nitrofurantoin (300 µg), Erythromycin (15 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), and Linezolid (30 µg).

Dilution methods, including broth microdilution, which determine the MIC of antibiotics, are considered the gold standard for phenotypic antimicrobial susceptibility testing. We typically used an automated system that performs this method whenever possible. Alternatively, we frequently employed the Kirby Bauer disk diffusion method on Mueller Hinton agar plates (Bio-Rad Laboratories, CA, USA), for manual antimicrobial susceptibility testing. Isolates tested using the disk diffusion method were also routinely assessed for inducible clindamycin resistance using the D-test.

MICs and inhibitory zone diameters were interpreted according to CLSI M100 guidelines [31]. Specifically, an *S. aureus* isolate was classified as methicillin-resistant (MRSA) if the oxacillin MIC was $\geq$8 µg/mL or if the inhibitory zone diameter around a 30-µg cefoxitin disk was $\leq$21 mm [32]. Vancomycin resistance was similarly assessed, although the disk diffusion method is no longer recommended. In cases of doubtful results or upon request by the physician, the MIC was measured.

After that, plates were automatically read by the ADAGIO system, which measures the diameters of the surrounding inhibition zones and automatically detects antibiotic disks on the agar using image equipment and management software.

2.7. Statistical analysis

The data were extracted from the laboratory information system and organized using Microsoft Excel for preliminary data management and cleaning. Statistical analysis was performed using IBM SPSS® Statistics version 24. This software was used to perform a wide range of analyses, including descriptive statistics, which summarized categorical data as frequencies and percentages. The chi-square (χ^2) test was employed to assess associations between categorical variables, with a predefined significance threshold of 5 % ($p < 0.05$). Antimicrobial susceptibility data were directly obtained from the ADAGIO automated zone size reader (Bio-Rad Laboratories, Hercules, CA, USA) and reported as percentages.

3. Results

This study aimed to assess the prevalence and antimicrobial resistance patterns of Gram-positive bacterial pathogens, specifically *Staphylococcus aureus*, coagulase-negative *Staphylococci* (CoNS), and *Enterococcus* spp., in clinical samples from Lebanese Hospital Geitaoui – UMC between 2017 and 2023. Our primary objective was to identify the most common infection sites for these pathogens and to evaluate their resistance to a variety of commonly used antibiotics. We hypothesized that the prevalence of multidrug-resistant strains would be significant given the global rise of antibiotic resistance. In this section, we present the findings regarding the distribution of these pathogens across different clinical samples, the antimicrobial resistance patterns observed, and the gender-specific trends associated with these infections.

3.1. Phenotypic characteristics of the recovered isolates

The phenotypic characterization of the recovered isolates was performed using a combination of macroscopic and microscopic observations, along with biochemical tests. The bacterial identification process began with gram staining, which revealed characteristic violet cocci for *Staphylococcus aureus* and Coagulase-negative *Staphylococci* (CoNS), while *Enterococcus* spp. appeared as gram-positive cocci arranged in pairs or short chains [26].

For *S. aureus*, colony morphology on agar plates was noted as golden yellow, smooth, and convex, while CoNS typically produced smaller, white or cream-colored colonies. The catalase test was performed on all isolates; a positive catalase result (bubbling upon hydrogen peroxide application) was observed for both *S. aureus* and CoNS, while *Enterococcus* spp. showed a negative catalase result. Coagulase testing was used to distinguish *S. aureus* (positive result) from CoNS (negative result) [33,34].

Further phenotypic tests for *Enterococcus* spp. included bile-esculin hydrolysis, which produced dark brown or black colonies, and the ability to grow in 6.5 % NaCl, which was indicative of *Enterococcal* species [35].

3.2. Prevalence of gram-positive cocci among different clinical samples

Among the 2676 gram-positive cocci (GPC) isolates under study, CoNS were the most frequent, accounting for 42.83 %, and most of the GPC were isolated from blood (29.04 %).

Among 777 positive blood cultures, CoNS were the predominant GPC (76.45 %). Out of 734 patients with UTI caused by GPC, 85.01 % were caused by *Enterococcus* spp. *S. aureus* was the most frequently identified organism (59.23 %) in wound and surgical site infections (see Fig. 2).

3.3. Gender prevalence

The incidence of GPC infections in male and female patients, as indicated in Fig. 3, exhibits distinct gender-specific trends (see Table 1).

3.4. Antimicrobial susceptibility pattern of gram-positive cocci isolates

Our study spanned seven years and examined the antimicrobial susceptibility of 2676 GPC isolates from January 2017 to December 2023. The findings in Tables 2–4 offer a detailed look at the antimicrobial drugs tested for CoNS, *S. aureus*, and *Enterococcus* species (Tables 2–4).

4. Discussion

S. aureus is a one of the major causes of hospital-acquired infections, including pneumonia, infection of the surgical sites and bloodstream [36]. After penicillin became available to treat severe *Staphylococcal* infections, strains of *S. aureus* and CoNS quickly developed resistance to this antibiotic by producing plasmid-mediated β-lactamase enzyme, which could break down the β-lactam bond in penicillin. By 2016, approximately 90 % of *S. aureus* isolates were penicillin resistant.

In the late 1950s, semisynthetic penicillins that are stable to penicillinase were developed, including methicillin, cloxacillin, and nafcillin, as well as cephalosporins like cephalothin and cefazolin. Among these, methicillin was one of the first drugs used in clinical practice. As early as 1961, MRSA strains began to be identified and spread widely across may countries [37], marking a significant progression in the issue of antibiotic resistance over time.

MRSA can be easily transmitted between patients through direct contact with asymptomatic healthcare workers and other carriers, leading to outbreaks of infections that have significant implications for

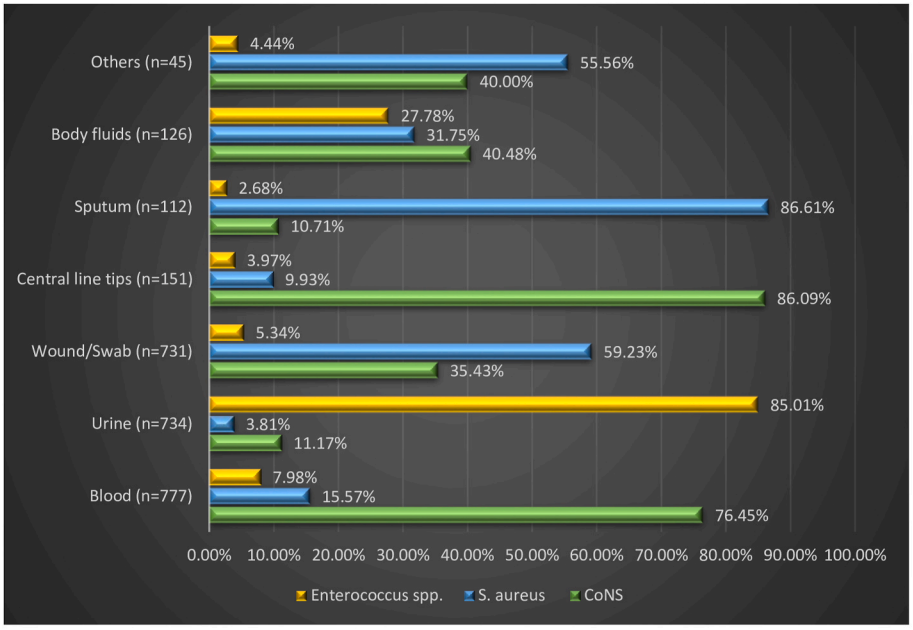

Fig. 2. Distribution of gram-positive cocci among infection sites.

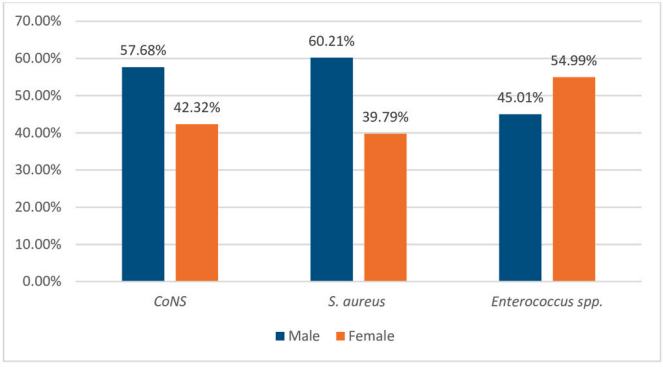

Fig. 3. Gender distribution of gram-positive cocci (p-value<0.01).

Table 1
Prevalence of gram-positive isolates (p-value<0.01).

Gram-positive isolate	Total number collected	Proportion (%)
Coagulase-negative *Staphylococcus*	1146	42.83 %
Staphylococcus aureus	759	28.36 %
Enterococcus spp.	771	28.81 %
Total	**2676**	100.00 %

healthcare facilities. For instance, the hands of medical staff may become colonized after taking care of a patient who is a carrier or infected with MRSA. The bacteria can spread to other patients without proper hand washing after such contact. Healthcare workers typically only carry the bacteria briefly, while individuals with skin conditions like dermatitis are more likely to carry it chronically. These factors have contributed to the spread of MRSA in healthcare settings [37]. After being discharged from the hospital, it was observed that MRSA carriage persisted, leading to an increased risk of infection with this organism in both community and hospital environments [38].

MRSA, similarly to susceptible strains of *S. aureus*, has the potential to cause life-threatening infections. Its clinical significance lies in its predictable resistance to all β-lactams, which include penicillins, cephalosporins, cephamycins, and carbapenems, as well as non-β-lactam antibiotics, including ciprofloxacin, gentamicin, erythromycin, clindamycin and trimethoprim-sulfamethoxazole [39].

Resistance to methicillin not only limits treatment options but also leads to longer hospital stays and increased treatment costs compared to infections caused by sensitive strains. There are few antibiotics that are effective against MRS species, and until recently, vancomycin (and teicoplanin in some countries) was the preferred treatment. The absence of an oral antibiotic as effective against MRSA as vancomycin likely played a significant role in driving up treatment costs, since vancomycin requires administration in a hospital or with the support of healthcare professionals at home or in a physician's office [40].

Chamoun et al. [41] found that the prevalence of MRSA increased significantly from 23.6 % in 2011 to 27.1 % in 2013, with an average of 26.7 %. Our current study spanning seven years shows a notably higher MRSA rate of 36.86 %, highlighting the challenges posed by the difficult to manage infections caused by healthcare workers. The susceptibility of *S. aureus* to vancomycin was 99.7 % in the study of Chamoun et al. [41]; however, out study did not report any vancomycin-resistant *S. aureus* strains.

In 2007, a study was conducted as part of the ARMed project to explore the prevalence of MRSA in Lebanon as well as in eight other Mediterranean countries. Hospital-specific variability in MRSA prevalence within the same country was exposed, requiring strengthening of infection control and responsible antibiotic use [42]. In this study, we aimed to determine the prevalence and antimicrobial resistance patterns of *S. aureus* and other Gram-positive organisms in a Lebanese hospital. Although localized, our finding provide a more recent assessment of resistance trends, highlighting the critical need for continuous surveillance and effective antibiotic stewardship to combat the spread of resistant strains [42].

On the other hand, Coagulase-negative *Staphylococci* are part of the normal microbiota of the skin and mucous membranes. Because they have traditionally been viewed as contaminants rather than primary infectious agents, there are limited comprehensive studies detailing their prevalence in human infections [43].

CoNS are the most commonly identified microorganisms in blood cultures. While they are a significant cause of healthcare-associated bloodstream infections, they are also frequently found as contaminants in blood cultures. Whereas contamination of blood cultures results in additional laboratory tests, unnecessary use of antibiotics

Table 2
Antimicrobial susceptibility pattern of coagulase-negative *Staphylococci* during the study period.

Antimicrobial category	Antimicrobial agent	2017 (n = 141)	2018 (n = 240)	2019 (n = 187)	2020 (n = 222)	2021 (n = 146)	2022 (n = 111)	2023 (n = 99)	Mean (n = 1146)
Aminoglycosides	Gentamicin	62.41 %	57.92 %	60.96 %	64.71 %	52.05 %	64.86 %	68.37 %	61.61 %
Ansamycins	Rifampin	87.14 %	85.42 %	85.03 %	87.84 %	75.34 %	86.49 %	83.67 %	84.42 %
Fluoroquinolones	Ciprofloxacin	46.81 %	42.92 %	41.18 %	49.10 %	39.73 %	51.35 %	54.08 %	46.45 %
Folate pathway antagonists	TMP-SMX	65.22 %	61.67 %	70.59 %	69.37 %	65.75 %	66.67 %	63.27 %	66.08 %
Glycopeptides	Teicoplanin	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %
	Vancomycin	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %
Lincosamines	Clindamycin	75.89 %	57.50 %	50.80 %	57.40 %	51.37 %	59.46 %	50.00 %	57.49 %
Macrolides	Erythromycin	36.88 %	22.08 %	22.99 %	29.73 %	18.49 %	35.14 %	28.57 %	27.70 %
Nitrofurantoins	Nitrofurantoin*	100.00 %	100.00 %	83.33 %	100.00 %	100.00 %	100.00 %	–	97.22 %
Oxazolidinones	Linezolid	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %
Penicillinase-stable penicillins	Cefoxitin	41.13 %	30.83 %	36.90 %	39.19 %	27.40 %	48.65 %	44.90 %	38.43 %
	Oxacillin	41.13 %	30.83 %	36.90 %	39.19 %	27.40 %	48.65 %	44.90 %	38.43 %
Tetracyclines	Tetracycline	74.29 %	79.17 %	73.80 %	79.73 %	75.34 %	73.87 %	70.41 %	75.23 %

TMP-SMX: Trimethoprim-sulfamethoxazole.
*For urine isolates only.

Table 3
Antimicrobial susceptibility pattern of *Staphylococcus aureus* during the study period.

Antimicrobial category	Antimicrobial agent	2017 (n = 67)	2018 (n = 133)	2019 (n = 171)	2020 (n = 117)	2021 (n = 95)	2022 (n = 95)	2023 (n = 81)	Mean (n = 759)
Aminoglycosides	Gentamicin	86.57 %	90.98 %	87.72 %	91.38 %	87.37 %	87.37 %	90.00 %	88.77 %
Ansamycins	Rifampin	92.54 %	94.74 %	91.23 %	98.28 %	94.74 %	95.79 %	97.50 %	94.97 %
Fluoroquinolones	Ciprofloxacin	67.16 %	73.68 %	78.36 %	79.31 %	77.89 %	72.63 %	81.25 %	75.75 %
Folate pathway antagonists	TMP-SMX	89.39 %	90.98 %	87.72 %	90.52 %	91.58 %	88.42 %	95.00 %	90.52 %
Glycopeptides	Teicoplanin	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %
	Vancomycin	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %
Lincosamines	Clindamycin	85.07 %	84.21 %	83.04 %	79.31 %	87.37 %	87.37 %	78.75 %	83.59 %
Macrolides	Erythromycin	76.12 %	75.19 %	76.61 %	75.86 %	78.95 %	80.00 %	71.25 %	76.28 %
Nitrofurantoins	Nitrofurantoin*	–	87.50 %	100.00 %	100.00 %	100.00 %	100.00 %	–	97.50 %
Oxazolidinones	Linezolid	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %
Penicillinase-stable penicillins	Cefoxitin	56.72 %	70.68 %	71.93 %	67.24 %	60.00 %	57.89 %	57.50 %	63.14 %
	Oxacillin	56.72 %	70.68 %	71.93 %	67.24 %	60.00 %	57.89 %	57.50 %	63.14 %
Tetracyclines	Tetracycline	76.12 %	76.69 %	78.36 %	82.76 %	81.05 %	86.32 %	90.00 %	81.61 %

TMP-SMX: Trimethoprim-sulfamethoxazole.
*For urine isolates only.

Table 4
Antimicrobial susceptibility pattern of *Enterococcus* spp. during the study period.

Antimicrobial category	Antimicrobial agent	2017 (n = 78)	2018 (n = 75)	2019 (n = 196)	2020 (n = 130)	2021 (n = 102)	2022 (n = 90)	2023 (n = 100)	Mean (n = 771)
Fluoroquinolones	Ciprofloxacin	56.41 %	43.55 %	52.73 %	27.16 %	37.35 %	40.54 %	63.10 %	45.83 %
Fosfomycins	Fosfomycin*	98.70 %	98.31 %	93.94 %	98.77 %	97.59 %	95.95 %	98.81 %	97.44 %
Glycopeptides	Teicoplanin	98.72 %	100.00 %	97.45 %	92.31 %	91.09 %	93.26 %	96.97 %	95.69 %
	Vancomycin	98.72 %	100.00 %	94.90 %	92.31 %	91.09 %	93.26 %	96.97 %	95.32 %
Macrolides	Erythromycin	34.62 %	13.33 %	21.43 %	17.69 %	16.83 %	17.98 %	16.16 %	19.72 %
Nitrofurantoins	Nitrofurantoin*	96.05 %	96.61 %	88.48 %	87.65 %	78.31 %	83.78 %	94.05 %	89.28 %
Oxazolidinones	Linezolid	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %	100.00 %
Penicillins	Ampicillin	87.18 %	80.00 %	83.67 %	76.92 %	80.20 %	76.40 %	80.81 %	80.74 %
Tetracyclines	Tetracycline	26.32 %	8.47 %	20.00 %	13.58 %	20.48 %	18.92 %	25.00 %	18.97 %

*For urine isolates only.

(particularly vancomycin), and longer hospital stays, but failure to promptly identify and treat true bacteremia can lead to increased morbidity and mortality [44]. Nevertheless, colonizing CoNS strains have been documented as causative agents of human infections, particularly in immunocompromised hosts [45] and neonates [46].

CoNS infections are typically treated with glycopeptides, such as vancomycin, but there are growing concerns regarding the emergence of resistance to these agents [45].

In a prior study conducted in Saudi Arabia from 2015 to 2019, the susceptibility of CoNS to vancomycin was reported at 99.7 % [47]. Our study has shown no evidence of resistance to vancomycin.

For nearly a century, *Enterococci* have been recognized as a major cause of endocarditis. In the late 1970s, they started to be acknowledged as a frequent cause of hospital-acquired infections, coinciding with the increased use of third-generation cephalosporins, to which *Enterococci* are naturally resistant [48]. In the United States, *Enterococci* are increasingly associated with hospital-acquired bacteremia, urinary tract and wound infections [49]. They succeed in surviving in hospital environments due to their intrinsic resistance to several commonly used antibiotics, as well as their ability to acquire resistance to nearly all currently available antibiotics through mutations or by acquiring foreign genetic material via the transfer of plasmids and transposons

[50].

Challenges in treating *Enterococci* were already evident in the 1950s when studies discovered that treating *Enterococcal* endocarditis exclusively with penicillin had significantly lower response rates compared to *Streptococcal* endocarditis [51]. Infections with *Enterococci* are considered problematic for antimicrobial therapy due to the longer duration of treatment and the increased toxicity of combination regimens compared to those used for *Streptococcal* endocarditis [51].

Until recently, vancomycin was essentially the only reliably effective antibiotic for treating infections caused by multidrug-resistant *Enterococci*. It had been in clinical use for more than 30 years without the emergence of significant resistance. Due to its activity against MRSA and other gram-positive bacteria, vancomycin has been widely used for therapy and prophylaxis against infections caused by these bacteria [52]. The first vancomycin-resistant *Enterococcus* strains were isolated in England in 1988 and reported by Uttley et al. [53]. Shortly after, investigators in the United Kingdom reported the first VRE isolates [54], as did hospitals in the eastern half of the United States [55]. Subsequently, resistance has spread with surprising speed and is now encountered in hospitals across most states [51].

In an earlier study carried out at 16 different tertiary care centers in Lebanon, the susceptibility rate of *Enterococcus* spp. to ampicillin was reported at 84.4 %, with low VRE rate at just 1 % [41]. Our study shows a slightly lower susceptibility rate to ampicillin at 80.74 %, while the VRE rate has increased to 4.68 %.

The antimicrobial resistance observed in our study is likely driven by well-documented resistance mechanisms. Resistance to methicillin in *S. aureus* is typically mediated by the *mecA* gene, which alters penicillin-binding proteins, reducing efficacy to β-lactams [56]. Similarly, vancomycin resistance in *Enterococcus* is commonly associated with *van* gene clusters, leading to modifications in cell wall precursors [57]. Macrolide and lincosamide resistance, frequently observed in *Staphylococcus* and *Enterococcus*, is often due to *erm* genes, which encode methylases that modify ribosomal targets [58]. While our study focused on phenotypic resistance patterns, these findings align with known genetic determinants reported in the literature.

The results of our study highlight the alarming prevalence of antimicrobial resistance among Gram-positive pathogens in Lebanon, emphasizing the critical need for improved surveillance and antibiotic stewardship programs. The high rates of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* observed in our study align with global trends and suggest a growing burden of resistant infections in hospital settings. Comparisons with previous studies from Lebanon and the broader Mediterranean region further emphasize the variability in resistance patterns, potentially influenced by differences in infection control measures, antibiotic prescribing practices, and healthcare infrastructure. Given the limited number of studies on this topic in Lebanon, our data provide crucial insights that can inform policy decisions and guide future research. Addressing antimicrobial resistance requires a multifaceted approach, including routine susceptibility testing, strict infection control measures, and the development of alternative therapeutic strategies to mitigate the spread of multidrug-resistant organisms.

4.1. Limitations

The study being conducted at just one medical center might have restricted how broadly its findings could apply to different settings or regions with diverse patient populations and medical procedures. Another limitation of this study is the absence of PCR-based detection of virulence and antimicrobial resistance genes, which could have provided deeper molecular insights. Consequently, we were unable to perform a correlation analysis between phenotypic and genotypic multidrug resistance, including estimating the correlation coefficient (r) between the identified resistance genes and the tested antimicrobial agents.

5. Conclusion

This study provides valuable insights into the prevalence and antimicrobial resistance patterns of Gram-positive bacterial pathogens, particularly *Staphylococcus aureus*, coagulase-negative *Staphylococci*, and *Enterococcus* spp., in a Lebanese hospital. Our findings highlight the widespread resistance among these organisms, emphasizing the urgent need for continuous surveillance and strict antimicrobial stewardship to curb the spread of resistant strains. Addressing this issue requires a multidisciplinary approach, integrating infection control strategies and judicious antibiotic use. Future research should focus on molecular resistance mechanisms and alternative therapeutic strategies to enhance the management of these infections in healthcare settings.

CRediT authorship contribution statement

Yara Khachab: Writing – original draft, Methodology, Formal analysis, Data curation. **Racha Khoumassi:** Writing – original draft, Methodology, Formal analysis, Data curation. **Elie Salem Sokhn:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization.

Ethical considerations

Ethical approval for this study was granted by the Institutional Review Board (IRB) of the Lebanese Hospital Geitaoui – UMC under code 2019-IRB-025.

Data availability statement

All data relevant to the study is included in the article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2025.101588.

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