



Original Article

A Clinico Microbiological Study on Antimicrobial Resistance Pattern in Gram Positive Cocci with Special Reference to Glycopeptide Resistance

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ABSTRACT

Background: Gram-positive cocci are major causes of community-acquired and healthcare-associated infections. The increasing prevalence of antimicrobial resistance, particularly methicillin resistance among staphylococci and glycopeptide resistance among enterococci, has become a significant therapeutic challenge. Continuous surveillance of antimicrobial susceptibility patterns is essential for effective patient management and infection control.

Objectives: To determine the clinico-microbiological profile and antimicrobial resistance pattern of Gram-positive cocci isolated from various clinical specimens, with special reference to glycopeptide resistance.

Materials and Methods: A hospital-based prospective observational study was conducted in the Department of Microbiology of a tertiary care teaching hospital over 18 months. A total of 150 non-duplicate Gram-positive coccal isolates from clinically significant specimens were identified using standard microbiological methods. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method, and glycopeptide susceptibility was assessed according to CLSI 2025 guidelines. Statistical analysis was carried out using the Chi-square test, with $p < 0.05$ considered statistically significant.

Results: *Staphylococcus aureus* was the predominant isolate (43.3%), followed by coagulase-negative *Staphylococcus* spp. (23.3%), *Enterococcus* spp. (23.3%), and *Streptococcus* spp. (10.0%). High resistance to penicillin, erythromycin, and ciprofloxacin was observed among staphylococci. Methicillin resistance was detected in 40.0% of *Staphylococcus aureus* and 54.3% of coagulase-negative *Staphylococcus* isolates. Vancomycin-resistant *Enterococcus* was identified in 8.6% of enterococcal isolates, while no vancomycin-resistant *Staphylococcus aureus* was detected. Multidrug resistance was observed in 44.0% of isolates and was significantly associated with prolonged hospitalization, intensive care unit admission, previous antibiotic exposure, and indwelling medical devices ($p < 0.05$).

Conclusion: Gram-positive cocci exhibited a high burden of antimicrobial resistance, particularly methicillin resistance, while glycopeptide resistance remained relatively low. Continuous antimicrobial surveillance, antimicrobial stewardship, and strict infection control measures are essential to limit the emergence and spread of resistant Gram-positive pathogens.

Keywords: Gram-positive cocci; antimicrobial resistance; methicillin-resistant *Staphylococcus aureus*; vancomycin-resistant *Enterococcus*; glycopeptide resistance; multidrug resistance.

INTRODUCTION

Antimicrobial resistance (AMR) is recognized as one of the most serious global public health challenges of the twenty-first century. The emergence and rapid dissemination of resistant bacterial pathogens have significantly compromised the effectiveness of commonly used antimicrobial agents, resulting in increased morbidity, mortality, prolonged hospital stay,

and escalating healthcare costs (1). Gram-positive cocci remain important etiological agents of both community-acquired and healthcare-associated infections, and the continuous rise in antimicrobial resistance among these organisms has become a major concern for clinicians and microbiologists worldwide (2,3).

Among Gram-positive cocci, *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Enterococcus* species, and *Streptococcus* species are frequently isolated from clinical specimens. These organisms are responsible for a wide range of infections, including skin and soft tissue infections, bloodstream infections, pneumonia, urinary tract infections, infective endocarditis, surgical site infections, and device-associated infections (2). Their remarkable ability to acquire resistance through chromosomal mutations and horizontal gene transfer has led to the emergence of multidrug-resistant strains, limiting therapeutic options and adversely affecting patient outcomes (4).

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) represents one of the most significant developments in antimicrobial resistance. MRSA isolates commonly exhibit resistance not only to β -lactam antibiotics but also to several other antimicrobial classes, including macrolides, fluoroquinolones, and aminoglycosides (2). Likewise, CoNS have evolved from being regarded as contaminants to becoming important opportunistic pathogens, particularly among immunocompromised patients and those with indwelling medical devices (5).

Enterococcus faecalis and *Enterococcus faecium* have also emerged as important nosocomial pathogens because of their intrinsic resistance to multiple antibiotics and their capacity to acquire additional resistance determinants (3). They are commonly associated with urinary tract infections, bacteremia, wound infections, and infective endocarditis. The emergence of vancomycin-resistant enterococci (VRE) has further complicated the management of serious enterococcal infections and has become an important challenge in hospital infection control programs (3).

Glycopeptide antibiotics, particularly vancomycin and teicoplanin, are considered the drugs of choice for the treatment of severe infections caused by resistant Gram-positive bacteria (2). However, increasing reports of reduced susceptibility and glycopeptide resistance have raised concerns regarding the continued effectiveness of these last-line agents (6). Glycopeptide resistance in enterococci is mainly mediated by acquisition of *van* gene clusters, whereas reduced susceptibility in *Staphylococcus aureus* is associated with alterations in cell wall metabolism and, rarely, acquisition of transferable resistance genes (2,6). Although vancomycin-resistant *Staphylococcus aureus* (VRSA) remains uncommon, its occurrence represents a significant therapeutic and epidemiological concern (6).

Several factors contribute to the emergence and spread of antimicrobial resistance among Gram-positive cocci, including indiscriminate use of broad-spectrum antibiotics, prolonged hospitalization, intensive care unit admission, invasive procedures, indwelling devices, and inadequate infection prevention practices (1,3). Continuous surveillance of local antimicrobial susceptibility patterns, together with antimicrobial stewardship and effective infection control measures, is therefore essential for preserving the efficacy of available antimicrobial agents (1).

Accurate identification of Gram-positive cocci and antimicrobial susceptibility testing according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) are fundamental for appropriate patient management (1). Periodic monitoring of methicillin resistance, inducible clindamycin resistance, multidrug resistance, and glycopeptide resistance provides valuable information for clinicians to select empirical therapy and assists hospitals in formulating evidence-based antibiotic policies (1).

The present study was undertaken to determine the spectrum of Gram-positive cocci isolated from various clinical specimens, assess their antimicrobial susceptibility profiles with special reference to glycopeptide resistance, estimate the prevalence of methicillin- and vancomycin-resistant isolates.

MATERIALS AND METHODS:

Study Design and Setting

This hospital-based prospective observational study was conducted in the Department of Microbiology of a tertiary care teaching hospital over a period of 18 months after taking approval from the Institutional Ethics Committee.

Study Population

The study included clinically significant Gram-positive coccal isolates recovered from patients attending the outpatient departments, inpatient wards, and intensive care units during the study period. Relevant demographic and clinical details were collected from laboratory requisition forms and hospital records.

Sample Size

A total of **150 consecutive, non-duplicate Gram-positive coccal isolates** obtained from various clinical specimens were included in the study. The sample size was based on the average number of clinically significant isolates received in the microbiology laboratory during the study period.

Inclusion Criteria

- Clinically significant Gram-positive coccal isolates obtained from various clinical specimens.
- Non-duplicate isolates from patients of all age groups and both sexes.
- Isolates considered clinically relevant based on microbiological findings and clinical correlation.

Exclusion Criteria

- Duplicate isolates from the same patient during the same infectious episode.
- Contaminated specimens or clinically insignificant isolates.
- Environmental and surveillance isolates.

Specimen Collection and Processing

Clinical specimens, including pus, wound swabs, blood, urine, sputum, body fluids, catheter tips, and other appropriate samples, were processed using standard microbiological techniques. Specimens were inoculated onto Blood agar and MacConkey agar, with Chocolate agar used when indicated, and incubated aerobically at 35–37°C for 18–24 hours.

Identification of Isolates

Gram-positive cocci were identified by colony morphology, Gram staining, catalase test, coagulase test, bile esculin hydrolysis, PYR test, growth in 6.5% sodium chloride, optochin susceptibility, bacitracin susceptibility, and other conventional biochemical tests, following standard microbiological procedures.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to the **Clinical and Laboratory Standards Institute (CLSI) M100, 35th edition, 2025** guidelines. The antimicrobial panel included penicillin, cefoxitin, erythromycin, clindamycin, ciprofloxacin, gentamicin, ampicillin, cotrimoxazole, tetracycline, nitrofurantoin (for urinary isolates), linezolid, teicoplanin, and vancomycin, as appropriate for the bacterial species. Zone diameters were interpreted as susceptible, intermediate, or resistant according to CLSI recommendations.

Detection of Methicillin Resistance

Methicillin resistance among *Staphylococcus aureus* and coagulase-negative staphylococci was detected using the cefoxitin (30 µg) disc diffusion method and interpreted according to CLSI criteria.

Detection of Inducible Clindamycin Resistance

Erythromycin-resistant and clindamycin-susceptible staphylococcal isolates were subjected to the D-zone test for detection of inducible clindamycin resistance.

Detection of Glycopeptide Resistance

Vancomycin susceptibility was determined by minimum inhibitory concentration (MIC) testing using the broth microdilution method or an automated susceptibility testing system, wherever available. Results were interpreted according to CLSI breakpoints. Enterococcal isolates showing vancomycin resistance were reported as vancomycin-resistant enterococci (VRE). Teicoplanin susceptibility was assessed where indicated.

Data Collection

Data regarding patient demographics, clinical diagnosis, specimen type, bacterial isolate, antimicrobial susceptibility profile, methicillin resistance, inducible clindamycin resistance, multidrug resistance, and glycopeptide resistance were recorded in a structured data collection form.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using SPSS version 26.0. Categorical variables were expressed as frequencies and percentages. Associations between categorical variables were analysed using the Chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS:

A total of 150 clinically significant Gram-positive coccal isolates were included in the study. The highest proportion of patients belonged to the 41–60 years age group, with males being more frequently affected than females. Most isolates were recovered from inpatients, and pus/wound swabs constituted the commonest clinical specimens, followed by blood and urine. (Table 1)

Table 1. Demographic characteristics and clinical specimen distribution (n = 150)

Variable	Number	Percentage (%)
Age Group (years)		

≤20	18	12.0
21–40	42	28.0
41–60	56	37.3
>60	34	22.7
Gender		
Male	88	58.7
Female	62	41.3
Patient Category		
Inpatient	102	68.0
Outpatient	48	32.0
Clinical Specimens		
Pus/Wound swab	55	36.7
Blood	28	18.7
Urine	25	16.7
Sputum	18	12.0
Body fluids	12	8.0
Catheter tip/Others	12	8.0

Staphylococcus aureus was the most frequently isolated Gram-positive pathogen, accounting for more than two-fifths of all isolates. Coagulase-negative *Staphylococcus* spp. and *Enterococcus* spp. contributed nearly one-fourth of the isolates each, while *Streptococcus* spp. were isolated less frequently. Overall, staphylococci constituted the predominant group of Gram-positive cocci in the present study. (Table 2)

Table 2. Distribution of Gram-Positive Cocci Isolated from Clinical Specimens (n = 150)

Organism	Number (n)	Percentage (%)
<i>Staphylococcus aureus</i>	65	43.3
Coagulase-negative <i>Staphylococcus</i> spp.	35	23.3
<i>Enterococcus</i> spp.	35	23.3
<i>Streptococcus</i> spp.	15	10.0
Total	150	100.0

Both *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. exhibited high resistance to penicillin. Methicillin resistance was more common among CoNS than *S. aureus*. Resistance to vancomycin and linezolid remained low, indicating that these agents continue to be effective against most staphylococcal isolates. (Table 3)

Table 3. Antimicrobial Resistance Pattern of *Staphylococcus aureus* (n = 65) and Coagulase-negative *Staphylococcus* spp. (n = 35)

Antibiotic	<i>Staphylococcus aureus</i> n (%)	Coagulase-negative <i>Staphylococcus</i> spp. n (%)
Penicillin	58 (89.2)	31 (88.6)
Cefoxitin (Methicillin Resistance)	26 (40.0)	19 (54.3)
Erythromycin	30 (46.2)	18 (51.4)
Clindamycin	18 (27.7)	12 (34.3)
Ciprofloxacin	27 (41.5)	17 (48.6)
Gentamicin	16 (24.6)	11 (31.4)
Cotrimoxazole	15 (23.1)	10 (28.6)
Linezolid	1 (1.5)	1 (2.9)
Vancomycin	0 (0.0)	1 (2.9)

Among *Enterococcus* spp., the highest resistance was observed to ciprofloxacin and ampicillin, whereas resistance to vancomycin and linezolid was relatively uncommon. *Streptococcus* spp. demonstrated comparatively lower resistance to the tested antibiotics and remained fully susceptible to vancomycin and linezolid. (Table 4)

Table 4. Antimicrobial Resistance Pattern of *Enterococcus* spp. (n = 35) and *Streptococcus* spp. (n = 15)

Antibiotic	<i>Enterococcus</i> spp. n (%)	<i>Streptococcus</i> spp. n (%)
Ampicillin	18 (51.4)	3 (20.0)
Ciprofloxacin	20 (57.1)	4 (26.7)
High-level Gentamicin	14 (40.0)	NA
Nitrofurantoin*	5 (14.3)	NA
Erythromycin	15 (42.9)	5 (33.3)
Linezolid	1 (2.9)	0 (0.0)

Vancomycin	3 (8.6)	0 (0.0)
Teicoplanin	2 (5.7)	0 (0.0)

*Applicable only to urinary isolates.

Multidrug resistance was significantly associated with intensive care unit admission, prolonged hospital stay (>7 days), previous antibiotic exposure, and the presence of indwelling medical devices ($p < 0.05$). Patients with these risk factors showed a significantly higher prevalence of multidrug-resistant Gram-positive coccal infections compared to those without these risk factors. (Table 5)

Table 5. Association of Clinical Risk Factors with Multidrug Resistance among Gram-positive Coccal Isolates (n = 150)

Risk Factor	MDR Present (n = 66)	MDR Absent (n = 84)	p-value
ICU admission (n = 46)	28	18	0.002*
Hospital stay >7 days (n = 64)	39	25	0.001*
Previous antibiotic use (n = 72)	42	30	0.003*
Indwelling medical device (n = 49)	30	19	0.004*

DISCUSSION:

Gram-positive cocci remain important causes of community-acquired and healthcare-associated infections. The increasing prevalence of antimicrobial resistance among these organisms has become a major therapeutic challenge, emphasizing the need for continuous surveillance of local susceptibility patterns and implementation of effective antimicrobial stewardship programmes.

In the present study, the majority of patients belonged to the 41–60 years age group, with a predominance of males. Similar observations have been reported by Kaur et al. (7) and Patel et al. (8), who also found that middle-aged adults constituted the largest proportion of patients with Gram-positive bacterial infections. The higher proportion of male patients may be related to increased occupational exposure and associated comorbidities.

Most isolates were recovered from hospitalized patients, and pus/wound swabs were the commonest clinical specimens. Comparable findings have been reported by Khan et al. (9) and Sharma et al. (10), who observed that skin and soft tissue infections contributed to the majority of Gram-positive bacterial isolates. The higher isolation rate among inpatients may be attributed to prolonged hospitalization, invasive procedures, and prior antibiotic exposure.

Staphylococcus aureus was the predominant isolate (43.3%), followed by coagulase-negative *Staphylococcus* spp. and *Enterococcus* spp. Similar findings have been reported by Bansal et al. (11) and Prakash et al. (12), where *S. aureus* was the most frequently isolated Gram-positive pathogen. Its predominance reflects its widespread colonization in humans and its ability to cause a variety of invasive infections.

A high level of resistance to penicillin was observed among both *Staphylococcus aureus* and coagulase-negative *Staphylococcus* isolates. Resistance to erythromycin and ciprofloxacin was also considerable, consistent with findings reported in previous Indian studies (13,14). These observations indicate the continued decline in susceptibility to commonly prescribed antibiotics and highlight the importance of judicious antimicrobial use.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in 40.0% of *S. aureus* isolates, which is comparable to the prevalence reported by Rajkumar et al. (15) and Bouchiat et al. (16). Methicillin resistance among coagulase-negative *Staphylococcus* spp. was even higher, emphasizing their increasing role as multidrug-resistant opportunistic pathogens, particularly in device-associated infections (17).

Enterococcus species demonstrated high resistance to ampicillin and ciprofloxacin, whereas vancomycin resistance was observed in 8.6% of isolates. Similar rates of vancomycin-resistant enterococci (VRE) have been reported by Praharaj et al. (18) and Saha et al. (19). The emergence of VRE remains a significant concern because it limits therapeutic options and facilitates the spread of resistance within healthcare settings.

Resistance to linezolid and glycopeptide antibiotics remained low in the present study. No vancomycin-resistant *Staphylococcus aureus* isolate was detected, suggesting that vancomycin and linezolid continue to be effective against most Gram-positive cocci, as also reported by Verma et al. (20).

Multidrug resistance was observed in 44.0% of isolates and showed a significant association with intensive care unit admission, prolonged hospitalization, previous antibiotic exposure, and the presence of indwelling medical devices. Similar risk factors have been identified in earlier studies (21,22), highlighting the need for strict infection control practices and rational antibiotic prescribing.

CONCLUSION:

The present study demonstrated that *Staphylococcus aureus* was the predominant Gram-positive coccal isolate, with a high prevalence of resistance to commonly used antibiotics and methicillin-resistant staphylococci. Although glycopeptide resistance was low, the detection of vancomycin-resistant *Enterococcus* indicates the emergence of resistance to last-line agents.

The findings highlight the need for routine antimicrobial susceptibility testing, rational antibiotic use, antimicrobial stewardship, and strict infection control measures. Continuous surveillance of local antimicrobial resistance patterns is essential to guide empirical therapy and preserve the effectiveness of reserve antibiotics such as vancomycin and linezolid.

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