

Infections caused by Gram positive cocci and their microbiological pattern in a tertiary care hospital

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KEYWORDS

ABSTRACT

Introduction: Gram-positive cocci are clinically important pathogens that cause infections and their development of antibiotic resistance continues to pose a severe threat to public health. Gram-positive cocci are included among some of the most significant human bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Strep. pneumoniae*, along with species of lower virulence such as *Staph. epidermidis*,

Staph. saprophyticus and *Enterococcus faecalis*. Isolation and identification of these organisms is one of the most important but also routine tasks performed in clinical microbiology. Therefore, this study aims to investigate the level of antimicrobial resistance among Gram-positive cocci isolated from different clinical samples.

Methods: From June 2023 to July 2024, a cross-sectional study was conducted at Microbiology Laboratory. clinical samples were received in the Laboratory inoculated into and Blood Agar and MacConkey agar. Bacterial identification was done by observing morphology of colonies, as well as Gram staining, catalase testing, and coagulase test after isolation of pure growth on culture media using the standard operating procedure and required biochemical tests were performed for bacterial identification. Drug susceptibility testing using kirby-bauer disc diffusion will be done. The information entry and analysis were performed by using SPSS version 20.

Results: In our study period we received 5747 samples and among them, total positive cultures are 2262 (39%). Among the total Gram-positive isolates 762 (34%) are Gram positive cocci. Among the Gram-positive cocci isolates staphylococcus aureus isolates are 484 (63.5%), Coagulase negative staphylococci are 141 (18.5%), streptococcus 95 (12.5%) and Enterococci are 42 (5.5%). Most of the isolates are MDR organisms

Conclusion: Importance of continuous monitoring of antimicrobial resistance patterns is crucial for selecting the suitable drug for treatment and infection prevention.

Introduction:

Due to high resistance shown by many micro-organisms there is a great burden to the public health. Even many of the patients who come for simple health issues are coming across these drug resistance bugs and suffering a lot physically and also economically. ^(1,2)

India has been termed as the “AMR capital” of the world. ⁽³⁾ Poor sanitary conditions, absence of effective infection-control policies, antibiotic overuse in humans and animals, and lack of

awareness among the masses about the infectious diseases⁽⁴⁾ have led to a gradual increase in AMR over time. When we come across both health care associated and community acquired infections, we can see trend of increasing infections by gram positive bacteria which are mainly *Staphylococcus spp* and *Streptococcus spp*.⁽⁵⁻⁸⁾

Staphylococcus aureus (causes wound infections) and *coagulase-negative staphylococci* (CoNS) (causes nosocomial bloodstream infections), and *Enterococci* are the most commonly isolated Gram-positive cocci from nosocomial infections.⁽⁹⁾

Among community-acquired infections, *Pneumococci* is a common cause of upper (sinusitis, otitis media) and lower (pneumonia) respiratory tract infections, meningitis, bacteremia, and other suppurative infections and *Streptococcus pyogenes* is the most common bacteria which causes pharyngitis.⁽⁹⁾ Prolonged usage of the available effective antibiotics against GPC coupled with their decreasing susceptibility rates have led to the emergence and spread of multidrug-resistant pathogens.⁽²⁾ Gram-positive cocci bacteria have shown the ability to acquire and express numerous antimicrobial resistance mechanisms which lead to a multidrug-resistant (MDR) phenotype that further complicates treatment efforts in addition to the innate virulence exhibited by bacteria like *S. aureus* and *S. pneumoniae*.⁽⁸⁾

Infections caused by MDR pathogens are an increasing problem and remain one of the main challenges in the treatment of infectious diseases. The ongoing increase of antimicrobial resistance among GPC infections continues to be a big threat to public health, which complicates antimicrobial therapy leading to treatment failures and imposing additional costs on health systems and patients. In addition, the emergence of antibiotic resistance in GPC has had a significant impact on patterns of prescribing and has created a clinical demand for effective novel therapeutic agents.⁸⁻¹³

The global increase of antimicrobial-resistant bacteria infection in both community and hospital settings is endangering the ability to effectively treat patients. Hence, recent data is needed for more appropriate antimicrobial prescribing, and careful infection control.

Aims/Objectives:

This study aims to find out the prevalence of Gram-positive cocci and their antibiotic susceptibility patterns among various routine samples received in the central laboratory in a tertiary care hospital.

Materials and methods:

This is a retrospective observational study of the specimens received for aerobic culture and sensitivity from June 2023 to June 2024 at a tertiary care hospital in Konaseema Institute of Medical Sciences and Research Foundation, Amalapuram, Andhrapradesh, India. The samples were processed using standard microbiological techniques. All the samples were plated on 5% sheep blood agar, Mac Conkey's agar. Chocolate agar was used for respiratory samples and body fluids like cerebrospinal fluid (CSF). Blood culture samples, Sterile body fluids and tissue were also inoculated in automated blood culture bottle and incubated in bactalert system. If growth is indicated by bactalert then it is cultured on to above mentioned agar plates.

After doing gram staining and manual tests like catalase and coagulase, identification and sensitivity were done using kirby bauer disc diffusion method. The antibiotic susceptibility results were reported as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.⁽¹¹⁻¹³⁾ All pathogens were reported with due clinical correlation. The commensals were reported only when they were grown in another specimen collected at a different time or from a different site.

Results:

We have received different samples for culture and sensitivity like blood, urine, respiratory samples like sputum, BAL fluid, Pleural fluid, ET secretions, body fluids like CSF, synovial fluid, Ascitic fluid, exudates like pus, swabs and drain fluids. So in our study period we received 5747 samples and among them, total positive cultures are 2262 (39%). Among the total Gram-positive isolates 762 (34%) are Gram positive cocci (fig 1). Among the Gram-positive cocci isolates staphylococcus aureus isolates are 484 (63.5%), Coagulase negative staphylococci are 141 (18.5%), streptococcus 95 (12.5%) and Enterococci are 42 (5.5%) (fig 2). Most of the Gram-positive cocci isolates are from medical ICU followed by ER and OP. The most of the GPC isolates are obtained from respiratory samples followed by blood cultures samples. The antimicrobial susceptibility testing pattern (AST) of the Gram-positive cocci are done according to CLSI guidelines and represented them in Fig 4.

Discussion:

In the present study the percentage of Gram-positive cocci isolates are 34%. In Seyoum Asres G et al study Gram-positive isolates constitute 45.2%⁽¹⁹⁾, in Mama M et al study 47% were Gram-positive⁽²⁰⁾, and in Hailu D et al study it was 52.6%⁽²¹⁾ in Amrishi Kohli et al study the Gram positive cocci isolates are 58%⁽²²⁾. But one of the study by Mekdes et al, the gram-positive cocci isolate rate was only 12.6%⁽²³⁾. So the percentage of the Gram positive cocci may vary according to the place and may also vary according to the hospital infection control practices.

Among the Gram-positive cocci isolates *Staphylococcus aureus* (63.5%) is the most common in this study. In the study by Mekdes et al also *S.aureus* is the most common (54%) gram-positive cocci⁽²³⁾ isolate in the others studies by Mama M et al(28.4%) and Wasihun AG et al (38%) also the most common isolate is again *S.aureus*.^(20,24)

The second common isolate is *CoNS* in this study. It is (18.5). In Swetha Shah et al study also the second most common isolate is *CoNS* (41%).⁽²⁵⁾ In others studies Mekdes et al, Mama M et al and Wasihun AG et al also the second most common isolate is *CoNS*.^(20,23,24)

In my study *Staphylococcus aureus* is resistant to most drugs like penicillin, 1st and 2nd generation cephalosporins and levofloxacin. But it is more sensitive for linezolid (95%), Teicoplanin (86%), Minocycline (85%), Vancomycin (83%), Doxycycline(80%). The drug susceptibility pattern for *S.aureus* is almost similar pattern in Swetha Shah et al study where they stated that All the *S. aureus* isolates were found to be highly sensitive toward vancomycin, linezolid, tigecycline, and teicoplanin.⁽²⁵⁾ Less than 1% of the strains were found to be sensitive to benzyl penicillin which is almost similar to our study.⁽²⁵⁾ In Seyoum Asres G et al study⁽¹⁹⁾ and Hailu D et al study⁽²¹⁾ also the resistant and sensitivity patterns for *S.aureus* was almost similar.

The *CoNS* isolates in my study showed resistance to penicillins, 1st and 2nd generation cephalosporins and also more vancomycin resistance (66%) compared to *Staphylococcus aureus* (83%). In Swetha Shah et al study also *CoNS* showed more resistance to Vancomycin i.e 7.1% more resistance than *S.aureus* which is almost similar to my study. Most of the *CoNS* isolates are Methicillin resistant which is similar to the studies done by Seyoum Asres G et al⁽¹⁹⁾ and Melkam birru et al.⁽²⁶⁾

In the present study *Streptococci* showing are more resistance to many drugs like cephalosporins, azithromycin but showing good sensitivity patterns for penicillin and

Linezolid. *Enterococci* are the most common emerging pathogens showing intrinsic resistance to many drugs and also showing high resistance to many drugs like ciprofloxacin, gentamycin. *Vancomycin resistant Enterococci* are about 24% which is almost similar to other studies like Mekdes et al ⁽²³⁾. Most of the *Enterococci* are also sensitive to linezolid which are also similar in studies like Seyoum Asres G et al. ⁽¹⁹⁾

Conclusion :

In conclusion, this study shows the steady increase in the emergence of highly resistant *S. aureus*, *CoNS*, and *Enterococcus species*. Although, in this study, a high efficacy against glycopeptides and linezolid was observed in the gram-positive isolates, however, the gradual increase in the resistance against these last line antibiotics for MDR gram-positive pathogens emphasizes the need for novel research antibiotics to combat these difficult-to-treat MDR pathogens. This suggests that the importance of continuous monitoring of antimicrobial resistance patterns is crucial for selecting the suitable drug for treatment and infection prevention.

Fig 1: Table showing Gram-positive and Gram-negative isolates

	Number	Percentage
Gram-positive cocci	762	34%
Gram-negative cocci	1500	66%
Total culture isolates	2262	100%

Fig 2: Table showing the details of Gram-positive cocci

Gram positive cocci	Number of isolates	Percentage
<i>Staphylococcus aureus</i>	484	63.5
<i>CONS</i>	141	18.5
<i>Streptococci</i>	95	12.5
<i>Enterococci</i>	42	5.5

Fig 3: Distribution of Gram-positive cocci area wise in the hospital

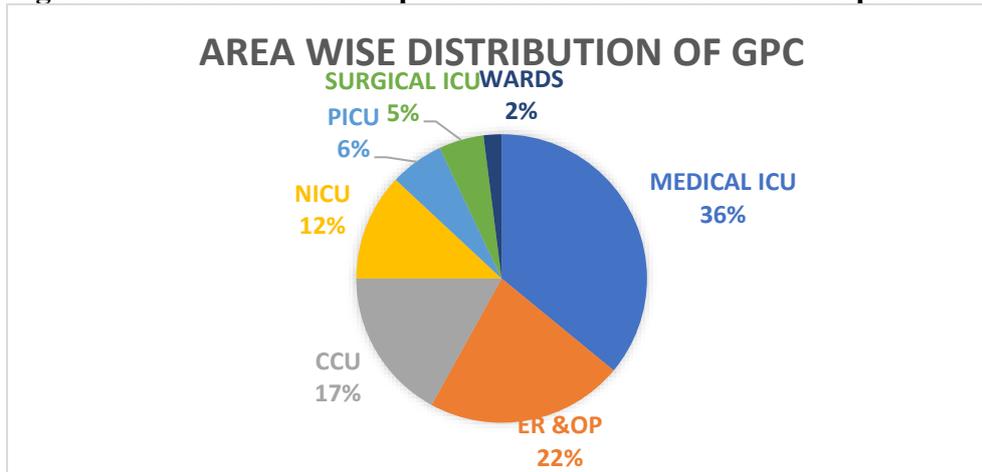


Fig 4: Antimicrobial susceptibility pattern of Gram-positive cocci

organism	No. of isolates	Ampicillin	Amoxiclav	Azithromycin	Ceftriaxone	Cefotaxime	Ciprofloxacin	Clindamycin	Cefoxitin	Gentamycin	levofloxacin	High level gentamycin	Co-trimoxazole	Doxycycline	Vancomycin	Linezolid	Minocycline	Teicoplanin	Nitrofurantoin	Norfloxacin
S.aureus	484	21%	58%	28%	35%	31%	25%	58%	16%	70%	30%	-	70%	80%	83%	95%	85%	86%	78%	32%
CONS	141	-	52%	36%	42%	36%	25%	54%	12%	74%	-	-	-	78%	66%	97%	52%	-	70%	41%
Streptococci	95	80%	68%	28%	50%	47%	32%	37%	5%	-	-	-	-	59%	64%	88%	58%	-	-	-
Enterococci	42	40%	IR	IR	IR	IR	13%	IR	IR	IR	-	53%	IR	46%	76%	89%	51%	-	70%	-
Total	762	35%	58%	30%	44%	40%	21%	58%	14%	69%	29%	53%	70%	76%	67%	93%	80%	86%	75%	40%

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