



The Prevalence and Antimicrobial Susceptibility Pattern of Gram-Positive Pathogens: Three-Year Study at a Tertiary Care Hospital in Mumbai, India

Sweta Shah¹ Ritika Rampal² Pooja Thakkar¹ Sushima Poojary¹ Shweta Ladi¹

¹Department of Laboratory Medicine, Kokilaben Dhirubhai Ambani Hospital and Research Institute, Mumbai, Maharashtra, India

²Department of Medical Affairs, Wockhardt Limited, Mumbai, Maharashtra, India

Address for correspondence Sweta Shah, MD, Department of Laboratory Medicine, Kokilaben Dhirubhai Ambani Hospital and Research Institute, Four Bungalows, Andheri West, Mumbai, Maharashtra 400053, India
(e-mail: shahsweta.microbiology@gmail.com).

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Abstract

Introduction The growing resistance pattern of the gram-positive pathogens along with a steady increase in minimum inhibitory concentration of the currently available antibiotics have led to an increase in morbidity and mortality rates in India. This study aims to access the shifting antibiotic susceptibility paradigm of the gram-positive pathogens in various infections at a tertiary care center.

Methods This is a 3-year retrospective observational study which was performed from January 2016 to December 2018 at a tertiary care hospital in Mumbai. All clinically significant gram-positive cocci isolated from a variety of clinical specimens were studied for their prevalence and antimicrobial susceptibility.

Results Out of 4,428 gram-positive isolates, *Staphylococcus aureus* (35.3%) was the commonly encountered pathogen, followed by *Enterococcus* spp. (32.1%) and coagulase-negative *Staphylococcus*(CoNS) (25.7%). *S. aureus* was majorly isolated from skin and soft tissue infections (60.3%), followed by patients with respiratory tract infections (18.2%) and blood stream infections (13%). Among *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), prevalence increased from 29.5% in 2016 to 35.1% in 2018, with an overall prevalence of 33.6%. All *S. aureus* isolates were 100% sensitive toward vancomycin, linezolid, tigecycline, and teicoplanin. However, the CoNS isolates showed a higher resistance rate with reduced susceptibility toward linezolid and teicoplanin. High prevalence of resistance was observed across gram-positive isolates with commonly used antibiotics such as ciprofloxacin, levofloxacin, and erythromycin. While the prevalence of linezolid-resistant enterococcus (LRE) was 3.6%, vancomycin (VRE) and teicoplanin resistance among the enterococcus species was as high as 7.7% and 7.5%, respectively.

Conclusion Rising methicillin resistance among the *Staphylococcal* species (MRSA and MR-CoNS) along with reduced susceptibility toward currently available anti-MRSA agents is a matter of serious concern as it limits the therapeutic options for treating multidrug resistant (MDR) gram-positive infections.

Keywords

- ▶ gram-positive pathogens
- ▶ *Staphylococcus aureus*
- ▶ vancomycin-resistant enterococcus
- ▶ coagulase-negative staphylococci
- ▶ MRSA

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Introduction

Antimicrobial resistance (AMR) is a serious global threat that alone would be responsible for 10 million deaths by 2050 and over USD 100 trillion economic losses if effective interventions against resistance are not made.¹ India has been termed as the “AMR capital” of the world.² 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 diseases³ have led to a gradual increase in AMR over time. With 10.7 units being consumed per person, India is the highest antibiotic consumer in the world.⁴ Among the low- and middle-income countries, India alone accounted to an increase of 103% in antibiotic consumption from 2000 to 2015.⁵

Gram-positive pathogens play a critical role in health care-associated and community-acquired infections due to its rapid ability to acquire resistance against the currently used antibiotics. Prolonged usage of the available effective antibiotics against these pathogens coupled with their decreasing susceptibility rates have led to the emergence and spread of multidrug resistant (MDR) *Staphylococcus* and *Enterococcus* spp., particularly methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), and vancomycin-resistant *Enterococcus* (VRE). MRSA is considered a life-threatening nosocomial pathogen as it is associated with worse clinical outcomes⁶ and increased complications⁷ than patients with methicillin-sensitive strains (MSSA), leading to a higher mortality rate.⁸ According to a recent Indian Council of Medical Research–Antimicrobial Resistance Surveillance Network (ICMR–AMRSN) report, the total MRSA prevalence in India escalated from 32.9% in 2017 to 38.6% in 2018, with North India reporting the highest MRSA prevalence of 52.8%, followed by West India (48.1%).⁹

Currently, the resistance rate of MRSA has significantly increased to non- β lactam antibiotics like ciprofloxacin, levofloxacin, and clindamycin as compared with MSSA isolates. Similarly, increasing minimum inhibitory concentration (MIC) to vancomycin, which has been a traditional “standard” therapy for the treatment of MDR gram-positive pathogens, has led to an inception of vancomycin intermediate *S. aureus* (VISA), VRSA, and VRE isolates. Therefore, the growing resistance pattern, mortality rate, increased burden on health care and community settings along with a shrinking antibiotic pipeline, has led WHO list MRSA and VRSA as a “high”-priority pathogen against which novel and effective antibiotics are needed.¹⁰

The shifting antibiotic paradigms and the need to select targeted antibiotic regimen against resistant pathogens prompted us to investigate the prevalence and the susceptibility pattern of the gram-positive pathogens in various infections at our tertiary care center.

Methods

This is a 3-year retrospective analysis of the specimens received for aerobic culture and sensitivity from January 2016 to December 2018 at a tertiary care hospital in

Mumbai, India. The samples were processed using standard microbiological techniques. All the samples were plated on 5% sheep blood agar, MacConkey's agar. Chocolate agar was used for respiratory samples and body fluids like cerebrospinal fluid (CSF). Sterile body fluids and tissue were also inoculated in automated blood culture bottle for enrichment. After doing gram staining and manual tests like catalase and coagulase, identification and sensitivity were done using Vitek2 (BioMérieux, France). 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

During the period of 3 years, a total of 4,428 gram-positive isolates were analyzed, out of which *Staphylococcus aureus* (35.3%) was the most dominant pathogen, followed by *Enterococcus* spp. (32.1%), coagulase-negative *Staphylococcus* (CoNS) (25.7%), and *Streptococcus* spp. (6.7%).

Gram-positive Isolates

A. aureus was majorly isolated from skin and soft tissue infections (60.3%) while it also represented 18.2% and 13% in respiratory tract infections (RTI) and blood stream infections (BSI), respectively; 4.6% of *S. aureus* isolates were from urinary tract infections (UTI).

CoNS were the major pathogens isolated from BSI (42.6%). While 40.5% of the CoNS pathogen were responsible for skin and soft tissue infections, only 4% and 3% were observed in RTI and UTI, respectively. *Staphylococcus epidermidis* (53.4%) was the most common species of CoNS, followed by *Staphylococcus haemolyticus* (25.8%), *Staphylococcus hominis* (14%), and *Staphylococcus capitis* (6.7%). Among CoNS, the most dominant pathogen in skin and soft tissue infections (SSTIs) and BSI was *S. epidermidis* (56.2% and 50.2%). Apart from *S. epidermidis*, both *S. haemolyticus* (19.7%) and *S. hominis* (20%) were observed to contribute equally in the development of BSI. In UTI, *S. haemolyticus* (72.7%) was the most common species of CoNS.

The *Enterococcus* spp. were the dominant causative pathogen behind UTI (51.5%), followed by SSTIs (26%), while only 9% and 6.7% were responsible for BSI and RTI, respectively. Both *Enterococcus faecalis* (49%) and *Enterococcus faecium* (51%) were equally prevalent among the *Enterococcus* spp. **Table 1** depicts the total prevalence of *S. aureus*, CoNS, and *Enterococcus* spp.

Among *Streptococcus* species, *Streptococcus agalactiae* (37.1%) was the most common isolate, followed by *Streptococcus pyogenes* (35.4%) and *Streptococcus pneumoniae* (14%).

Antimicrobial Susceptibility Pattern Among the Gram-Positive Isolates

An antibiotic sensitivity pattern for ciprofloxacin, erythromycin, clindamycin, tetracycline, levofloxacin, gentamycin,

Table 1 Total prevalence of *S. aureus*, coagulase-negative *Staphylococcus* (CoNS), and *Enterococcus* spp.: 2016 to 2018

Infection	SSTIs	Respiratory tract infection	Blood stream infection	Urinary tract infection
Pathogen				
<i>Staphylococcus aureus</i> (n = 1,566)	944, 60.3%	285, 18.2%	204, 13%	Nil
Coagulase-negative <i>Staphylococcus</i> (CoNS) (n = 1,141)	486, 42.6%	Nil	462, 40.5%	Nil
<i>Enterococcus</i> spp. (n = 1,422)	370, 26%	Nil	128, 9%	733, 51.5%

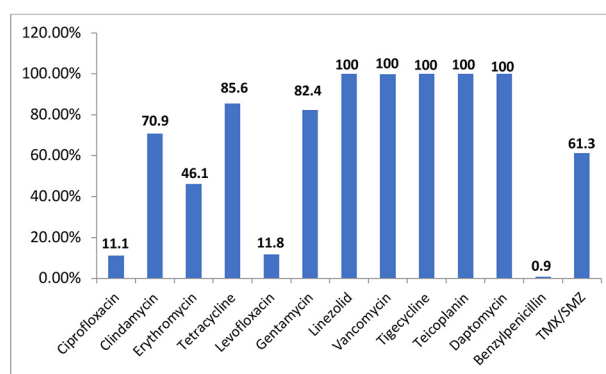
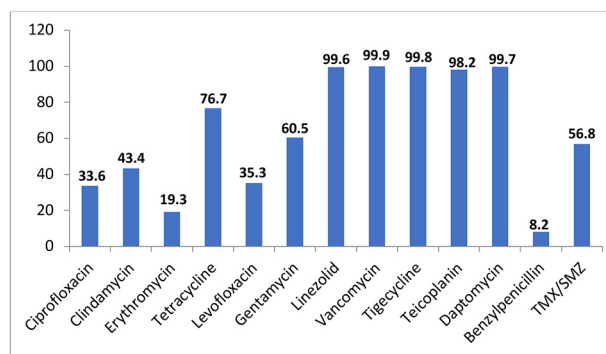
linezolid, vancomycin, daptomycin, tigecycline, teicoplanin, benzylpenicillin, and trimethoprim (TMX)/sulfamethoxazole (SMZ) were determined for *Staphylococcal* spp., *Enterococcus* spp., and *Streptococcus* spp.

The overall prevalence of MRSA over the period of 3 years was 33.6% (►Table 2). Moreover, the MRSA prevalence steadily increased from 29.5% in 2016 to 35.1% in 2018.

►Fig. 1 shows the total sensitivity pattern of *S. aureus* isolates against various tested antimicrobials. The *S. aureus* isolates showed lower sensitivity toward ciprofloxacin (11.1%), levofloxacin (11.8%), erythromycin (46.1%), benzylpenicillin (0.9%), TMX/SMZ (61.3%), clindamycin (70.9%), and gentamycin (82.4%). All the *S. aureus* isolates were found to be highly sensitive toward vancomycin, linezolid, tigecycline, and teicoplanin (100%). Less than 1% of the strains were found to be sensitive to benzyl penicillin.

The prevalence of methicillin resistance among CoNS was 78.7%. ►Fig. 2 shows the sensitivity pattern in the CoNS isolates against various antibiotics tested. In this study, vancomycin remained highly sensitive toward the CoNS species as no vancomycin-resistant (VR-CoNS) isolate was documented. There was only one isolate of CoNS which showed intermediate susceptibility (VI-CoNS). The total teicoplanin sensitivity was 98.2% wherein 8 isolates were resistant and 12 isolates were teicoplanin intermediate. Four isolates of linezolid-resistant CoNS (LR-CoNS) were also encountered. The total resistance prevalence was observed to be higher in *S. haemolyticus* than *S. epidermidis* and *S. hominis*. In *S. haemolyticus*, resistance to cefoxitin was 90.5%, ciprofloxacin 82.2%, levofloxacin 70.2%, erythromycin 91.9%, and clindamycin 67.3%, while in *S. epidermidis*, the resistance to cefoxitin was 82.2%, ciprofloxacin was 53.2%, levofloxacin 39.6%, erythromycin 77%, and clindamycin in 55.6% of the isolates.

Although the prevalence of *E. faecium* and *E. faecalis* is similar, a decrease in susceptibility among the antimicrobials is more dominant in *E. faecium* than in *E. faecalis* (►Fig. 3). *E. faecium* demonstrated increased resistance in ciprofloxacin (92.9%), levofloxacin (92.4%), erythromycin (87.8%), and gen-

**Fig. 1** Antibiotic sensitivity pattern among *S. aureus* isolates.**Fig. 2** Total sensitivity pattern of CoNS isolates against various tested antibiotics.

tamycin (76.1%), as compared with *E. faecalis* which showed a resistance rate of 65.1% in ciprofloxacin, 58.7% in levofloxacin, 66.6% in erythromycin, and 49.4% in gentamycin. Further, vancomycin (VRE) and teicoplanin resistance was as high as 14.6% and 14.4%, respectively, in *E. faecium*. Linezolid-resistant enterococcus (LRE) was also found to be 3.6%. Tigecycline was highly sensitive to *Enterococcus* spp. except one isolate in *E. faecium* group that was found to be resistant. ►Fig. 3 elucidates

Table 2 Prevalence of methicillin-resistant *S. aureus* (MRSA) among *S. aureus*: 2016 to 2018

Total <i>S. aureus</i> isolates	2016 (n = 386)		2017 (n = 783)		2018 (n = 393)		Total prevalence
	n	%	n	%	n	%	
MRSA	114	29.5	274	35	138	35.1	33.6%
MSSA	272	70.4	509	65	255	64.9	66.3%

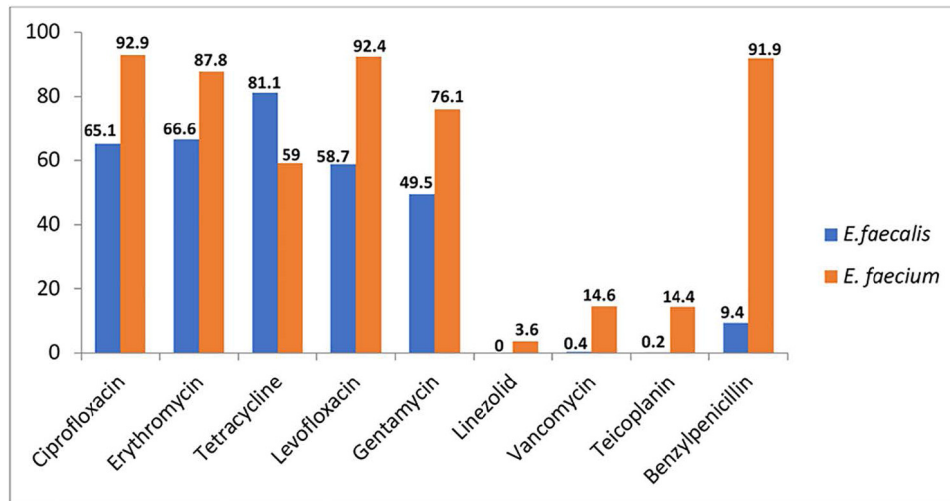


Fig. 3 Total antibiotic resistance pattern of *Enterococcus* species.

the resistance pattern against the tested antimicrobials in both *E. faecium* and *E. faecalis*.

The streptococcus species were highly sensitive (100%) to vancomycin, teicoplanin, tigecycline, and linezolid. While *Streptococcus* species were susceptible to benzylpenicillin (90.5%) and ciprofloxacin (98%), it exhibited reduced sensitivity toward erythromycin (69.1%), tetracycline (41.7%), and moxifloxacin (75.8%).

Discussion

India has the highest burden of MDR pathogens, with Centre for Disease Dynamics, Economics and Policy (CDDEP) predicting two million deaths in India due to AMR by 2050.¹⁴ WHO listed resistant gram-positive pathogens such as MRSA and VRSA as “high”-priority pathogens due to their ability to swiftly acquire resistance which eventually imposes an increased mortality burden.¹⁰ Among the *S. aureus*, the overall MRSA prevalence in our study was 33.6% which was lower than studies from various centers across India where the prevalence rates vary from 38.6% to 52%.^{15–17} Moreover, we observed that the MRSA prevalence rose from 29.5% in 2016 to 35% in 2018. This pattern was very similar to the recent reports which highlighted that over a decade, the MRSA prevalence has gradually escalated from 29% in 2009¹⁸ to 39% in 2018.¹⁵ This is a challenging scenario for clinicians as a recent study from south India reported that patients infected with MRSA have worse clinical outcomes in terms of longer hospital and intensive care unit (ICU) stay, greater number of complications, and increased mortality.⁷

Previous surveillance reports have shown *S. aureus* to be the major causative pathogen in SSTIs. Our study also reflected the same results with 60.3% of *S. aureus* being isolated from SSTIs caused by gram-positive organisms. Similar to previous studies, *S. aureus* isolates exhibited multidrug resistance as evident by their decreased sensitivity toward levofloxacin, ciprofloxacin, and erythromycin. An increase in the resistance rate against erythromycin is a huge concern for the clinicians as it mechanistically confers cross-

resistance to clindamycin which is a preferred oral antibiotic for the treatment of SSTIs.

CoNS are a major nosocomial pathogen wherein *S. epidermidis* and *S. haemolyticus* are the most common species involved. CoNS have been reported to exhibit higher levels of MDR as compared with *S. aureus*, which makes their treatment challenging. The same thing is highlighted in our study where the methicillin resistance among CoNS was 78.7%, which is almost double than the methicillin resistance in *S. aureus*. Out of all the CoNS spp., *S. haemolyticus* has been shown to display the highest level of resistant antimicrobial pattern.^{19,20} The ICMR-AMRSN study (2016–2018) has also shown the *S. haemolyticus* nonsusceptibility rate of 93% to penicillin and 81% to ciprofloxacin.²¹ This pattern was reflected in our study also, where the prevalence of resistance encountered in *S. haemolyticus* was much higher than *S. epidermidis*.

Vancomycin is the choice of drug to treat MRSA infections. A surge in studies reporting vancomycin minimum inhibitory concentration (MIC) creep and reduced vancomycin susceptibility suggests its therapeutic limitation in severe infections. A recent study by Amberpet et al reported the rate of hVISA to be as high as 12.4%²² while the prevalence of VRSA varies from 2 to 7.1% in India.^{23,24} However, it was heartening to note that in our study, the *Staphylococcal* species were highly sensitive toward vancomycin with no resistant isolate being documented in *S. aureus* and CoNS isolate in a span of 3 years.

While there was no teicoplanin resistance among the *S. aureus*, CoNS demonstrated an evident rise in teicoplanin resistance (8 resistant and 12 intermediate isolates), especially in *S. epidermidis* isolates. These results were in line with previous studies^{25–27} where teicoplanin-resistant CoNS species were reported. Though 98% of the CoNS isolates in our study are susceptible to glycopeptides, however, the rising methicillin and glycopeptide resistance among the CoNS species is a grave concern as it limits the currently available therapeutic options to treat MDR CoNS infections.

In India, the VRE strain first emerged in 1999.²⁸ Our study showed overall VRE prevalence of 7.7%, which was similar to

previous reports from India where the prevalence varied from 2 to 11.9%.^{21,29-31} *E. faecium* is associated with a higher antimicrobial resistant pattern and attributable mortality^{21,32} as compared with *E. faecalis*. A recent study by Purohit et al reported that among the VRE prevalence (22.8%), the prevalence of VR *E. faecium* was as high as 33.9% while that of *E. faecalis* was 2.4%.³³ These isolates were also found to be resistant to teicoplanin. The ICMR-AMRSN report also emphasizes on the rising concern of the overall glycopeptide resistance which has escalated from < 15% to as high as 30%.²¹ Although the rate of glycopeptide (vancomycin and teicoplanin) resistance was less in our study than the ICMR report, *E. faecium* displayed higher levels of glycopeptide resistance (14.5%) than *E. faecalis* (< 1%) and formed 98.1% of the total glycopeptide resistance in the *Enterococcus* spp.

Apart from vancomycin, linezolid is the most preferred drug which is approved for the treatment of resistant gram-positive infections. Linezolid was found to be highly sensitive against *S. aureus* and CoNS spp., wherein only four LR-CoNS were encountered. These observations reflect the Indian scenario with sporadic case studies which collectively report less than 1% of linezolid-resistant *S. aureus* isolates. The first report of linezolid resistant CoNS in India was reported in 2012,³⁴ after which there were many reports that document linezolid resistance in the CoNS species.³⁵⁻³⁷ Recently, Mittal et al observed that the linezolid-teicoplanin resistant *S. haemolyticus* strain that was identified was not due to prior exposure to these antibiotics but due to the pathogen's ability to horizontally transmit resistant genes across species, thereby resulting in highly virulent nosocomial pathogen.³⁶

Further, in India, the LRE isolates were first reported in 2014.³⁸ Our study showed high prevalence of LRE which was similar to a recent study from Mumbai which reported a high LRE prevalence of 4.1%.³¹ Linezolid is the preferred drug in the treatment of infections caused by MRSA and VRE. Therefore, overuse of linezolid in patients where vancomycin treatment has failed might have led to an antibiotic selection pressure and subsequent co-occurrence of resistant strains.

In conclusion, this study demonstrates 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. Therefore, this study provides an in-depth snapshot of the shifting susceptibility patterns of gram-positive pathogens, thereby facilitating the clinicians in choosing the appropriate antibiotic regimen which will help in reducing the selection pressure and subsequently controlling the rising AMR.

Authors' Contribution

R.R. S.S.: Data analysis, drafting of the manuscript, and critical revision. P.S., L.S.: Data generation and data entry. S.S., T.P.: Study concept and design, study supervision,

data interpretation, critical revision, and final approval of the manuscript.

Conflicts of Interest

Authors Sweta Shah, Pooja Thakkar, Sushima Poojary, and Shweta Ladi have nothing to declare. Author Ritika Rupal is an employee of Wockhardt Limited.

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