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Microbiological profile and antibiotic susceptibility pattern of Gram-positive isolates at a tertiary care hospital

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Abstract:

OBJECTIVES: Gram-positive infections such as those by *Staphylococcus aureus* have contributed to the disease burden by increasing the morbidity and mortality rates in India. This study aims to determine the prevalence and the antibiotic susceptibility pattern of Gram-positive pathogens at a tertiary care hospital, Mumbai, Maharashtra, India.

MATERIALS AND METHODS: This retrospective cross-sectional study was carried out from January, 2015 to December, 2017, at a tertiary care hospital in Mumbai, India. The clinical isolates were cultured, and identification was done using Vitek 2 culture system. The antibiotic susceptibility testing was done as per the Clinical Laboratory Standard Institute guidelines.

RESULTS: Out of 2132 (29%) Gram-positive isolates, *S. aureus* (49%) was the most common encountered pathogen, followed by *Enterococcus* spp. (24.5%) and coagulase-negative *Staphylococcus* (16%). Majority of the *S. aureus* were observed in patients with skin and soft-tissue infections (61.2%) followed by those suffering from respiratory (41%) and bloodstream infections (35%). Among the infections caused by *S. aureus*, the prevalence of methicillin resistance was 30%. While the MRSA isolates showed lower sensitivity toward co-trimoxazole (39%), clindamycin (30%), erythromycin (23%), and ciprofloxacin (10%), they showed higher susceptibility to linezolid (98%), vancomycin (98%), and teicoplanin (98%). All the isolates were found to be sensitive to daptomycin and tigecycline. While vancomycin-resistant enterococci (VRE) formed 7.5%, the linezolid-resistant enterococcus species was as high as 4.1%.

CONCLUSION: The study showed a high prevalence of MRSA and VRE, thereby emphasizing the increasing antimicrobial resistance pattern of the Gram-positive pathogens. Therefore, there is an urgent need for novel antimicrobial stewardship to restrict the ongoing resistance rate among the isolates.

Key words:

Antibiotic susceptibility pattern, Gram-positive pathogen, methicillin-resistant *Staphylococcus aureus*, prevalence, vancomycin-resistant enterococci

Introduction

The 1918 “Spanish flu” is one of the few examples of an infectious disease that has plagued humans and has prominently marked itself in history. More than a century now, infectious diseases are still the leading causes of disability, morbidity, and mortality worldwide.^[1,2] In India

too, high rate of infections is a public health threat which is not only associated with increased mortality rate, but also responsible for disability and prolonged hospital stay.^[3] The crude mortality rate in India is 417/100,000 persons.^[4] Over the years, the poor antimicrobial stewardship in India has led to an increase in multidrug-resistant (MDR) pathogens in both community and hospital settings.^[4,5]

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Gram-positive pathogens have played a crucial role in common infections such as sepsis, pneumonia, and urinary tract infections. *Staphylococcus aureus* is the major Gram-positive pathogen related to clinical infections ranging from bacteremia, skin and soft-tissue infections to device-related infections.^[6] Methicillin Resistant *S. aureus* (MRSA) is a life-threatening nosocomial pathogen whose resistance pattern is contributed by either multiple mutations or by horizontal gene transfer. Moreover, according to national guidelines on antimicrobial use, patients infected with MRSA have a high mortality rate^[7] which could be due to various factors such as late detection, manifestation of the underlying susceptibility to infections, and past antibiotic overuse. A recent multicentric study has reported the overall MRSA prevalence to be 37.3%;^[8] however, other studies have reported the prevalence to range from 20.2% to 80.4% depending on the geographical region.^[9-12]

Apart from MRSA, another potential threat is the emergence of MDR enterococci, especially vancomycin-resistant enterococci (VRE). *Enterococcus* species consisting of *Enterococcus faecalis* and *Enterococcus faecium* are the most common causative pathogens of urinary tract infections followed by bloodstream infections (BSIs).^[13] Indian studies have shown VRE prevalence to vary from 1% to 9%, which has raised concerns for clinicians to treat difficult enterococcal infections in patients.^[14-17] The shift in the antibiotic paradigm has, therefore, made it necessary to investigate the susceptibility pattern of Gram-positive pathogens in order to select an appropriate antibiotic regimen for patients with resistant pathogens. Therefore, the aim of this study is to investigate the prevalence and the antibiotic sensitivity pattern of Gram-positive pathogen in various infections in a tertiary care hospital in Mumbai.

Materials and Methods

This retrospective cross-sectional study was carried out from January, 2015 to December, 2017, at a tertiary care hospital in Mumbai, India. The patient samples were processed using standard laboratory techniques and were further cultured overnight on Columbia sheep blood agar (CBA) (BioMérieux, France) and on MacConkey's agar (Himedia, India). After colony characterization and Gram staining, the Gram-positive pathogens were processed and distinguished on the basis of catalase test, slide coagulase test, and tube coagulase test. Further, organism identification and antibiotic susceptibility test was done using the automated VITEK 2 compact system (BioMérieux, France). For streptococci identification, CBA plates were inoculated and Kirby-Bauer disc diffusion testing was done. The antibiotic susceptibility testing was reported as per the Clinical and Laboratory Standards Institute criteria.^[18,19]

Results

Prevalence of Gram-positive isolates

A total of 7393 bacterial isolates were analyzed from 2015 to 2017. Of the 2132 Gram-positive pool (29%), *S. aureus* (48.8%) formed the major encountered pathogen followed by *Enterococcus* spp. (24.6%) and coagulase-negative *Staphylococcus* (CoNS) (15.7%). Table 1 summarizes the Gram-positive prevalence from 2015 to 2017.

Majority of *S. aureus* (61.2%) were isolated from patients with skin and soft-tissue infections (SSTIs) followed by respiratory infections (41.1%) and BSIs (35.1%). Out of the total Gram-positive pathogens, 310 (14.5%) were MRSA isolates. Further, the MRSA isolates formed 29.7% of the total *S. aureus* identified, whereas the total methicillin-sensitive *S. aureus* (MSSA) prevalence was 67.3% from 2015 to 2017. Table 2 summarizes the MRSA prevalence from 2015 to 2017. Table 3 summarises system wise infection prevalence of gram positive infections.

Apart from *S. aureus*, the second most common infection in blood was caused by CoNS pathogens (39.7%). While 9.2% of the CoNS species were responsible for infection in patients with SSTIs, no role of these pathogens was observed in respiratory or urinary tract infections. Further, *Staphylococcus epidermidis* was the most common CoNS species in both BSI and SSTIs, representing 62.4% and 68% of the total CoNS species identified, respectively. *Staphylococcus haemolyticus* represented 12.4%. Other rare CoNS species identified included *Staphylococcus hominis* (6.5%), *Staphylococcus lugdunensis* (4.5%), *Staphylococcus warneri* (2%), *Staphylococcus xylosus* (1.5%), *Staphylococcus capitis* (1.2%),

Table 1: Total prevalence of Gram-positive pathogens in our hospital

Gram-positive pathogens	2015 (n=707)	2016 (n=711)	2017 (n=714)	Total prevalence (%)
<i>Staphylococcus aureus</i>	361	338	342	48.8
<i>Enterococcus</i> spp.	186	175	163	24.6
CoNS	81	123	131	15.7
<i>Streptococcus</i> spp.	76	74	78	10.7

CoNS=Coagulase-negative *Staphylococcus*

Table 2: Total methicillin-resistant *staphylococcus aureus* and methicillin-susceptible *staphylococcus aureus* prevalence in Gram-positive isolates from 2015 to 2017

Total Gram-positive isolates	2015 (n=707)	2016 (n=711)	2017 (n=714)	Total prevalence (%)
Total <i>S. aureus</i>	361	338	342	28.8
MRSA	124	81	105	29.7
MSSA	223	255	225	67.3

S. aureus=*Staphylococcus aureus*, MSSA=Methicillin-susceptible *S. aureus*, MRSA=Methicillin-resistant *S. aureus*

Table 3: Total prevalence of *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Streptococcus* spp., and *Enterococcus* spp.

Pathogen	Infection	2015	2016	2017	Total prevalence (%)
<i>S. aureus</i>	BSI (n=435)	83	44	26	153 (35.10)
	SSTIs (n=1355)	262	268	300	830 (61.2)
	RTI (n=68)	3	16	9	28 (41.1)
CoNS	BSI (n=435)	51	64	58	173 (39.7)
	SSTIs (n=1355)	21	43	61	125 (9.2)
<i>Streptococcus</i> spp.	BSI (n=435)	26	20	10	56 (12.8)
	SSTIs (n=1355)	35	50	45	130 (9.6)
	RTI (n=68)	13	3	16	32 (47)
<i>Enterococcus</i> spp.	BSI (n=435)	14	22	15	51 (11.7)
	SSTIs (n=1355)	93	88	86	267 (19.7)
	UTI (n=175)	69	55	43	167 (95.4)

CoNS=Coagulase-negative *Staphylococcus*, BSI=Bloodstream infection, SSTIs=Skin and soft-tissue infections, RTI=Respiratory tract infection, *S. aureus*=*Staphylococcus aureus*

Staphylococcus urealyticus (<1%), *Staphylococcus sciuri* (<1%), and *Staphylococcus saprophyticus* (<1%).

Streptococcus spp. were the most dominant pathogen isolated in patients with respiratory tract infection (RTI) (47%), whereas in BSI and SSTIs, it was 12.9% and 9.6%, respectively. While *Streptococcus pneumoniae* (37.5%) was the most common *Streptococcus* spp. in RTI patients, patients with BSI and SSTIs also had *Streptococcus pyogenes* (12.2%), *Streptococcus agalactiae* (10%), *Streptococcus mitis* (1.8%), *Streptococcus dys equisimilis* (<1%), *Streptococcus anginosus* (<1%), and *Streptococcus pasteurianus* (<1%).

On the other hand, the total prevalence of *Enterococcus faecalis* was 30.8% while that of *E. faecium* was 8.4% of the total isolated *Enterococcus* species. In addition, *Enterococcus* spp. were the major causating pathogen behind urinary tract infections (95.4%), whereas only 19.7% and 11.7% were responsible for SSTIs and BSI, respectively.

Antibiotic sensitivity pattern in *Staphylococcus aureus* and *Enterococcus* spp.

An antibiotic sensitivity pattern for ciprofloxacin, erythromycin, clindamycin, co-trimoxazole, linezolid, vancomycin, daptomycin, tigecycline, and teicoplanin was determined. MRSA isolates showed lower sensitivity toward ciprofloxacin (10.2%), erythromycin (24%), clindamycin (30.2%), and co-trimoxazole (39.2%) as compared to MSSA isolates [Figure 1].

Further, MRSA was shown to be highly susceptible toward linezolid (98%), vancomycin (97.7%), and teicoplanin (98.3%). All MRSA and MSSA isolates tested were found to be sensitive to daptomycin and tigecycline (100%). Moreover, none of the isolates were found to be sensitive to benzyl penicillin.

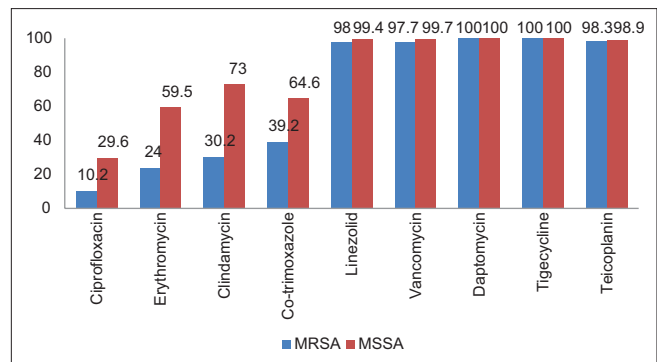


Figure 1: Antibiotic sensitivity pattern in methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* isolates

We also investigated the sensitivity pattern in *Enterococcus* spp., where they were highly susceptible to linezolid (96%), vancomycin (92%), and teicoplanin (93.3%) while being resistant to erythromycin and ciprofloxacin. Further, the prevalence of VRE was found to be 7.5%. Figure 2 shows the sensitivity pattern for *Enterococcus* spp.

Discussion

MDR bacterial infections have today become the biggest threat in the world due to the irrational use of antimicrobial agents. The European Prevalence of Infection in Intensive Care 2 study showed that, out of 47% of the Gram-positive isolates, 20% were *S. aureus* which was further associated with longer ICU stay.^[20] In India too, Gram-positive infections, particularly MRSA, have been reported to increase exponentially from 29% in 2009 to 47% in 2014.^[21] The overall MRSA prevalence in our study was 29.7%, which was lower than the recently reported prevalence of 37.3% by ICMR-AMRSN^[8] and 40% as reported by INSAR study.^[22] Furthermore, the prevalence varies among different centers across India ranging from 20.2% in Gujarat to 80.4% in Odisha. This variation could be attributed to the fact that different centers have different patient profiles and local antibiotic regimens that could influence the overall prevalence.

The ICMR-AMRSN study reported *S. aureus* to be the most frequently isolated organism in SSTI patients (73.7%).^[8,23] Similarly, our study also showed similar results with *S. aureus* isolates to be 61.2% in SSTI patients. Further, we also report a high prevalence of *S. aureus* (35.1%) in BSI patients. Recently, apart from SSTIs, *S. aureus* has also emerged to be a critical pathogen in BSIs.^[24] This is further supported by a study conducted by Tak *et al.* where the crude mortality rate in patients with MRSA BSI was 31%.^[25]

In 2010, India was the highest antibiotic consumer, with 10.7 units being consumed per person. Among BRICS countries, India registered an increase of 23% in the retail sale volume.^[26] Therefore, easy availability

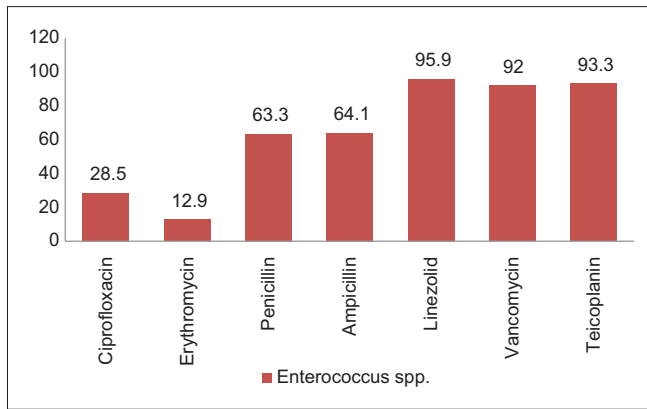


Figure 2: Antibiotic sensitivity pattern of *Enterococcus* species

and inappropriate antibiotic abuse has resulted in the development of antimicrobial resistance, which is difficult to treat and has become a national health disaster, today. In the present study, the MRSA isolates showed higher resistance toward ciprofloxacin, erythromycin, co-trimoxazole, and clindamycin as compared to MSSA isolates. Similar observations were also seen in previous multicentric studies.^[8,22] These findings justify the increased mortality rates^[27] among patients infected with MRSA infections due to the ability of the pathogen to display varied resistance patterns toward various antibiotics. Moreover, both daptomycin and tigecycline remained the most effective antibiotics against *S. aureus* because there was no resistant isolate observed. This effect could be attributed to two reasons, with the higher cost of the drugs to be the primary reason due to which these drugs are not used very commonly. Second, both tigecycline and daptomycin are not the antibiotic of choice for treating BSIs and lung infections, both of which are the most common sources of infection in our hospital.

Vancomycin has been regarded as the first-line drug for the treatment of MRSA and MDR *Enterococcus* spp. Vancomycin was highly effective against MRSA isolates as we documented <1% of vancomycin-intermediate strains in them. However, a meta-analysis has shown vancomycin-intermediate *S. aureus* (VISA) and hetero-VISA prevalence to be 15.09% and 12.41%, respectively.^[28] While we observed only three (<1%) vancomycin-resistant isolates, a study by Singh *et al.* has reported 13% of VRSA isolates.^[29]

The first vancomycin-resistant *E. faecalis* and *E. faecium* was reported in 1988 in England.^[30] Subsequently, the first case of VRE was reported from New Delhi in 1999^[31] because of unscrupulous use of vancomycin for *Enterococcus* treatment. In India, the prevalence of VRE infection is increasing with every passing year. In 2003, the VRE prevalence was 1%^[14] which in 2013 has risen up to 8.7%.^[17] In the present study too, a total of 39 (7.5%) VRE isolates were obtained. Moreover, eight

isolates (1.5%) of *E. gallinarum* and *E. casseliflavus*, which have inherent resistance to vancomycin, were also observed. However, out of the eight isolates, one isolate was vancomycin, linezolid, and teicoplanin resistant.

Linezolid is the only oral antibiotic available as compared to its other counterparts for the treatment of resistant staphylococcus species. Furthermore, linezolid is used as a reserved drug for the treatment of difficult extremely drug-resistant tuberculosis and extensively drug-resistant tuberculosis.^[32] However, linezolid overuse in the management of Gram-positive pathogens, which are otherwise easy to treat, has caused an emergence of linezolid-resistant strains. Globally, there are not much reports about the prevalence of linezolid-resistant *S. aureus* (<1%). A study from Rajasthan showed 12% of the linezolid-resistant *S. aureus*;^[33] however, we show that 98% of the MRSA isolates were sensitive to linezolid, making it a potent drug for activity against resistant staphylococcus organisms. Further, in case of *Enterococcus*, linezolid is a successful therapeutic option if vancomycin treatment fails. Kumar *et al.* first reported linezolid-resistant enterococci (LRE) in 2014.^[34] However, there are no further reports which document the prevalence of LRE, thereby making this the first study to show a high LRE prevalence of 4.1%. The high prevalence of LRE in our hospital may have resulted due to the increased MRSA prevalence which further corresponds to an increase in the use of linezolid empirically. Due to this, the antimicrobial stewardship program in the hospital has taken measures and has tightened the program in order to keep a check on the increasing resistant strains.

Conclusion

This study demonstrates MRSA and VRE as a growing health problem in India. While, vancomycin and linezolid remain the preferred choice for MRSA and multiresistant *Enterococcus* treatment, the rise in both vancomycin- and linezolid-resistant strains has caused treatment dilemmas for clinicians. Therefore, the data generated provide in-depth knowledge of the changing antibiotic regimen against the difficult pathogens, thereby helping the physicians to make the right antibiotic choice at the start of the treatment. Further, there is an urgent need for new research molecules to combat the difficult MDR pathogens.

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Conflicts of interest

There are no conflicts of interest.

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