PREVALENCE AND ANTIBIOTIC SENSITIVITY PATTERN OF STAPHYLOCOCCUS AUREUS FROM ALL CLINICAL SAMPLES WITH EMPHASIS ON MRSA IN A TERTIARY CARE HOSPITAL

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ABSTRACT

BACKGROUND

S. aureus is a versatile pathogen that causes human diseases ranging from mild infections to life-threatening sepsis. Therefore, to prevent *S. aureus* infections, effective infection control practices and antibiotic policies should be formulated. The present study was conducted to study the prevalence and antibiotic sensitivity pattern of *Staphylococcus aureus* (*S. aureus*) isolated from various clinical samples in a tertiary care hospital.

MATERIAL AND METHODS

A descriptive study was conducted in Microbiology Department. In the present study, out of 1194 clinical isolates, 206 *S. aureus* isolates were identified and processed using standard microbiology procedures. The antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method as per CLSI guidelines.

RESULTS

The prevalence of *S. aureus* was reported as 206 (17.25%) and MRSA was reported as 99 (48.0%). High resistance was seen in case of Penicillin whereas resistance less than 50% was reported in Methicillin, Ciprofloxacin, Gentamicin, Erythromycin; and Clindamycin. Vancomycin, Teicoplanin and Linezolid were found to be 100% sensitive.

CONCLUSION

S. aureus is a common cause of skin and soft tissue infections in hospitals. There should be a regular surveillance on the changing patterns of antibiotic sensitivity pattern of MRSA and antibiotic policy should be formulated to reduce the increasing levels of infections in the hospitals.

KEYWORDS

Staphylococcus aureus, MRSA, Antibiotic Sensitivity Pattern.

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BACKGROUND

Staphylococcus aureus is recognised as a causative agent of nosocomial and community-acquired infections in almost every region of the world.^[1] Staphylococci are the leading pathogens which are known to cause skin and soft tissue infections (SSTIs).^[2] Antibiotic resistance in *S. aureus* has become an increasing problem among outpatients, hospitalised patients and even in health care persons.^[3]

S. aureus are Gram-positive cocci approximately 1 µm in diameter and are arranged in grape like clusters. It expresses many potential virulence factors which include production and secretion of toxins and enzymes; alpha, beta, gamma and delta toxins act on the cell membrane of host and mediates cell destruction. Some exotoxins like exfoliative, toxic shock syndrome toxin (TSST) and some enterotoxins are also produced.

Clumping factor, coagulase and hyaluronidase enhances invasion and its survival in the tissues.^[4]

In humans, *Staphylococcus aureus* causes a variety of suppurative (pus-forming) infections. It causes skin and soft tissue infections such as furuncles (boils), folliculitis, abscess (particularly breast abscess), wound infections; infections such as pneumonia, meningitis and urinary tract infections; musculoskeletal infections such as osteomyelitis, arthritis and endovascular infections such as bacteraemia, septicemia, endocarditis. *S. aureus* also causes hospital-acquired (nosocomial) infections that are associated with medical indwelling devices and surgical wounds. It also releases enterotoxins that causes food poisoning and also cause toxic shock syndrome by releasing superantigens into the blood stream.^[4]

The emergence of resistance to antimicrobial agents among staphylococci was increasing. Then introduction of penicillin in 1941 tackled the problem. In 1961, Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported and since then, it has become a major nosocomial pathogen.^[5,6] Methicillin resistance in *S. aureus* is mediated by production of altered penicillin binding protein 2a (PBP-2a) which is encoded by a gene called *mecA*.^[7]

The glycopeptide antibiotic vancomycin was first released in 1958, as the drug of choice for MRSA. Subsequently,

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vancomycin has been used for treatment of serious infections caused by MRSA for many years. Initial reports of reduced susceptibility to vancomycin in *S. aureus* from Japan in 1997 from various clinical samples generated significant concern in the medical community.^[8] There is renewed interest in the use of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics due to increase in frequency of Staphylococcal infections and change in patterns of antimicrobial resistance.^[9]

The aim of the present study was to determine the antibiotic sensitivity pattern of strains of *S. aureus* isolated from various clinical samples.

MATERIALS AND METHODS

A descriptive study was conducted in Department of Microbiology, AIMSR, Bathinda, Punjab. All clinical samples like pus, sputum, urine, blood, genital specimens (high vaginal swab, semen), body fluids (cerebrospinal fluid, pleural fluid, synovial fluid, etc.), devices (endotracheal tube, tracheostomy tube, drain, etc.) were included in the study.

The samples were collected under aseptic conditions and were further processed and identified using standard microbiological procedures. The specimens were cultured on the Nutrient Agar (NA), Blood Agar (BA) and MacConkey Agar (MA), incubated overnight at 37°C in incubator.^[10]

Gram staining was performed on colonies that were suggestive of *S. aureus* by colony morphology followed by microscopy. The samples showing Gram-positive cocci in clusters were processed further for confirmation by biochemical reactions whether the organisms were *S. aureus*. Tests like catalase, slide coagulase and tube coagulase were carried as confirmatory tests for *S. aureus*.^[10]

The antibiotic susceptibility testing was done on Mueller-Hinton Agar by Kirby-Bauer disk diffusion method. A 0.5 McFarland turbidity inoculum was prepared by sub-culturing 4-5 colonies in nutrient broth and incubated at 37°C for 4 hrs. in incubator. Lawn culture was made on MH agar and antibiotic sensitivity of isolates was checked using penicillin (10 µg), cefoxitin (30 µg), cephalixin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), amoxicillin + clavulanic acid (20/10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), azithromycin (15 µg), erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg), teicoplanin (30 µg) and linezolid (15 µg) as per CLSI guidelines.^[11] The culture plates were then incubated at 37°C for 24 hrs. in incubator.

RESULTS

In the present study which was carried out in microbiology lab, AIMSR, out of 1194 clinical isolates, 206 were *S. aureus* and the prevalence of *S. aureus* from all clinical samples being

17.25%. Out of the 206, MRSA were 99 (48%) and MSSA were 107 (51.9%) as shown in Table I.

Isolate	Prevalence
MRSA	48% (99)
MSSA	52% (107)

Table I. Prevalence of MRSA and MSSA

Out of 206 *S. aureus* isolates, 101(49.0%) were from OPD, 83 (40.3%) were from IPD and 22 (10.7%) were from ICU as shown in Table II.

Department	% Isolation (n=206)	% age of MRSA in each Dept.	% of MSSA in each Dept.
OPD	49.0% (101)	48.5% (49)	51.5% (52)
IPD	40.3% (83)	48.2% (40)	51.8% (43)
ICU	10.7% (22)	45.5% (10)	54.5% (12)

Table II. Prevalence of *S. aureus* from Various Departments

Out of 206 *S. aureus* isolates, the percentage isolation from urine was 33.4%, from pus 45.1%, in blood 4.8%, sputum 2.4%, body fluids 3.3% and from various devices 10.6% as shown in Table III. Maximum number of *S. aureus* isolates were from pus samples.

Specimens	Percentage of Age Isolation of <i>S. aureus</i> (n=206)	Percentage Isolation of MRSA in each Specimen	Percentage Isolation of MSSA in Each Specimen
Urine	33.4% (69)	50.7% (35)	49.3% (34)
Pus	45.1% (93)	47.4% (44)	52.6% (49)
Blood	4.8% (10)	40% (4)	60% (6)
Sputum	2.4% (5)	100% (5)	0
Body fluid	3.3% (7)	28.5% (2)	71.5% (5)
Devices	10.6% (22)	41% (9)	59% (13)

Table III. Prevalence of *S. aureus* from Various Clinical Samples

The antibiotic resistance pattern of all 206 *S. aureus* isolates towards following antibiotics was: Penicillin 178 (86.4%), Cefoxitin 99 (48.05%), Cephalixin 99 (48.05%), Cefuroxime 99 (48.05%), Amoxicillin + Clavulanic Acid 99 (48.05%), Gentamicin 75 (36.40%), Ciprofloxacin 83 (40.29%), Levofloxacin 27 (13.10%), Azithromycin 29 (14.07%), Erythromycin 67 (32.52%), Clindamycin 36 (17.47%) and there were no strains which were found resistant to Vancomycin, Teicoplanin and Linezolid as shown in Table IV.

Antibiotic	Resistance	Sensitive	Susceptibility pattern of MRSA strains	Susceptibility pattern of MSSA strains
Penicillin	86.4% (178)	213.6% (28)	0	26.2% (28)
Cefoxitin	48.1% (99)	51.9% (107)	0	100%
Cephalixin	48.1% (99)	51.9% (107)	0	100%
Cefuroxime	48.1% (99)	51.9% (107)	0	100%
Cefotaxime	48.1% (99)	51.9% (107)	0	100%
Amoxicillin+ Clavulanic Acid	48.1% (99)	51.9% (107)	0	100%
Gentamicin	36.4% (75)	63.6% (131)	51.5% (51)	74.8% (80)
Ciprofloxacin	40.3% (83)	59.7% (123)	27.3% (27)	89.7% (96)

Azithromycin	14.1% (29)	15.9% (177)	82.8% (82)	88.8% (95)
Erythromycin	32.5% (67)	67.5% (139)	40.4% (40)	92.5% (99)
Clindamycin	17.5% (36)	82.5% (170)	77.8% (77)	86.9% (93)
Vancomycin	0	100%	100%	100%
Teicoplanin	0	100%	100%	100%
Linezolid	0	100%	100%	100%

Table IV. Prevalence of Antibiotic Susceptibility Pattern

DISCUSSION

Among the Gram-positive pathogens, *S. aureus* is a common cause of skin and soft tissue infection in community and as well as hospitalised patients. The prevalence of *S. aureus* in the present study was 17.25% from all clinical samples. Other studies reported prevalence as 6%, 24.5%, 42.73% and 48.14%.^[12,13,14,15]

In the present study, the prevalence of *S. aureus* isolates in OPD, IPD and ICU was 49.0%, 40.3% and 10.7% respectively. The prevalence of MRSA was 48.5% in OPD, 48.2% in IPD and 45.5% in ICU. In a study conducted by INSAR group, India, the prevalence of MRSA was 28.4%, 42.3% and 43.6% from OPD, IPD and ICU respectively in 2008 and 27%, 49% and 47% from OPD, IPU and ICU respectively in 2009.^[16] The increased rate of isolation from OPD could be because our institute is a tertiary care hospital where patients are referred after prior treatment failure and over-the-counter empirical treatment.

The prevalence of *S. aureus* from various clinical specimens in this study were 33.4% from urine, 45.1% from pus, 4.8% in blood, 2.4% in sputum, 3.3% in body fluids and 10.6% from devices. In another two studies, the prevalence of pus was maximum i.e. 39.48%^[17] and 68.7%.^[15] Thus, our study is in accordance to most other studies where prevalence of *S. aureus* is maximum from pus sample.

The prevalence of MRSA in this study from various specimens was 50.7% in urine, 47.4% in pus, 40% in blood, 100% in sputum, 28.5% in body fluids and 41% in devices. In a study conducted INSAR group, India the prevalence was 41%, 36%, 44% and 34% in urine, pus, blood and body fluids respectively in 2008. Whereas in 2009 the prevalence showed a slight change and the results found were 52%, 40%, 48%, 54% in urine, pus, blood and body fluids respectively.^[16]

Cefoxitin is the indicator drug for methicillin-resistant *S. aureus* by disc diffusion method as per the CLSI guidelines.^[11] All isolates that are Cefoxitin resistant are also resistant to all Penicillins, Cephalosporins, Beta lactam group + Beta lactamases inhibitor combination and carbapenems. Hence, overall prevalence of MRSA in this study was found to be 48.1%. Our study is in accordance with the study conducted in Jaipur by Gupta et al in 2015 which showed MRSA prevalence of 40%.^[18] Similarly researches conducted in Benin, Bengaluru South East Nigeria showed a prevalence similar to the ongoing study.^[12,15,13]

A study was conducted by Indian network for Surveillance of Antimicrobial Resistance (INSAR) group, India in different leading hospitals of India to study the prevalence and susceptibility pattern of MRSA. The overall prevalence observed was 42% in 2008 and 40% in 2009 for MRSA.^[16]

The variation may be due to difference in the methods of detection, infection control practices and usage of antibiotics

which vary from hospital to hospital. This could be the reason of non-uniform epidemiology of MRSA in India.^[18] Very low prevalence of 5.26% and 6% were seen in studies from Peshawar in 2014^[19] and Benin in 2011^[12] respectively.

In our study, 178 (86.4%) isolates out of 206 were resistant to Penicillin. A similar prevalence rate of resistance against Penicillin was observed in studies from South East Nigeria^[13] and Jaipur.^[17] Dhanalakshmi TA et al showed 85.6% *S. aureus* isolates were resistant to Penicillin.^[20] In our study, all the MRSA strains were reported resistant to Penicillin whereas, 73.8% MSSA strains were resistant and 26.2% were sensitive. All the *S. aureus* isolates in the present study were sensitive to Vancomycin, Teicoplanin and Linezolid. This finding is similar to the studies conducted by Arora et al in 2010^[21] and Sharma M et al in 2013^[17] where Vancomycin and Linezolid were found to be 100% sensitive. Our study showed 36.4% resistance towards Gentamicin which was similar to a study conducted in South East Nigeria in 2016 by Chijioke A Nsofor et al where Gentamicin resistance was 31.7%.^[13] Two other similar studies conducted by Dhanalakshmi TA et al^[20] and Sina H et al^[12] showed 33.6% and 42.27% resistance respectively towards Gentamicin. On the other hand, in present study, the resistance pattern for Ciprofloxacin was found to be 40.3%. Similar results were seen in studies from South East Nigeria^[13] and Jaipur,^[17] where the resistance was 38.5% and 41.53% respectively.

In case of macrolides that include Azithromycin and Erythromycin, the resistance in our study was found to be considerably low. Azithromycin resistance was 14.1% in the present study whereas in other studies the prevalence was 32.8%^[20] and 45.12%^[17] that is comparatively higher than our study. In case of Erythromycin, the resistance in our study was 32.5%. A study from Benin has reported resistance similar to our study i.e. 35.77%.^[12] Some other researches have reported high resistance against Erythromycin.^[13,17,20,21] In the ongoing study, 59.6% MRSA isolates and 7.5% MSSA isolates showed resistance against erythromycin. A study carried out by INSAR group, India reported higher resistance in MRSA and MSSA isolates.^[16]

In the present study, the prevalence of resistance against Clindamycin was 17.5% among *S. aureus* isolates. Two other studies showed a similar resistance pattern, Dhanalakshmi TA et al^[20] reported 14% resistance and Sharma M et al^[17] reported 9.72%. The prevalence of resistance in our study was 22.2% in MRSA isolates and 13.1% in MSSA. In a study conducted by INSAR group, India, the prevalence of resistance was reported as 46.6% and 14.7% in MRSA and MSSA isolates respectively.^[16]

CONCLUSION

In conclusion, adequate preventive and control measures should be taken to reduce MRSA infections by formulating antibiotic policies and effective infection control practices.

The progressive increase in positivity of MRSA in *S. aureus* isolates calls for routine testing of methicillin resistance by using Cefoxitin disc diffusion method or Minimum Inhibitory Concentration (MIC) detection method. Vancomycin, Teicoplanin and Linezolid are still the drugs of choice for MRSA infections. Thus, there is a need of in vitro susceptibility testing for all the isolated clinical samples for selection of drugs that are appropriate for treatment of the infection, thereby limiting the overdose of powerful antibiotics which might help in changing the trends of antibiotic susceptibility pattern.

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