Isolation And Antimicrobial Susceptibility Pattern Of Staphylococcus Aureus.

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Abstract: Introduction- Staphylococcus aureus is one of the principal human pathogens that colonizes healthy individuals as well as causes severe infections in hospitalized patients. They have differential ability to spread and cause outbreaks in hospitals. The study was done to isolate S.aureus from various clinical samples and to know their antibiogram. Methodology- The study included a total of 312 Staphylococcus aureus strains isolated from various clinical specimens. The specimens received were processed further for identification by standard microbiological procedures like direct microscopic examination, culture and various standard identification methods. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per CLSI guidelines. Result- A total of 312 isolates of S. aureus were obtained from various clinical samples. Male to female ratio was 1.6:1. Infection rate was more in age group 46-60 years. Staphylococcus aureus isolates were more commonly obtained from surgery department (38.78%) followed by orthopedics. All the strains were 100% sensitive to Vancomycin, Netilmicin, Linezolid and Teicoplanin. Higher resistance was noted against Penicillin(94.87%), Trimethoprim/Sulfamethoxazole(72.44%).Lower resistance was noted against Nitrofurantoin(13.33%). Conclusion- The determination of the anti-microbial susceptibility is also crucial for an optimal therapy, for epidemiological purposes and for infection control measures. [Kulkarni VL NJIRM 2016; 7(4):33-38]

Key word: Antimicrobial Susceptibility, Staphylococcus Aureus

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Introduction: Staphylococcus aureus, a Gram positive bacterium from Micrococcaceae family has been recognized as an important cause of human disease for more than 100 years.[1] It is one of the principal human pathogens that colonizes healthy individuals as well as causes severe infections in hospitalized patients, especially in high risk areas like ICUs, burn, surgical and skin wards. They have differential ability to spread and cause outbreaks in hospitals.[2]

In the preantibiotic era, mortality from Staphylococcus aureus disease was high and introduction of penicillin had a dramatic impact which was short lived due to the emergence of penicillinase producing Staphylococcus aureus. Later resistance was spread to semisynthetic penicillins such as Methicillin and Oxacillin, macrolides, tetracyclines and aminoglycosides.[3] This has made the staphylococcal disease a global challenge.

Methicillin resistant Staphylococcus aureus (MRSA) has been reported with increasing frequency worldwide.[4] Life threatening sepsis, endocarditis, postoperative wound infections, skin & soft tissue infections and osteomyelitis caused by MRSA have been reported since 1961 from various parts of the world.[5] These strains are not only resistant to routinely used antibiotics but also act as a reservoir for multidrug-resistance development.

The determination of the anti-microbial susceptibility is also crucial for an optimal therapy, for epidemiological purposes and for infection control measures.[6] The present study was undertaken to isolate staphylococcus aureus from various clinical samples and to know the antibiogram of the isolates to the commonly used antibiotics.

AIMS & OBJECTIVES:

1. To study the isolation pattern of staphylococcus aureus from various clinical samples
2. To know the antibiogram of clinical isolates of Staphylococcus aureus to commonly used antibiotics.

Methods: The present study was carried out in the department of Microbiology at a tertiary care hospital. The study included a total of 312 non-duplicate Staphylococcus aureus strains which were isolated from various clinical specimens.

Study design: Prospective study

Duration of study: Oct 2012 to Sep 2014.

Ethical clearance from institutional ethical committee was obtained.

Inclusion criteria:

1. Specimen from both indoor as well as outdoor patients of all age groups and both sexes

2. Staphylococcus aureus isolated from various clinical specimen.

Exclusion criteria: Species other than Staphylococcus aureus were excluded

History taking of patient: A history was taken with reference to name, age and sex. Clinical history was recorded on a predesigned proforma.

Collection and transport of specimens:

The specimens were collected using strict aseptic precautions and immediately transported to the laboratory.

Processing of specimens: All the specimens received were processed further for identification by standard microbiological procedures like direct microscopic examination, culture and various standard identification methods.[7]

1. **Direct microscopic examination:**Smears were prepared from specimens and Gram staining was done. It was examined under the oil immersion lens to see the presence of bacteria and to study their morphology.

In case of sputum samples smears were examined first under the low power to determine adequacy of specimen.
For non purulent fluid, the specimen was centrifuged and smear made from well mixed sediment.

Wet mount of urine was done to find out whether pus cells, red blood cells and epithelial cells are present.

2. **Culture:** The samples were inoculated onto nutrient agar, blood agar and MacConkey agar plates. All plates were incubated aerobically at 37˚C and observed for growth after 18-24 hours.

Blood culture- Blood inoculated into brain heart infusion broth was incubated at 37˚C. The bottles were examined for turbidity and subcultures were made at regular intervals on Blood agar and MacConkey agar and any growth present was further processed for identification.

3. **Identification:**[8] Isolates of Staphylococcus aureus were identified on the basis of colony characteristics on nutrient agar and blood agar.

Smears were prepared from the colonies and Gram stain was done. Cluster forming Gram positive cocci were further confirmed as S. aureus by catalase, coagulase test, fermentation of mannitol and battery of biochemical tests.

Antimicrobial susceptibility testing:
Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Detection of Methicillin resistance was done by Cefoxitin disc diffusion technique, which was performed according to CLSI guidelines.

Staphylococcus aureus ATCC 25923 was used as a control strain.

All the antibiotic discs were procured commercially from Hi-Media Laboratories Pvt. Ltd, Mumbai. The diameter of zone of inhibition
was measured and interpreted according to CLSI guidelines.[9]

Statistical Methods: The data obtained was analyzed by applying appropriate statistics wherever needed.

RESULTS: During study period, a total of 312 isolates of S. aureus were obtained from various clinical samples

Table-1: Sex wise distribution of patients with S. aureus infection:

Sex	No. of Cases	Percentage (%)
Male	192	61.54
Female	120	38.46
Total	312	100

In the present study, the number of males (61.54%) was more than females (38.46%). Male to female ratio was 1.6:1. (table-1)

Table-2: Age wise distribution of patients with S. aureus infection:

Age Group (in years)	No. of Cases	Percentage (%)
< 15 years	25	8.01
16-30	82	26.28
31-45	68	21.79
46-60	108	34.62
>60	29	9.30
Total	312	100

Infection rate was more in age group 46-60 years. Minimum infection rate was found in age group of below 15 years.(table-2)

Table-3: Distribution of Indoor and OPD patients with S. aureus infection:

Type	No. of cases	Percentage (%)
Indoor patients	211	67.63
Outdoor patients	101	32.37
Total	312	100

(P value < 0.05; Two proportion Z test)

Majority of S. aureus isolates were obtained from patients admitted in various wards than the patients who were attending outpatient department.This difference was found to be statistically significant with p value <0.05. (table-3)

Table-4: Department wise distribution of patients with S. aureus infection:

Department	No. of cases	Percentage (%)
Surgery	121	38.78
Orthopedics	58	18.59
ENT	41	13.14
Pediatrics	30	9.62
Medicine	28	8.97
Obstetrics- Gynecology	20	6.41
ICU	14	4.49
Total	312	100

Staphylococcus aureus isolates were more commonly obtained in patients from surgery department (38.78%) followed by orthopedics (18.59%) and ENT department (13.14%).(table-4)

Table-5: Sample wise distribution of S. aureus isolates:

Type of Specimen	No of samples	Percentage (%)
Pus	192	61.54
Sputum	68	21.79
Blood	30	9.62
Urine	15	4.81
Others*	7	2.24
Total	312	100

*-Others include tracheal aspirate, pleural fluid, peritoneal fluid, cerebrospinal fluid.

Maximum number of Staphylococcus aureus isolates were obtained from pus (61.54%), followed by sputum (21.69%).(table-5)

Table 6: Antibiotic sensitivity pattern of Staphylococcus aureus:

Antibiotics	Staphylococcus aureus n=312	
	Sensitive	Resistant
Penicillin G	16 (5.13)	296 (94.87)
Cefoxitin	198 (63.46)	114 (36.54)
Amoxicillin/ Clavulanic acid	163 (52.24)	149 (47.76)
Gentamicin	231 (74.04)	81 (25.96)
Amikacin	227 (72.76)	85 (27.24)
Netilmicin	312 (100)	0 (0)
Erythromycin*	148 (49.83)	149 (50.17)
Clindamycin*	211 (71.04)	86 (28.96)
Ciprofloxacin	162 (51.92)	150 (48.08)
Nitrofurantoin**	13 (86.66)	2 (13.33)
Tetracycline	212(67.95)	100 (32.05)
Trimethoprim/ Sulfamethoxazole	86 (27.56)	226 (72.44)
Vancomycin	312 (100)	0 (0)
Linezolid	312 (100)	0 (0)
Teicoplanin	312 (100)	(0)

*-not tested against urine isolates. **-tested only against urine isolate.

All the strains were 100% sensitive to Netilmicin, Linezolid and Teicoplanin. Higher resistance was noted against Penicillin (94.87%), Trimethoprim/Sulfamethoxazole (72.44%).

Discussion: The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. Unfortunately, the emergence of antibiotic resistant bacteria, is threatening the effectiveness of many antimicrobial agents.

In our study, a total of 312 isolates of Staphylococcus aureus were obtained from various clinical specimens.

Age and sex wise distribution of patients in various studies: In the present study, S. aureus infection rate was more in males than females. Similar observations were shown in studies done by Siddiqui et al[10] (2002), Anandi et al (2004)[11], Sasirekha B et al (2012)[12]. Sex wise distribution of cases in present study has followed natural epidemiological pattern. The increased rate of infection among males could be due to their outdoor occupation, more prone to injuries and due to exposure to contaminated environment.

In the present study, among 312 cases with S. aureus infection, most were in the age group 46-60. Similar observations were made by Sasirekha B et al (2012)[12] and Kahsay et al (2014) [13].

Distribution of Indoor and outdoor patient-In the present study, majority of Staphylococcus aureus isolates were obtained from patients admitted in various wards (67.63%) than OPD patients (32.37%). Similarly Joshi et al (2013)[14] reported that isolation rate of Staphylococcus aureus was higher among indoor patients (65.51%) than from patients attending OPDs (34.49%), while Tiwari et al (2008)[15] has reported higher isolation rate in OPD (64.24.%) patients than indoor patients (35.76%).

Many workers have expressed their view that the duration of hospital stay is directly proportional to the higher infection rate since the rate of isolation of organisms is higher in indoor than outdoor patients. Practicing frequent hand washing to prevent spread of organisms should be encouraged.

Department wise distribution of Staphylococcus aureus isolates in various clinical samples: In the current study, rate of isolation of Staphylococcus aureus was highest from surgery department (38.78%) followed by orthopedic department (18.59 %) and ENT (13.14%). Similar observation was made by Sanjana RK et al (2008)[16] who showed highest isolation from surgery department (24%) followed by orthopedic units (16%), while in another study by Loveena Oberoi et al (2012)[2], maximum isolation was from orthopedic department (28.86%), followed by surgery department (21.65 %).

Acquisition of these types of organisms is typically associated with particular settings i.e. surgical interventions, prolonged duration of hospitalization and postoperative surgical wounds. Resistant strains of S. aureus are very notorious and have a tendency to spread indiscriminately in the hospital settings.

Isolation of Staphylococcus aureus from various clinical samples: In the present study, Staphylococcus aureus was most commonly isolated from pus (61.54 %) followed by sputum samples (21.79 %). This finding is comparable with study done by Sasirekha et al (2012)[12] who reported 71.89 % of S. aureus isolates from pus followed by sputum samples (8.49 %). In a study by Sachin Kishore et al (2012)[17], 49.23 % of S. aureus isolates were from pus followed by urine samples (17.30 %).

The wide spectrum of diseases caused by Staphylococcus aureus includes, infections affecting skin and soft tissues, surgical site infection, ventilator associated pneumonia, infections of bones & joints and bacteremia associated with intravenous devices.[18] This might be the reason for higher isolation of S. aureus from pus samples.

Antibiotic resistance pattern of Staphylococcus aureus: Staphylococcus aureus is a common nosocomial pathogen with propensity to develop resistance to large number of antimicrobials. Therefore the knowledge of current antibiotic resistance pattern of S. aureus strains is necessary in selection of appropriate empirical treatment.[19] In our study, we determined the antimicrobial resistance pattern of S. aureus isolates against several antimicrobials.

In our study, among total S. aureus isolates, 94.87 % showed resistance to Penicillin G and 47.76 % showed resistance to Amoxicillin/Clavulanic acid. Dhanalakshmi TA et al (2012)[6] and Al-Dahbi et al (2013)[20] reported 85.6% and 100 % resistance to Penicillin respectively. Efuntoye MO et al (2012)[21] and Dhanalakshmi TA et al (2012)[6] reported 76.9 % and 42.8% isolates were resistant to Amoxicillin/Clavulanic acid.

Rate of MRSA using Cefoxitin disc diffusion method was 36.54%. Dhanalakshmi TA et al (2012)[6] and KB Anand et al (2009)[22] have reported rate of MRSA by Cefoxitin disc diffusion method as 32% and 64% respectively.

Resistance to Gentamicin and Amikacin was 25.96 % & 27.24 % respectively. Dhanalakshmi TA et al (2012)[6] reported comparable resistance pattern against Gentamicin and Amikacin. Al-Dahbi et al (2013)[20] and Farhadian A et al (2014)[23] reported 29.3 % and 39 % resistance against Gentamicin respectively. No resistance was reported against Netilmicin in our study, while Loveena Oberoi et al (2012)[2] reported 3.1% resistance to Netilmicin.

In our study, Erythromycin and Clindamycin resistance was 50.17% and 28.96% respectively which is

comparable with study done by Farhadian A et al (2014)[23] who reported 42 % and 33% resistance to Erythromycin and Clindamycin respectively.

Resistance shown against Ciprofloxacin was 48.08 % which is mirrored with the findings of Efuntoye MO et al (2012)[21] (46.2 %), while Al-Dahbi et al (2013)[20] and Farhadian A et al (2014)[23] have reported low resistance to Ciprofloxacin (29.2 % and 29% respectively).

Low resistance was noted against Tetracyclin (32.05 %) in present study which was in concordance with study done by Al-Dahbi et al (2013)[20] who showed 34.9% resistance to Tetracyclin.

High resistance was noted against Trimethoprim/Sulfamethoxazole (72.44 %) in our study. Similarly Dhanalakshmi TA et al (2012)[6] reported 68.8% resistance, while Al-Dahbi et al (2013)[20] reported 50 % resistance to Trimethoprim/Sulfamethoxazole.

All isolates in present study were sensitive to Teicoplanin and Linezolid and vancomycin. Loveena Oberoi et al (2012)[2]also reported the similar findings.

Conclusion: Antibiotics have traditionally been known as miracle drugs, but there is growing evidence that they are becoming overworked miracles. Although the development of antibiotic resistance may be inevitable, the rate at which it develops may be reduced by the rational use of antibiotics.
The present study was conducted to study the pattern of S. aureus infection at a tertiary care center and to know their antibiogram. It was found that the S. aureus isolates were resistant to the commonly prescribed antibiotics. Irrational and inappropriate use of antibiotics is responsible for emergence of Methicillin and multidrug-resistance in S. aureus. The emergence and the dissemination of resistance can be controlled by a heightened awareness of the issues, by encouraging proper personal hygiene, early intervention, and by the monitored use of antimicrobial agents based on the susceptibility data.

REFERANCES:

1. Rao BN, Prabhakar T. Reduced susceptibility of Vancomycin in Methicillin Resistant Staphylococcus aureus (MRSA) in and around Visakhapatnam, Andhra Pradesh. J Pharm Biomed Sci 2011;10(6):1–4.
2. Oberoi L, Kaur R, Aggarwal A. Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-Resistant Staphylococcus aureus (MRSA) in a Rural Tertiary Care Hospital in North India. Int J Appl Biol Pharm Technol 2012;3(1):200–5.
3. Reddy PCM, Thati VB, Shivannavar CT, Gaddad SCM. Vancomycin resistance among methicillin resistant Staphylococcus aureus isolates in Rayalaseema region Andhra Pradesh , South India. 2012;2(1):6–8.
4. Pujol M, Pena C, Pallares R, Ariza J, Ayats J, Dominguez A. Nosocomial Staphylococcus aureus bacteremia among nasal carriers of methicillin-resistant and methicillin susceptible strains. Am J Med 1996;100:509–16.
5. Majumdar D, Sarma Bardaloi J N, Phukan A C, Mahanta J. Antimicrobial susceptibility pattern among methicillin resistant Staphylococcal isolates in Assam. Ind J Med Microbiol 2001;19(3):138–40.
6. Dhanalakshmi T A, Umapathy B L, Mohan D R. Prevalence of Methicillin , Vancomycin and Multidrug Resistance among Staphylococcus aureus. J Clin Diagnostic Res 2012;80:1–4.
7. Tille PM. Baily & Scott's Diagnostic Microbiology. 13th ed. St Louis, Missouri: Elsevier; 2014.
8. Ananthanarayan PC. Staphylococcus In: Textbook of Microbiology. 9th ed. Hyderabad: Universities Press; 2013.
9. Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI Document M100-S22, Clinical and Laboratory Standard Institute, 950 West Valley Rd, Suite 2500, Wayne,. Wayne PA 19087 USA: 2012.
10. Siddiqi F, Masood MB, Saba N, Samad A, Qayyum M, Qazilbash AA. Antibiogram sensitivity pattern of methicillin resistant Staphylococcus aureus isolates from pus samples. Pakistan J Biol Sci 2002;5(4):491–3.
11. Anandi C, Alaguraja D, Natarajan V, Ramanatham M, Subramaniam CS Al, Thulasiram M et al. Bacteriology of diabetic foot lesions. Ind J Med Microbiol 2004;22(3):175–8.
12. Sasirekha B, Usha MS, Amruta AJ, Ankit S, Brinda N, Divya R. Evaluation and Comparison of Different Phenotypic Tests to Detect Methicillin Resistant Staphylococcus aureus and their Biofilm

Production. Int J Pharma Tech Res 2012, 4 2012;4(2):532–41.
13. Kahsay A, Mihret A, Abebe T, Andualem T. Isolation and antimicrobial susceptibility pattern of Staphylococcus aureus in patients with surgical site infection at Debre Markos Referral Hospital , Amhara Region , Ethiopia. 2014;72(1):1–7.
14. Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Gautam V, et al. Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence & susceptibility pattern. Indian J Med Res 2013;(February):363–9.
15. Tiwari HK. High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. 2008;57–62.
16. Sanjana RK, Shah R, Chaudhary N, Singh YI. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus (MRSA) in CMS-teaching hospital: a preliminary report. J Coll Med Sci 2010;6(1):1–6.
17. Kishore S., Siddique M.E., Mahmood,Verma D. Levels of Vancomycin Susceptibility Amongst Isolated Staphylococcus aureus Strains in a Tertiary Care Hospital. Int J Microbiol Res 2012;4(5):223–6.
18. Peacock S J. Staphylococcus. In: Topley and Wilson's Microbiology and Microbial Infections. 10th ed. London: Edword Arnold Ltd; 2005.
19. Mehta A P, Rodrigues C, Sheth K, Jani S, Hakimiyan A, Fazalbhoy N. Control of Methicillin resistant Staphylococcus aureus in tertiary care centre, a five year study. Ind J Med Microbiol 1998;16(1):31–4.
20. Al-dahbi AM, Al-mathkhury HJ. Distribution of Methicillin Resistant Staphylococcus aureus in Iraqi patients and Healthcare Workers. Iraqui J Sci 2013;54(2):293–300.
21. Efuntoye M O, Omotosho O O, Ashidi J S. Prevalence of Methicillin-Resistant Staphylococcus aureus And Coagulase Negative Staphylococci Among Male Students In A Private Tertiary Institution And Their Enteritoxin- producing Potential. Asian J Pharm Hea Sci 2012;2(1):231–4.
22. Anand KB, Agrawal P, Kumar S, Kapila K. Comparison of Cefoxitin disc diffusion test , Oxacillin Screen Agar , and PCR for mecA gene for detection of MRSA. Indian J Med Micro 2009;27(1):27–9.
23. Farhadian A, Nejad Q B, Peerayeh S N, Rahbar M, Vaziri F. Determination of Vancomycin and Methicillin Resistance in Clinical Isolates of Staphylococcus aureus in Uranian Hospital. Br Microbiol Res J 2014;4(4):454–61.

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