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Antibiotic Susceptibility Profile of Staphylococci Isolated from Clinical Samples

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Abstract:

Background: Staphylococcal species are versatile pathogens responsible for the increasing morbidity and mortality worldwide. Treating infections, especially nosocomial infections caused by *Staphylococus aureus* is a global concern now due to their constantly changing antibiotic susceptibility patterns**.**

Objective: To understand the current status of antibiotic susceptibility pattern of Staphylococcal isolates from various clinical specimens sent for laboratory workup.

Materials and methods: Various clinical specimens were collected and processed for routine culture and antibiotic sensitivity tests by Standard Microbiology Techniques. Antibiotic susceptibility testing (AST) was done on Mueller Hinton agar (HiMedia M173) by modified Kirby Bauer disc diffusion method.

Results: Out of 4538 samples received for microbiological examination, 534 were Staphylococcal species with *Staphylococcus aureus* accounting for 315 isolates and 219 for Coagulase Negative Staphylococcus species (CONS). They were isolated maximally from exudate samples. Isolation rate for *Staphylococcus aureus* was more from males especially among the age group 51-60 years whereas in CONS, isolation rate was equal from both sexes and more among the age group 51-60 years. MRSA prevalence was found to be 18%. Vancomycin and linezolid susceptibility was high. Increased resistance was shown to penicillin. Nitrofurantoin susceptibility (S.aureus -85%, CONS-94%) was found to be high among urine isolates. However, the susceptibility patterns showed variations among different specimens that were analysed (i.e Exudate, Blood, Urine) and also depending on the species (i.e. *Staphylococcusaureus* or Coagulase Negative Staphylococcal species).

Conclusion: From this study, it can be concluded that vancomycin , linezolid and nitrofurantoin (for urinary infections) are the leading drugs of choice for treatment of Staphylococcal infections. Prolonged or broad spectrum antibiotic therapy leads to the development of antibiotic resistant strains.Hence, there is a need for the development, adoption, and enforcement of appropriate control policies in hospital wards and departments where there is no existing or effective MRSA control.

Keywords:Antibiotic susceptibility pattern, nosocomial infections, MRSA, *Staphylococus aureus.*

INTRODUCTION:

Staphylococcal species are commonly carried on the skin or in the nose of healthy individuals. *Staphylococcusaureus* is an important human pathogen causing illness ranging from minor skin infections &abcesses to life-threatening diseases such as pneumonia, endocarditis, septicaemia, meningitis and toxic shock syndrome. It is challenging to treat because of its resistance to antimicrobial agents. Infections caused by Methicillin Resistant *Staphylococcus aureus* (MRSA) are mainly nosocomial and are being increasingly reported worldwide. As MRSA strains have become resistant to many different classes of antimicrobial drugs, second and third line antimicrobial resistance is a growing concern. Methicillin was first introduced in the 1960s for the treatment of infections caused by penicillin resistant *Staphylococcus aureus*, but within a few years, MRSA emerged. Methicillin resistance in Staphylococci is mediated by mecA gene, which encodes for the penicillin binding protein (PBP2A) resulting in reduced affinity for the beta lactam antibiotics including the penicillinase resistant penicillin. MRSA has become a major hospital pathogen in human medicine. However, vancomycin resistant *Staphylococcus aureus* is not widely seen even though low resistance to vancomycin is being reported. The risk factors which contribute to MRSA are excessive antibiotic usage, prolonged hospitalisation, intravascular catheterisation and hospitalisation in intensive care units.

OBJECTIVE:

To understand the antibiotic susceptibility pattern of Staphylococcal isolates from various clinical specimens sent to Microbiology laboratory.

MATERIALS AND METHODS:

A retrospective study was conducted at the Clinical Microbiology Laboratory of Saveetha Medical College and Hospital for a period of 6 months (July 2018 – December 2018). The study included both outpatient and inpatient samples.

Inclusion Criteria*:* All *Staphylococcus aureus* strains collected from variousclinical specimens were screened for their susceptibility to various antibiotics.

Exclusion Criteria:Cases of wound infection which did not yield the growth of Staphylococci, but yielded growth of other bacteria, fungal, commensal growth and mixed infection were excluded.

The samples collected were properly evaluated in terms of acceptability, proper labelling (full name, age, sex, serial number of the patient and date of collection). The specimens were cultured on blood agar and MacConkey agar plates and incubated aerobically at 37˚C for 24 hours. Staphylococcal species were identified by standard microbiology tests such as colony characteristics, gram staining, biochemical tests such as catalase test, coagulase test and mannitol fermentation test. Colonies that were mannitol fermenters (yellow coloured) & coagulase positive were taken to be *Staphylococcus aureus* while those that were mannitol non-fermenters (pink) & coagulase negative were considered to be other Staphylococci.

Antibiotic susceptibility testing (AST) was done on Mueller Hinton agar (HiMedia M173) by modified Kirby Bauer disc diffusion method. The diameter of the zone of inhibition was measured and interpreted according to the CLSI (Clinical and Laboratory Standards Institute) guidelines 2018. The antibiotics tested include

gentamycin(10mcg), erythromycin(15mcg), clindamycin(2mcg), vancomycin(30mcg), linezolid(30mcg), tetracycline(30mcg), cotrimoxazole(25mcg), penicillin(10 units), ofloxacin(5mcg), ciprofloxacin(5mcg), cefoxitin(30mcg), cefazolin(30mcg), nitrofurantoin(300mcg) and nalidixic acid(10mcg). In this study ATCC 25923 was used as control.

RESULTS:

A total of 4538 samples were collected (exudate, blood, urine). Out of 1068 (24%) culture positive samples, 534 (12%) samples were positive for Staphylococcal species. Among them, 315 (59%) isolates were*Staphylococcus aureus* and 219 (41%) isolates were Coagulase-Negative Staphylococcal species (CONS).

Isolation rate of *Staphylococcus aureus* and CONS from exudate samples was 84% and 46% respectively. The samplewise distribution of Staphylococcal isolates is illustrated in figure 1.

Thesample wise prevalence of methicillin resistance among Staphylococcal isolates is shown in table 1.

Staphylococcusaureus was isolated more from males in the age group 51-60 years whereas Coagulase Negative Staphylococcal species were isolated almost equally from male and females and in the age group51-60 years. Table 2 shows the gender and agewise prevalence of Staphylococcal isolates.

Exudate Samples:

*Staphylococcus aureus*strains from exudate samples showed 100% susceptibility to linezolidand vancomycin followed by 95% susceptibility to tetracycline. Almost equal number of *Staphylococcus aureus* isolates showed susceptibility and resistance to erythromycinand cotrimoxazole. They showed 95% and 84% resistance to penicillin and ciprofloxacin respectively. Similarly,Coagulase-Negative Staphylococcal species (CONS) showed100% susceptibility to linezolid and vancomycin. The isolates showed 67% resistance to penicillin and erythromycin and 65% resistance to cotrimoxazole.Antibiotic susceptibility pattern of Staphylococcal isolates from exudate samples is shown in table 3.

Blood Samples:

Among the *Staphylococcus aureus* isolates obtained from blood culture,100% susceptibility was shown to linezolid and vancomycin and 80% susceptibility to gentamicin.Many of the isolates showed resistance to ciprofloxacin (68%) followed by penicillin(64%). Coagulase-Negative Staphylococcal species (CONS) showed similar antibiotic susceptibility profile as *Staphylococcus aureus*(i.e. all strains susceptible to vancomycin (100%), linezolid (100%)). Considerable erythromycin resistance (83%) was observed among CONS isolates. Antibiotic susceptibility pattern of Staphylococcal isolates from blood samples is shown in table 4.

Urine Samples:

Among the *Staphylococcus aureus*isolates obtained from urine culture, susceptibility to linezolid was 100% and vancomycin was 100%. Most of the isolates showed resistance to ciprofloxacin (76%) and penicillin(68%). *Staphylococcus aureus* and CONS showed 88% and 98% susceptibility to nitrofurantoin respectively. They also showed 68% and 74% resistance to nalidixic acid respectively. All CONS isolates were susceptible to linezolid (100%) and vancomycin (100%) and 84% resistance was shown to penicillin.Antibiotic susceptibility pattern of Staphylococcal isolates from urine samples is shown in table 5.

TABLE 1: Sample wise prevalence of Methicillin susceptibility among Staphylococcal isolates.

	STAPHYLOCOCCUS AUREUS		COAGULASE NEGATIVE STAPHYLOCOCCI	
	MSSA	MRSA	MSCONS	MRCONS
EXUDATE	222	43	81	19
BLOOD	18	7	66	10
URINE	19	6	37	6

MSSA- Methicillin Sensitive Staphylococcus aureus; MRSA- Methicillin Resistant Staphylococcus aureus; MSCONS- Methicillin Sensitive Coagulase Negative Staphylococci; MRCONS- Methicillin Resistant Coagulase Negative Staphylococci

TABLE 2: Gender and age wise prevalence of Staphylococcal isolates

AGE GROUP	STAPHYLOCOCCUS AUREUS		COAGULASE NEGATIVE STAPHYLOCOCCI	
	MALES	FEMALES	MALES	FEMALES
Newborn	1	1	1	1
1-10	5	3	2	4
11-20	15	7	5	5
21-30	30	32	14	27
31-40	28	14	9	16
41-50	42	19	19	17
51-60	56	17	30	18
61-70	16	11	16	15
71-80	3	9	10	7
81-90	2	2	3	-
91-100	1	1	-	-
TOTAL	199	116	109	110

TABLE 3 : Antibiotic susceptibility pattern of Staphylococcal isolates from exudate samples

EXUDATE				
ANTIBIOTIC	STAPHYLOCOCCUS AUREUS		COAGULASE NEGATIVE STAPHYLOCOCCI	
	SENSITIVE (%)	RESISTANT (%)	SENSITIVE (%)	RESISTANT (%)
Gentamycin	201(76%)	64 (24%)	57 (57%)	43 (43%)
Erythromycin	133 (50%)	132 (50%)	33 (33%)	67 (67%)
Clindamycin	158 (60%)	107 (40%)	41 (41%)	59 (59%)
Vancomycin	265 (100%)	-	100 (100%)	-
Linezolid	265 (100%)	-	100 (100%)	-
Tetracycline	253 (95%)	12 (5%)	80 (80%)	20 (20%)
Cotrimoxazole	124 (47%)	141 (53%)	35 (35%)	65 (65%)
Penicillin	13 (5%)	252 (95%)	33 (33%)	67 (67%)
Ofloxacin	51 (19%)	214 (81%)	55 (55%)	45 (45%)
Ciprofloxacin	43 (16%)	222 (84%)	46 (46%)	54 (54%)
Cefoxitin	222 (84%)	43 (16%)	81 (81%)	19 (19%)

TABLE 4 : Antibiotic susceptibility pattern of Staphylococcal isolates from blood samples

BLOOD				
ANTIBIOTIC	STAPHYLOCOCCUS AUREUS		COAGULASE NEGATIVE STAPHYLOCOCCI	
	SENSITIVE (%)	RESISTANT (%)	SENSITIVE (%)	RESISTANT (%)
Gentamycin	20 (80%)	5 (20%)	46 (61%)	30 (39%)
Erythromycin	10 (40%)	15 (60%)	13 (17%)	63 (83%)
Clindamycin	12 (48%)	13 (52%)	25 (33%)	51 (67%)
Vancomycin	25 (100%)	-	76 (100%)	-
Linezolid	25 (100%)	-	76 (100%)	-
Tetracycline	14 (56%)	11 (44%)	40 (53%)	36 (47%)
Cotrimoxazole	13 (52%)	12 (48%)	33 (43%)	43 (57%)
Penicillin	9 (36%)	16 (64%)	26 (34%)	50 (66%)
Ofloxacin	13 (52%)	12 (48%)	46 (61%)	30 (39%)
Ciprofloxacin	8 (32%)	17 (68%)	28 (37%)	48 (63%)
Cefoxitin	18 (36%)	7 (60%)	66 (87%)	10 (13%)

TABLE 5: Antibiotic susceptibility pattern of Staphylococcal isolates from urine samples

URINE				
ANTIBIOTIC	STAPHYLOCOCCUS AUREUS		COAGULASE NEGATIVE STAPHYLOCOCCI	
	SENSITIVE (%)	RESISTANT (%)	SENSITIVE (%)	RESISTANT (%)
Gentamycin	19 (76%)	6 (24%)	33 (77%)	10 (23%)
Erythromycin	12 (48%)	13 (52%)	18 (42%)	25 (58%)
Clindamycin	14 (56%)	11 (44%)	16 (37%)	27 (63%)
Vancomycin	25 (100%)	-	43 (100%)	-
Linezolid	25 (100%)	-	43 (100%)	-
Tetracycline	14 (56%)	11 (44%)	24 (56%)	19 (44%)
Cotrimoxazole	12 (48%)	13 (52%)	21 (49%)	22 (51%)
Penicillin	8 (32%)	17 (68%)	7 (16%)	36 (84%)
Ofloxacin	8 (32%)	17 (68%)	14 (33%)	29 (67%)
Ciprofloxacin	6 (24%)	19 (76%)	16 (37%)	27 (63%)
Cefoxitin	19 (76%)	6 (24%)	37 (86%)	6 (14%)
Nitrofurantoin	22 (88%)	3 (12%)	42 (98%)	1 (2%)
Nalidixic acid	8 (32%)	17 (68%)	11 (26%)	32 (74%)

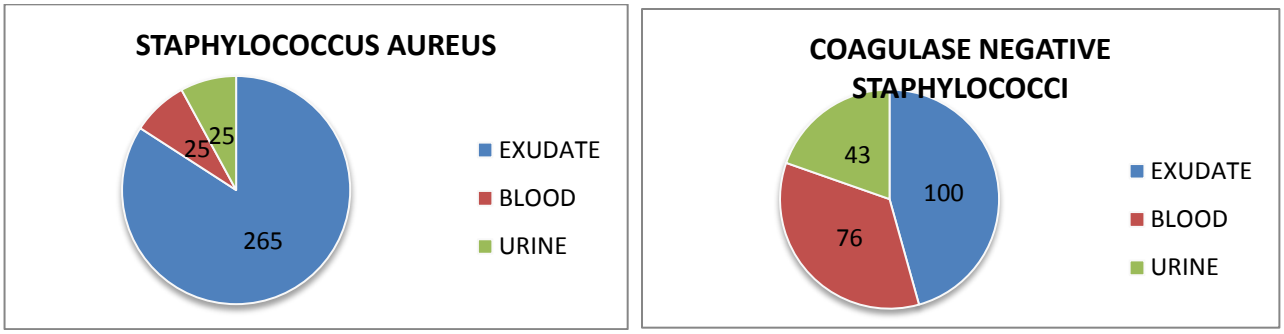

FIGURE 1:Samplewise distribution of Staphylococcal isolates

DISCUSSION:

A total of 4538 samples were processed, out of them 1068 (24%) were positive for bacterial growth. 534 (12%) samples were Staphylococcal isolates. Among them, *Staphylococcus aureus* accounted for 59% (315) whereas CONS accounted for 41% (219) of the isolates.In our study, isolation rate of *Staphylococcus aureus* from exudate samples was 84% which was much higher compared to blood and urine samples. Similarly, isolation rate of 74% from pus was reported by Baral R et al[1]. Analysis of gender and age prevalence of *Staphylococcus aureus*revealed more isolation from males (63%) and among the age group 51-60 years (23%). In contrast, Bhatt CP et al[2] has reported higher isolation from females and more prevalence among the age group 0-10 years (24%). Higher colonization among age group 0-10 could be due to their frequent contact with respiratory secretions or may be due to sanitary reason. Also, their immunity is not properly developed to combat bacterial infections and hence they get infected easily.Among the isolates of*Staphylococcus aureus*, 18% were found to be methicillin resistant (MRSA). This is almost consistent with the study conducted by Tahnkiwale et al[3] which reported 19.5% of S.aureus strains were MRSAand much lower than the reports from the study conducted by Vaez et al[4]which concluded that 42.9% of the strains were MRSA.

Amongthestrainsof*Staphylococcus aureus*, susceptibility to vancomycin and linezolid is high. Similar observations were made on vancomycin(100%susceptibility) by Bhatt CP et al[2] and on linezolid (100% susceptibility) by Mahmood K et al[5] .Therewas novancomycin resistance found during this study. In contrast, LK Khanal et al[6] reported 21% resistance to vancomycin. This indicates the emergence of vancomycin resistant *Staphylococcus aureus*(VRSA) strains.In this study, penicillin and ciprofloxacin resistance were high. This finding almost correlates with the reports from the study conducted by LK Khanal[6] et al which states 97% and 70% resistance to pencillin and ciprofloxacin respectively.Many studies have documented that increased resistance to penicillin was due to destruction of the lactam ring by a lactamase produced by *Staphylococcus*aureus. In our study, no attempt was done to detect the production of a lactamase. Therefore, it is difficult to comment on the nature of resistance.Cotrimoxazole is an effective antibiotic to which resistance is currently on the rise. Whether the resistance observed is due to their inherent genetic propensity to acquire resistance or due to erratic prescription of antibiotics could not be classified as previous antibiotic intake data were not available.Baral R et al[1] has reported 64% resistance to cotrimoxazole in his study.

In our study, isolation rate of Coagulase Negative Staphylococcal species (CONS) from exudate samples was found to be 46% which was much higher compared to blood and urine samples. In contrast, study conducted by Murad Ehsan et al[7] has reported maximal isolation rate from blood samples (45.9%). Among CONS, sensitivity pattern was similar to that of *Staphylococcus aureus*strains showing 100% susceptibility to vancomycin and linezolid .This almost coincides with the reports from the study done by Shamsadh Begum et al[8] which also states 100% susceptibility to both of these drugs.*Staphylococcus aureus* and CONS showed 88% and 98% susceptibility to nitrofurantoin respectively. High susceptiblity to nitrofurantoin suggests that it could be an effective antibiotic against Coagulase Negative Staphylococcal infections. Similar observation was done by Ibrahim Ali Al Tayyar et al[9] in his study and he has reported that 92.8% susceptibility to nitrofurantoin. Bora P et al[10] has reported 57% resistance to ciprofloxacin and 50% resistance to cotrimoxazole which is almost consistent with our study as well.CONS strains analysed in our study showed moderate resistance to penicillin. In contrast,Shamsadh Begum et al[8] reported high degree of resistance (91%) to penicillin in her study. Exudate and blood isolates in our study showed increasing resistance to erythromycin as well. Mohan Uet al[11] reported in his study that S.epidermidis followed by S.saprophyticus were commonly isolated among Coagulase Negative Staphylococcal species. However, this could not be commented upon because species categorisation was not a part of our study.

CONCLUSION:

From this study, it can be concluded that vancomycin , linezolid and nitrofurantoin (for urinary infections) are currently the leading drugs with high degree of susceptibility that can be employed to treat Staphylococcal infections. But their judicial use is of prime importance in this changing world.

Despite the introduction of antimicrobial therapy and the recent improvements in medical services, MRSA is recognised as a major cause of nosocomial infections which can result in significant morbidity and mortality rates. Prolonged or broad spectrum antibiotic therapy predisposes patients to infections with antibiotic resistant strains[12]. Hence, there is a need for the development, adoption, and enforcement of appropriate control policies in hospital wards and departments where there is no existing or effective MRSA control.

Increasing prevalence of MRSA, leads to extensive use of vancomycin. Vancomycin resistance could be a global health concern in the near future.[13,14] It could lead to serious clinical and public health consequences because currently very few licensed alternatives are available to treat vancomycin resistant *Staphylococcus aureus* infections.

Conflict of interest:

There is no conflict of interest.

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