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Prevalence and Antimicrobial Susceptibility of *Staphylococcus aureus* strain from Clinical Isolates at Gwalior Medical College Hospital

KEYWORDS

Staphylococcus aureus, antimicrobial resistance, hospital infection, clinical isolates.

Saroj Kothari
Department of Pharmacology, G.R.Medical College, Gwalior (M.P.), India.

Anubhuti Khare
Department of Pharmacology, G.R.Medical College, Gwalior (M.P.), India.

Vaibhav Misra
Department of Microbiology, G.R.Medical College, Gwalior (M.P.), India.

ABSTRACT

Present study was undertaken to find the prevalence & antimicrobial sensitivity of *Staphylococcus aureus* in clinical isolates in tertiary care hospital. Various samples were collected for isolation & antimicrobial susceptibility testing of *S.aureus. Out* of 5000 samples collected 1684 showed growth in culture media. A total of 255 *Staphylococcus* were isolated & subjected to antimicrobial susceptibility. High antimicrobial resistance was seen with common antimicrobial agents such as erythromycin, ampicillin, cefixime & ceftazidime+clavulinic acid as 78%, 71%, 71% & 63% respectively. Mean highest sensitivity to linezolid (84%), vancomycin (83%) & doxycycline (82%), whereas mean intermediate antibiotic sensitivity to amikacin (77%), levofloxacin (72%), amoxiclav (64%) & piperacillin (56%) were observed. Present study showed that *Staphylococcus aureus* (15%) were resistant to commonly used antibiotics & did not show 100% sensitivity to linezolid & vancomycin. Thus, there is urgent need to regulate use of antibiotics in hospital for preventing the spread of antimicrobial resistance.

INTRODUCTION

The genus Staphylococcus includes pathogenic organisms in which *Staphylococcus aureus* is most important. *Staphylococcus aureus* is a gram-positive coccal bacterium and is frequently found in the nose, respiratory tract, and on the skin. *S.aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis.[1] It is still one of the five most common causes of hospital-acquired infections and is often the cause of postsurgical wound infections.[1]

Both community-associated and hospital-acquired infections with *Staphylococcus aureus* have increased in the past 20 years, and the rise in incidence has been accompanied by a rise in antibiotic-resistant strains, in particular, methicillin-resistant *S.aureus* (MRSA) and more recently, vancomycin-resistant strains[2]. The development of resistance to multiple antibiotics and control of disease transmission by MRSA isolates in hospitals/communities have been recognized as the major challenges as the bacterial population that expresses the resistance phenotype varies according to the environmental conditions[3]. Therefore, present study is planned to determine current antibiotic sensitivity and resistance pattern of *Staphylococcus aureus* that would help in laying down recommendations for empirical regimen for treating such infections.

MATERIALS AND METHODS

An observational, prospective, survey based study was conducted in the department of Pharmacology & department of Microbiology, J.A group of hospitals, G.R. Medical College, Gwalior (M.P.) from March 2015 to February 2016. A total of 5000 samples of urine, blood, pus, CSF & miscellaneous (which included ascitic, pleural & peritoneal fluid; ear, throat & vaginal swab; sputum & stool) were collected from indoor and outdoor patients of various departments and hospital units for isolation and antimicrobial susceptibility pattern of *S.aureus.*

Identification of Bacteria

The bacteria were cultured on Mac *Conkey's* agar, Nutrient agar, blood agar and other selective media followed by the identification of the isolates based on their cultural characteristics, gram staining, motility and reactions in standard biochemical tests.

Antimicrobial agents

The isolates were tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion technique on Muller Hinton Agar by Filter Paper disks impregnated with antibiotics (Span diagnostics limited, Surat, India): Penicillins: Ampicillin(10mcg), Amoxicillin(10mcg), Amoxicillin-Clavulanic acid(20/10 mcg), Piperacillin(75mcg); Cephalosporins: Cefixime(30mcg), Ceftazidime+ clavulinic acid(30/10mcg); Aminoglycosides: Amikacin(30 mcg); Quinolones: Levofloxacin(5mcg), Ofloxacin(5mcg); Tetracyclines: Doxycycline(30mcg); Macrolides: Erythromycin(15mcg), Azithromycin(15mcg); Vancomycin(5-10mcg); Linezolid(15mcg); Fusidic Acid (10mcg). A pre-diffusion time of 30 min was allowed at room temperature and the plates were incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured and compared to that of standard strain and the results were interpreted as sensitive, or resistant, based on Clinical Laboratory Standard Institute 2014 guidelines[4].

The percentage antimicrobial susceptibility of the isolated microorganism against different antimicrobials tested was calculated and interpreted as sensitive and resistant.

The study was conducted after an approval from the Institutional medical ethical committee.

RESULTS

Out of total 5000 samples 1684 showed growth in the culture media including *S.aureus, E.coli, K.pneumoniae, P.aeruginosa, Acinetobacter, Enterococci, Streptococcus, Proteus, Citrobacter,* etc. Of these *Staphylococcus aureus being* 255 (15.14%) (Fig.1).

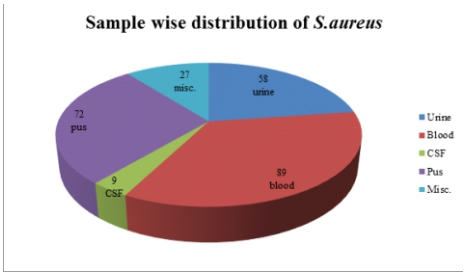

Figure 1. Showing the distribution of *S.aureus* isolates among different types of samples.
Misc.- Miscellaneous.

Volume - 7 | Issue - 3 | March - 2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

Prevalence of *S.aureus* was 11%, 13%, 5%, 38%, & 27% in urine, blood, CSF, pus & miscellaneous group respectively. *S.aureus* was highest in pus (72 isolates out of 190samples) & miscellaneous group (27 isolates out of 101 samples). (Table 2)

Table 2 Prevalence of *S.aureus* in different samples

Sample (No.)	Samples showed growth	*S.aureus* isolates
Urine (1936)	526	58 (11%)
Blood (1505)	667	89 (13%)
CSF (782)	195	9 (5%)
Pus (491)	190	72 (38%)
Miscellaneous (286)	101	27 (27%)
Total (5000)	Total: 1684	Total: 255

Sensitivity pattern of ***S.aureus***

The highest mean sensitivity of *S.aureus* was towards linezolid, vancomycin, doxycycline as 84%, 83% and 82% respectively. Intermediate sensitivity for amikacin, levofloxacin, amoxicillin+ clavulinic acid, piperacillin was 77, 72, 64 and 56% respectively.

The sensitivity of *S.aureus* for fusidic acid in urine samples was 91% (i.e. 43 samples were sensitive out of the total 47 tested).

Highest mean resistance of *S.aureus* was for erythromycin, ampicillin, cefixime, Ceftazidime+clavulinic acid & as azithromycin 78, 71, 71, 63 and 58% respectively. (Table 3)

Table 3 Sensitivity pattern of *Staphylococcus aureus*

Antibiotic S/R%(x/y)	Samples					Mean of sensitivity (%)
	Urine	Blood	CSF	Pus	Miscellaneous	
Amikacin	60/40 (3/5)	56/44 (5/9)	_	91/9 (10/11)	100/00 (1/1)	77
Ampicillin	27/73 (9/33)	27/73 (17/63)	29/71 (2/7)	48/52 (23/48)	16/84 (3/19)	29
Amoxycillin+ Clavulinic acid	58/42 (28/48)	58/42 (48/83)	50/50 (4/8)	84/16 (53/63)	70/30 (16/23)	64
Cefixime	20/80 (2/10)	30/70 (8/27)	00/100 (0/4)	43/57 (15/35)	53/47 (8/15)	29
Linezolid	87/13 (40/46)	88/12 (66/75)	67/33 (4/6)	87/13 (46/33)	92/8 (22/24)	84
Ofloxacin	100/00 (2/2)	_	_	_	00/100 (0/1)	50
Fusidic acid	91/9 (43/47)	_	_	00/100 (0/1)	100/00 (1/1)	64
Ceftazidime +clavulinic acid	63/37 (5/8)	12/88 (3/26)	00/100 (0/2)	83/17 (20/24)	25/75 (1/4)	37
Doxycycline	67/33 (10/15)	82/18 (9/11)	100/00 (1/1)	100/00 (16/16)	60/40 (3/5)	82
Azithromycin	36/64 (15/42)	39/61 (30/77)	38/62 (3/8)	56/44 (34/61)	42/58 (8/19)	42
Piperacillin	52/48 (13/25)	35/65 (22/63)	67/33 (6/9)	60/40 (28/47)	67/33 (12/18)	56
Vancomycin	92/8 (44/48)	82/18 (70/85)	63/37 (5/8)	89/11 (55/62)	87/13 (20/23)	83
Levofloxacin	63/37 (5/8)	56/44 (9/16)	75/25 (3/4)	92/8 (11/12)	75/25 (3/4)	72
Erythromycin	17/83 (4/23)	28/72 (15/54)	00/100 (0/4)	42/58 (13/31)	25/75 (4/16)	22

S/R% - sensitivity/resistance(%) . (x/y) – x: no. of samples tested sensitive ; y: total no. of samples on which antibiotic susceptibility test was applied.

DISCUSSION

Present study revealed the prevalence of *S.aureus* infections to be 15%. This was in accordance with I.Ginawi et al 2014 (nosocomial infections by *S.aureus* - 34.8%) [5]. We also found that in pus samples the prevalence of *S.aureus* was highest as was the case with other two studies.[6,7]

In our study, the sensitivity to the penicillin and cephalosporins showed poor efficacy. These results point out towards the increasing prevalence of methicillin resistant staphylococcus aureus (MRSA) which are defined as bacterias resistant to all the penicillinase-resistant penicillins and cephalosporins.[8] MRSA contains an additional high-molecular-weight penicillin binding protein with a very low affinity for β-lactam antibiotics[9] *S.aureus* was found to have highest sensitivity to linezolid (84%), vancomycin (83%) & doxycycline (82%) in our study. Ideally in case of MRSA the first line agents are linezolid & vancomycin[10], but the sensitivity to these is decreasing in our region which is a matter of great concern. ***Staphylococcus aureus*** continues to be a dangerous pathogen for both community-acquired as well as hospital-associated infections. *S. aureus* resistant to methicillin were reported soon after its introduction in October 1960[11]. Methicillin resistant S.aureus (MRSA) is now endemic in India. The incidence of MRSA varies from 25 per cent in western part of India12 to 50 per cent in South India[13]. Community acquired MRSA has been increasingly reported from India[14]. Thus, the result of our study indicates that for MRSA isolates also the resistance is found even for the most susceptible antibiotics (16%-linezolid, 17%-vancomycin & 18%-doxycycline).

The resistance to linezolid antibiotic could be due to point mutation of the 23S rRNA in staphylococcal bacteria[9]. Resistance of *S.aureus* for vancomycin may be intermediate or high level. Intermediate strains produce an abnormally thick cell wall, and resistance may be due to false targets for vancomycin. Several genetic elements & multiple mutations are involved, and many of the genes that have been implicated encode enzymes of the cell-wall biosynthetic pathway. The high-level vancomycin-resistant S.aureus strain harbours a conjugative plasmid into which the Van A transposon, Tn1546 was integrated as a consequence of an interspecies horizontal gene transfer from *E.faecalis* to a methicillin- resistant strain of S.aureus[9].

Resistance to doxycycline could be due to: decreased accumulation of drug as a result of decreased antibiotic influx or acquisition of an energy- dependent efflux pathway; production of a ribosomal protection protein that displaces the drug from its target; and by enzymatic inactivation of the drug.

In the aminoglycoside group our study showed 77% sensitivity to amikacin while others showed variable results as 61%[15] & 95%[16] sensitivity in different parts of India. The genes encoding aminoglycoside-modifying enzymes are acquired primarily by conjugation and transfer of resistance plasmids. These enzymes phosphorylate, adenylate, or acetylate specific hydroxyl or amino groups. Amikacin is a suitable substrate for only a few of these inactivating enzymes; thus, strains that are resistant to multiple other aminoglycosides tend to be susceptible to amikacin.[9]

The susceptibility to macrolide antibiotic erythromycin was very low in our study (22%).The INSAR group showed 42%[17] & a study by Chetan Mandelia et al showed 52% sensitivity[18]. While some other studies showed good results[15,16]. This shows that there is high resistance to this antibiotic in our region which could be due to drug efflux by an active pump mechanism (encoded by mrsA in staphylococci)[9].

The flouroquinolone agent ofloxacin showed only 50% sensitivity but levofloxacin showed good efficacy (72%). This was in little discordance with the other two studies which showed 21%[17] & 18%[18] sensitivity for ciprofloxacin. Results similar to our were shown by Lahari Saikia et al (48%-ciprofloxacin)[15] & Vidya Pai et al (75%-ciprofloxacin)[16]. Resistance to quinolones may develop during therapy via mutations in the bacterial chromosomal genes encoding DNA gyrase or topoisomerase IV or by active transport of the drug out of the bacteria. Resistance has increased after the introduction of fluoroquinolones, especially in Pseudomonas and staphylococci.[9]

The study we conducted showed an increasing number of MRSA and their increasing trend of resistance towards the various antibiotics and

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also towards the most efficacious ones like linezolid and vancomycin i.e. 16% & 17% respectively. This might seem a negligible percentage at present, but it could become a grave danger in future if proper steps to curtail the infections & to prevent the resistance are not taken immediately. Thus, the results of this study suggest a poor & unsatisfactory antibiotic susceptibility pattern of *S.aureus* in this region. Immediate steps must be taken to curtail the problem of antibiotic resistance, otherwise it will take no time for us to move to pre-antibiotic era which would be a huge step back for human community. However, the study had its share of limitations. It was done in our hospital only over a short period of time. Studies like this should be done on a regular basis covering every hospital & medical care centers so as to get updated & thus use the antibiotics rationally according to the local pattern of resistance.

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