IJPSR (2012), Vol. 3, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 August, 2012; received in revised form 11 November, 2012; accepted 25 November, 2012

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* STRAINS FROM PATIENTS IN ETHIOPIA

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Keywords:
S. aureus, Antibiotic resistance, Multidrug resistance, Methicillin resistant *S. aureus*, Ethiopia

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QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

ABSTRACT

Staphylococcus aureus (*S. aureus*) is one of the most serious gram-positive bacteria causing several infections. Because of its intrinsic ability to develop resistance to many antibiotics, nowadays only few drugs can be confidently mentioned for the treatment of *S. aureus* infections. The study aimed to investigate the antibiotics susceptibility pattern of *S. aureus* among patients. A cross-sectional study was conducted antibiotic susceptibility pattern of *S. aureus* among patients. A total of 323 wound and nasal swab specimens were collected and inoculated on mannitol salt agar (Oxoid) and incubated at 37^{o}C for 18 to 24 hours. For primary cultures with bacterial growth, Gram-staining and specific biochemical tests (catalase and coagulase) were used to identify the study organism. Sensitivity of the isolates to ten commonly used antibiotics was determined by modified Kirby-Bauer antibiotic sensitivity testing method. The isolates were found to be highly resistant to penicillin G and cephalothin (98.8%; each). Chloramphenicol, amoxicillin/clavulanic acid and oxacillin were also resisted by 53.1%, 50.6% and 38.3% of the isolates, respectively. Least resistance was obtained for ciprofloxacin, vancomycin and gentamycin. There was higher *S. aureus* prevalence for inpatient isolates than outpatients'. The isolates showed high resistance and multidrug resistance pattern to several combinations of the tested antibiotics.

INTRODUCTION: Antibiotics have revolutionized modern medicine and the treatment of many infectious diseases. But the battle against pathogenic bacteria is by no means over; many organisms have become resistant to certain antibacterial agents and this requires continuing search for new drugs and modification of those already in use. Although many antibiotic structures have been subjected to intensive chemical manipulation to yield novel derivatives with extended activity spectra, resistance to such antibiotics has usually emerged quickly [1]. The development of resistance to antimicrobial agents by many bacterial pathogens has compromised traditional therapeutic regimens, making treatment of infections difficult and expensive [2]. Antimicrobial resistance is becoming an important public health problem for both hospital and community acquired infections. In the hospital, infections caused by multiply antibiotic resistant pathogens including staphylococci have resulted in increasing morbidity, mortality and cost because of prolonged hospitalization and the use of more expensive antimicrobial agents [3].

Antibiotic resistance may be intrinsic or acquired; the later being more important. Once resistance has occured, the continuing presence of an antibiotic exerts a selective pressure in favor of the resistant organisms [4]. Bacteria can become resistant to antibiotics through three main types of mechanisms: antibiotic destruction, target modifications and mechanisms that reduce antibiotic concentration inside the microorganism. Resistance due to reduced antibiotic concentration secondary to efflux is being increasingly described as a cause of antibiotic resistance in gram-positive organisms (staphylococci, streptococci, Bacilli, etc) [5].

Staphylococcus aureus (*S. aureus*) and other staphylococci were inherently sensitive to many antimicrobial agents (penicillins, cephalosporins, aminoglycosides, tetracyclines, macrolides, lincosamides, glycopeptides, rifampicin, fusidic acid, trimethoprim, chloramphenicol, carbapenems, temocillin, polymyxins). Benzylpenicillin was among the most active, but about 90% of those found in hospitals are now resistant. Resistance to penicillin depends on the production of the enzyme penicillinase [6].

In Ethiopia, studies have shown the importance of *S. aureus* in diseases and the emergence of multidrug resistant strains [7]. A review of bacterial pathogens susceptibility studies in Ethiopia, as compared with data from other countries, showed a higher antimicrobial resistance rate and *S. aureus* was amongst the most frequently isolated strains [8]. So, this study was aimed at investigating the current antibiotics susceptibility pattern of *S. aureus* among patients in Jimma University Specialized Hospital, Ethiopia.

MATERIALS AND METHODS

Study setting, design and population: The study was conducted in Jimma University Specialized Hospital (JUSH), which is in Jimma town located 346km from Addis Ababa, South West of Ethiopia. Since it is the only specialized hospital in the area, it gives service to about 15 000 000 people living in South West Ethiopia including the South Sudanese refuges. In addition, it is a training center for about 1 000 health sciences students, yearly. The study was conducted from February to April, 2008.

A cross-sectional study was conducted to determine the prevalence and antibiotic susceptibility pattern of *S. aureus* in both inpatients and outpatients. The research proposal was submitted to Jimma University Medical Faculty Ethics Committee and ethical approval of the study was obtained. All inpatients and outpatients who came to the study hospital for medical help during the study period were taken as source population. Samples were taken from 323 patients who were selected by systematic sampling technique from the source population. A systematic sampling technique using independent sampling interval for the different departments was used to select the 323 patients included in the study.

Sample size and Sampling Technique: The sample size was determined to be 323 by the using the single population proportion formula; employing Z-score of 1.96 (at 95% confidence interval); assuming the proportion of *S. aureus* carriers in the general population to be 30% [12] and allowing 5% sampling error.

A systematic sampling technique [13] was used to select the 323 patients included in the study. Depending on previous years' service records from the hospital, the six wards included in the study would give service to an average of 73,725 patients annually. Based on this, it was expected that the wards would serve about 3780 patients in the study period. Thus to select 323 samples from 3780 patients by systematic sampling technique, the sampling fraction would be 11. Therefore, each day the first sample was being selected randomly from among the first 11 patients encountered and every 11^{th} (example 22^{nd}, 33^{rd}, 44^{th}, etc) patients were included in the study.

Specimen collection, handling and identification *S. aureus* **isolates:** Cotton wool swabs were prepared and sterilized by steam autoclave in open test tubes at $121^{o}C$ for 15 minutes. Screw capped test tubes with racks, normal saline in a vial with sterilized dropper and gloves were taken to the hospital for specimen collection. After securing oral consent and patient information recording, the cotton swabs were moistened with few drops of the normal saline. In case of nasal swab collection, the moistened cotton swabs were carefully inserted in to both anterior nares of the patient and the swab was taken.

For wound swabs, pus was taken just before dressing by the hospital staff. These swabs were put in to the test tubes carefully and closed immediately and transported to the study laboratory, with in 30 minutes of collection with care to prevent it from direct sun light.

Before specimen collection, mannitol-salt-agar (MSA) (HIMEDIA) plates were prepared according to the procedure instructed by the manufacturer of the agar powder. The specimens collected were inoculated on the MSA plates with sterilized wire loop and incubated at 37^{o}C for 18-24 hours. The next day, suspicious colonies were Gram- stained; and catalase and coagulase tests were done for gram-positive cocci to differentiate between *S. aureus* and other microorganisms [6]. Primary cultures that showed *S. aureus* growth were stored in a refrigerator until the nutrient broth and Mueller-Hinton-Agar (MHA) (manufacturer's company) medium were prepared for the susceptibility study.

Antibiotic Sensitivity Testing: Susceptibility of the *S. aureus* isolates was checked by modified Kirby-Bauer disk diffusion method [9]. After the nutrient broth (HIMEDIA) and MHA (Oxoid) were prepared and made ready, 3-4 well grown *S. aureus* colonies were taken and inoculated in the nutrient broth and incubated at 37^{o}C for about 4 hours.The MHA plate was inoculated with in 15 minutes after the inoculum suspension was adjusted. Suspension adjustment was made by comparing the turbidity of the inoculated and diluted bacterial suspension with a control, non-inoculated nutrient broth and 0.5 Mc Farland. A sterile cotton swab was used to inoculate the bacterial suspension on the MHA plate according to the recommended procedure [9].

With in 15 minutes after the plates were inoculated, the antibiotic disks were applied evenly on the surface of the agar with about 24 millimeters between their centers. After 24 hours incubation growth inhibition zone produced by these antibiotics was measured using a sliding metal caliper. Standard strain of *S. aureus* (ATCC- 25923) was used as a control [9].

The antibiotic disks were penicillin G (10 units), amoxycillin/clavulanic acid (30µg), oxacillin (5µg), cephalothin (30µg), erythromycin (15µg), gentamicin (10g), vancomycin (30µg), chloramphenicol(30µg), sulphamethoxazole/trimethoprim (25µg), ciprofloxacin (5µg). With in 15 minutes after the disks were applied, the plates were incubated at 37^{o}C for 24 hours.

Data analysis and Interpretation: All the necessary laboratory results obtained were recorded and documented. This record was used for data analysis and interpretation. All the *S. aureus* isolates obtained were tabulated under categories titled susceptible, intermediate and resistant for all antibiotics tested based on the recorded zone of inhibition diameters. This was done using interpretive charts of the antibiotic disks' manufacturers (Oxoid and HIMEDIA). Isolates that fall in the intermediate category were recognized as resistant.

RESULTS: A total of 323 patient specimens (wound swabs and nasal swabs) were collected and investigated in the study period. As presented in Table 1, among the total study subjects, 165 (51.1%) were females and 158 (48.9%) were males with a sex ratio of 1:0.96. The age of the sampled patients ranges from two months to 86 years with a mean of 32.7 years.

TABLE 1: DISTRIBUTION OF SAMPLED PATIENTS BY AGE AND SEX, JUSH, FEBRUARY TO APRIL, 2008

		Age (years)				
		≤5	5-18	19-45	>45	Total (%)
Sex	Male	11	41	59	47	158(48.9)
	Female	13	33	86	3	165(51.1)
Total (%)		24(7.4)	74(82.9)	145(44.9)	50(15.5)	323(100)

Antibiotic resistance among *S. aureus* isolates for penicillin G (98.8%), cephalothin (98.8%), Amoxicillin/clavulanic acid (50.6%), oxacillin (38.3%) and chloramphenicol (53.1%) was high. While the isolates showed lowest resistance to ciprofloxacin (2.5%) and vancomycin (1.2%). Inpatient isolates were more resistant than outpatient isolates to all the tested antibiotics except erythromycin (**Table 2**).

TABLE 2: ANTIBIOTIC RESISTANCE PATTERN OF *S. AUREUS* ISOLATES AMONG SAMPLES FROM INPATIENTS AND OUTPATIENTS, JUSH, FEBRUARY TO APRIL, 2008

Patient category	Antibiotics (% resistance)									
	P	AMC	Ox	KF	E	G	C	SXT	Cf	V/M
Inpatients	65(100)	35(53.9)	26(40.0)	64(98.5)	32(49.2)	14(21.5)	36(55.4)	11(16.9)	2(3.1)	1(1.5)
Outpatients	15(93.8)	6(37.5)	5(31.5)	14(87.5)	9(56.3)	2(12.5)	7(43.7)	1(6.3)	0(0.0)	0(0.0)
Total	80(98.8)	41(50.6)	31(38.3)	80(98.8)	41(50.6)	16(19.7)	43(53.1)	12(14.8)	2(2.5)	1(1.2)

P = Penicillin G, Cf = Ciprofloxacin; AMC = Amoxicillin/clavulanic acid; C = Chloramphenicol; Ox = Oxacillin; KF = Cephalothin; E = Erythromycin; G = Gentamycin; V/M = Vancomycin; SXT = Sulphamethoxazole/trimethoprim

The most frequent multidrug resistance pattern consisting of two drugs is exhibited for penicillin-chloramphenicol, with a resistance of 26 (32.1%) of the isolates. Fourty five (55.5% of the isolates) were resistant to different combinations of two of the tested antibiotics (**Table 3**).

TABLE 3: ANTIBIOGRAMS OF THE TOTAL *S. AUREUS* ISOLATES FORM THE DIFFERENT DEPARTMENTS OF THE HOSPITAL, JUSH, FEBRUARY TO APRIL, 2008.

Antibiotics	Resistant strains	
	Number	%
P, C	26	32.1
P, SXT	12	14.8
P, G	7	8.6
P, AMC, Ox, KF, E	12	14.8
P, Ox, KF, C, Cf	2	2.5
P, AMC, Ox, KF,C	8	9.9
P, Ox, KF, C, V/M	1	1.2
P, AMC, Ox, KF, SXT	1	1.2
P, AMC, Ox, KF, E, C	4	4.9
P, Ox, KF, E, C, SXT	1	1.2
P, KF, G, C, SXT, V/M	1	1.2
P, OX, KF, E, C, G, SXT	1	1.2
P, AMC, Ox, KF, E, G, C	1	1.2
P, AMC, Ox, KF, E, C, SXT	1	1.2
P, AMC, Ox, KF, E, G, C, Cf	1	1.2
P, AMC, Ox, KF, E, C, G, SXT	1	1.2
P, AMC, Ox, KF, E, G, C, Cf, V/M	1	1.2
Total	81	100

DISCUSSION: The *S. aureus* isolates showed a very high resistance to penicillin and cephalothin (98.8%, each) and chloramphenicol (43%). Studies conducted in Tikur Anbessa Hospital (Ethiopia) reported resistance of 56% for penicillin and 37% for chloramphenicol in 1997 [19]; 63.1% for ampicillin and 37% for penicillin in 2001 [20]. The highest resistance rates observed for penicillins could be due to an increased number of β-lactamase producing strains of *S. aureus* and other possible intrinsic factors.

This finding indicated that 50.6% of the isolates were found to be resistant to amoxicillin/clavulanic acid while 38.3% of the *S. aureus* isolates tested here were resistant to oxacillin. The number of oxacillin resistant isolates identified in this study is unusually high than previous studies [28, 29]. These differences might be due to several possible reasons such as poor nosocomial infection control in this hospital or MRSA become increasingly prevalent over time and counting of the intermediate isolates as resistant might also contribute for the increased resistance obtained.

In this study, intermediate bacterial isolates were counted as resistant because previous studies made a similar categorization and following them would increase the comparability of the findings; infections caused by them can't be treated with the normal dosage of the drug/s and the invivo effect of the drugs might be even worse than the invitro and thus it is safer to consider these isolates as resistant.

Detection of mecA gene and PBP2' may be a further requirement to justify this exceptionally high level of oxacillin resistant isolates which is not included in the present study.

Increased resistance rates of *S. aureus* observed in the present study against penicillinase resistant penicillins, cephalothin and chloramphenicol could be due to genetic variations in the strains, presence of more isolates whose resistance is mediated by mechanisms different from penicillinase production and continued increment of resistant strains from time to time.

Irrational prescribing, availability of antibiotics with out prescriptions and wide spread self-medication practice of antibiotics in the area [23, 24] might also have significant contribution for the higher rates of antibiotic resistance obtained in this study.

Gentamycin and sulphmthoxazole/trimethoprim were among the drugs that showed lower resistance: 19.7% and 12%, respectively. These drugs showed higher efficacy against *S. aureus* when compared with most previous studies.

In the present study, ciprofloxacin and vancomycin were the least resisted antibiotics. This agrees with most previous studies. A very high susceptaiblity of *S. aureus* to ciprofloxacin [25], vancomycin [2, 22, 26] or both was reported from Saudi Arabia, eight African hospitals, Antananarivo/Madagascar. Even though vancomycin was found to be the second most effective drug in this study, it can't be relied up on for treatment of all infections caused by *S. aureus*. This is because its usefulness is limited due to its pharmaceutical and pharmacokinetic properties, i.e. it is available only as injectable dosage form and it has least penetration to central nerveous system (CNS) so that it can't be used for CNS infections caused by *S. aureus* [18].

Ciprofloxacin was found to be the most effective antibiotic against *S. aureus.* Although the floroquinolones are not new antibiotics, many studies are still being conducted to assess their uses. Important features of this drug class include excellent bioavailability after oral administration, achievement of high tissue concentrations and a broad spectum of activity. Ingeneral fluoroquinolones are active against many gram-positve bacteria. They don't appear to be affected by β-lactamase enzymes or altered penicillin binding proteins. The quinolones have a unique mechanism of action; they inhibit two bacterial enzymes, DNA gyrase and topoisomerase IV that are essential for bacterial DNA synthesis. Because they target bacterial sites distinct from the site of action of other antibiotics, resistance is less likely to occur or slower to develop [22].

S. aureus was found to be more susceptible to sulphamethoxazole/trimethoprim, genatamycin and vancmycin than that observed in other studies, even in the same study area [5,7]. This may be because of the difference in study group of population or other factors such as some degree of bacterial resistance reversibility i.e., reducing the usage of an antibiotic that is resisted may help in combating resistance development; but this is not always guaranteed [22].

In the study a wide spread existence of multidrug resistance by the isolates was observed. It was found that multidrug resistance was higher for inpatient isolates than the outpatients'. There are several different combinations of the tested antibiotics which give very huge numbers of multidrug resistance patterns by the organism. The drug combinations resisted by the isolates contain two up to nine of the antibiotics tested.

CONCLUSION: In this study the *S. aureus* isolates exhibited very high degree of resistance to the antibiotics penicillin G, cephalothin, cloxacillin, oxacillin and chloramphenicol. The isolates were also multidrug resistant to several combinations of the tested antibiotics. Ciprofloxacin, vancomycin, gentamycin and sulphamethoxazole/trimethoprim had relatively lower resistance.

ACKNOWLEDGEMENTS: We would like to express our deepest gratitude to the study participants, Mr. Mi'o Ayana, Mr. Wakijra Kebede, Mrs. Tirfe Beyene, Mrs. Selamawit Tilahun (patient specimen collectors and laboratory assistants). We also like to acknowledge Jimma University Community Based Education Office for its financial support.

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How to cite this article:
Wubishet BL, Sabe ZS, Alemu HM and Z/Mariam FD: Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Strains From Patients In Ethiopia. *Int J Pharm Sci Res*. 3(12); 4889-4894.