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Staphylococcus aureus and its Antimicrobial Susceptibility Pattern in Patients, Nasalcarage of Health Personnel, and objects at Dessie referral hospital, Northern Ethiopia

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Abstract

Introduction: Staphylococcus aureus is one of the most common causes of healthcare and communityassociated infections. Its remarkable ability to acquire antimicrobial resistance mechanisms and advantageous pathogenic determinants has contributed to emergence of infections in both nosocomial and community settings. Objective: To determine prevalence of Staphylococcus aureus and antibacterial susceptibility patterns in patients, nasal carriage of health personnel and objects of Dessie Referral Hospital. Methods: Cross sectional study was conducted at Dessie Referral Hospital from February 01 to May 30, 2013. Using a convenient sampling technique, 180 specimens of pus, blood, nasal swab and swab from hospital objects were collected and cultured by standard procedure. Growth identification was based on colony morphology, Gram staining and results of biochemical tests. Antibacterial susceptibility testing was done by disk diffusion method on Mueller-Hinton agar. Result: Overall prevalence of Staphylococcus aureus was 40.5

Index terms— staphylococcus aureus, antimicrobial susceptibility, ethiopia.

catalase and coagulase positive and causes diseases through the production of toxins and enzymes and through direct invasion and destruction of tissues (1). It is one of the most common causes of healthcare-and community-acquired infections, such as localized cutaneous and life threatening systemic infections. Although most community infections are treated in the outpatient setting, some invasive infections, including bacteremia, septic arthritis, toxic shock syndrome, osteomyelitis, and endocarditis, have devastating complications and may require hospitalization (2, 3). Hospitalized patients are unusually at high risk of infection for various reasons, and tend to be more susceptible to infections. S. aureus causes more sever diseases in immunocompromised patients than in immune competent ones (4).

S. aureus is one of the most successful and adaptable human pathogens. Its remarkable ability to acquire antibiotic-resistance mechanisms and adventageous pathogenic determinants has contributed to emergence of infections in both nosocomial and community settings. However, because of different selective pressures, several notable differences exist between nosocomial-and community-acquired strains (5). Healthcare workers are potential source of hospitalacquired infections. Pathogens are transmitted by carriage on hands from inanimate objects present in the hospital setting (6). However, the role of fomites and the inanimate hospital environment (e.g. surfaces and medical equipment) in the transmission of healthcare associated infections is controversial (7). Nasal carriage of S. aureus plays a key role in the development of S. aureus infections. It is a major risk for the development of infection in patients undergoing hemodialysis, continuous ambulatory peritoneal dialysis, surgical patients, and patients with intravascular devices (8).

Multidrug-resistant strains of S. aureus, particularly methicillin resistant S. aureus (MRSA), pose a major clinical and epidemiological problem in hospitals, as they are easily transferred among hospital staff and patients (9). Antimicrobial resistance among nosocomial pathogens is a significant problem in many countries with severe

consequences including increased medical costs, morbidity and mortality of patients (10). Since the first isolation of MRSA in the United Kingdom in 1961 (11), increasing rates of methicillin resistance among S. aureus strains have been a cause for concern, especially in developed countries. Until recently, vancomycin was believed to have retained activity against all strains of MRSA; therefore, the spread of MRSA has led to increased vancomycin usage and hence increased selective pressure for the development of resistance (12). The first report of MRSA in Ethiopia was made from 1987-1988 and the overall MRSA isolation rate was 31% while 71% out of the MRSA strains were multiple drug resistant (13). Nosocomial infection causes substantial morbidity and mortality, prolong hospital stay of patients, and increase direct patient-care costs. Widespread uses of antibiotics, together with length of time over which they have been available have led to major problems of resistant organisms. S. aureus as a cause of various nosocomial infections has not been recognized in Dessie Referral Hospital. Studying staphylococcal nosocomial infections in the area is essential for early prevention and control, correct diagnosis and treatment, and reducing morbidity and mortality of hospitalized patients owing to this infection. The aim of this study was therefore to assess prevalence of S. aureus and its susceptibility pattern to antimicrobials in inpatients isolates, nasal carriage of hospital personnel and hospital objects of Dessie Referral Hospital.

1 II.

2 Material and Method a) Study area

The study was conducted in Dessie, capital of South Wollo Zone in Amhara Regional State Northern Ethiopia, located 401 km far from Addis Ababa, on the way to Asmara. This town has a latitude and longitude of 11 o 8N 39 o 38E/11.133 o N 39.633 O E with an elevation of between 2,470 and 2,550 meter above sea level. The town has an estimated total population of 151,094 of whom, 78,203 are women (14). Dessie has one referral hospital, three general hospitals (private), three health centers, five higher clinics (private) and one regional health research laboratory where culture and susceptibility tests are performed.

3 b) Study Design and period

A cross sectional study was conducted from February 01 to May 30, 2013.

4 III.

5 Population a) Source population

All patients visited Dessie referral hospital, all health personnel who were working in this hospital and Objects (blankets, floor and cupboards) which were being used by patients in the hospital.

6 b) Study population

All patients who were admitted to Dessie referral hospital and who had developed signs and symptoms of hospital acquired infection after 48hs of admission during the study period, all health personnel who were working in inpatient wards of the hospital and who were willing to participate in the study and the objects (blankets, cupboards and floor) which were being used by patients in the hospital.

7 c) Inclusion criteria

Patients who had signs and symptoms of hospital acquired infection after 48 hours of admission to hospital, and health personnel who had not antimicrobials within seven days of specimen collection during the study period.

8 Data Collection and Laboratory Methods

a) Socio-demographic data collection Data on socio-demographic characteristics from each study participant was collected using pretested questionnaire guided interview. b) Specimen collection Specimens were collected from the study participants using the standard operational procedures. Thirty six swabs of wound secretions were aseptically obtained from patients after patients were diagnosed as wound sepsis by a physician. The specimens were collected with sterile cotton swabs before the wound was cleaned with an antiseptic solution and 10ml of four blood samples were aseptically collected from each patient, and mixed into blood culture bottle containing 90ml of nutrient broth. Nasal swabs were taken from 35 health personnel with sterile cotton swab. A separate sterile cotton swab was passed into the anterior nares of both the nostrils and rotated in both directions and then

9 c) Sample size determination and sampling technique

Convenient sampling technique was used. All the 40 patients who had developed signs and symptoms of hospital acquire infection during the study period were included in the study. Thirty five volunteer health personnel in five inpatient wards (medical, surgical, gynecology, pediatric and orthopedic) were also included. In addition, 105 specimens were taken from Objects (blanket, cupboards and floor) that could be touched with hands of health personnel and patients within the five wards. placed into sterile test tube. One hundred five specimens were collected from Objects (blanket, cupboards and floor). Sterile cotton swabs moistened with normal saline was

rotated against the surface of objects to obtain specimens. All collected specimens were labeled and transported to Dessie Regional Health Research Laboratory for culturing and antimicrobial susceptibility testing. c) Bacterial isolation and identification Swab specimens were cultured onto mannitol salt agar and incubated at 35-37 o c for 24 hrs. Each culture was read and then sub-cultured onto blood agar and incubated at 35-37 o c for 24 hrs. Blood samples were incubated at 35-37 o c for 7-14 days (until growth was seen) and growth was sub-cultured on mannitol salt agar. Identification of growth was based on colony morphology, Gram staining and appropriate biochemical test. S. aureus is a gram positive, beta hemolytic, catalase, and coagulase positive.

10 d) Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates was performed using disk diffusion method on Muller-Hinton agar plates as per the National Committee for Clinical Laboratory standards (15). Single colony was selected and emulsified in 3ml sterile normal saline solution in a sterile test tube. The turbidity of the suspension was then adjusted to the density of a barium chloride standard (0.5 McFarland) in order to standardize the size of inoculums. A sterile cotton swab was dipped into the standardized suspension of the bacterial culture, squeezed against the sides of the test tube to remove the excess fluid and inoculated onto Mueller-Hinton agar and allowed to dry the flood. Thereafter, antimicrobial discs were placed on the agar with forceps and gently pressed down to ensure contact. The plates were then allowed to stand for 30 minutes for diffusion of active substance of the agents. Plates were inverted and incubated at 35-37 o c for 24 hrs. An inhibition zone diameter of each antimicrobial was then measured and interpreted as 'Resistant', 'Intermediate' and 'Sensitive' by comparing with recorded diameters of a control organism, ATCC25923 (16). Antimicrobials used, include oxacillin (1?g), vancomycin (30 ?g), penicillin G (10IU), tetracycline (30?g), sulphamethoxazole (25 ?g), chloramphenicol (30?g), gentamicin (10?g), ciprofloxacin (5?g), nalidixic acid (30?g), amoxicillin (10?g), ceftriaxone (30?g) and kanamycin (30 ?g). All media and antibiotics used were Oxoid, UK products. e) Quality control Pre-tested questionnaire guided interview was used for data collection on socio-demographic characteristics. Specimens were collected and processed according to the standard operating procedure. Sterility of culture media was checked by incubating 5% of the batch at 35-37 o c overnight and observed for bacterial growth and the standard reference strains, S aureus ATCC25923 (16) was tested weekly as controls on the biochemical tests and agar plates including Mueller Hinton agar with antimicrobial discs to assure testing performance of the potency of antimicrobial discs. f) Data processing and analysis Data was checked for its completeness and entered and analyzed using SPSS statistical software version 16.0. Results were explained in words and tables. Proportions for categorical variables were compared using chi-square test. In all cases P-value less than 0.05 was taken as statistically significant.

11 g) Ethical consideration

The project was started after ethical clearance was obtained from the Ethical Clearance Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Written informed consent was obtained from the study participants. Permission was obtained from Dessie Referral Hospital. For each confirmed infection cases, the responsible clinician of the participant was informed and treatment was started as per the culture result and antimicrobial susceptibility pattern. Confidentiality of information of the participants was maintained. Different antimicrobials showed different antimicrobial susceptibility patterns in different study participants. Resistance pattern of isolates for nalidixic acid (91.3%), penicillin G(100%) and amoxicillin (100 %) were demonstrated in inpatient, whereas, in health personnel, the level of resistance were 85.7% for nalidixic acid, 92.9% penicillin G and 78.6% amoxicillin. In objects, the level of resistance for nalidixic acid, penicillin G and amoxicillin were 97.2% 83.3% and 75% respectively (table5). was recorded in 79 (95.9 %) of S. aureus isolates. About half, 39(53.4%) of the isolates were demonstrated resistant to at least five antibacterials. Four (5.5%), 2

12 VI.

13 Results

(2.7%), 17 (23.3%) and 11(15.1%) of the S. aureus were found to be resistant for one, two, three and four antibacterials respectively. None of the S. aureus isolates were susceptible for all tested antibacterials (table6).

14 Discussion

Results of previous studies which are also confirmed in this study had shown that S. aureus is the common cause of nosocomial infection. Overall prevalence of S. aureus infection in this study (table1) is comparable to other study done elsewhere in the world (37.3%) (17). The present study also showed that the frequency of S. aureus isolated from hospital objects of different wards (table2) is consistent with studies done in Gondar and Nigeria (17,18). One of the important sources of S. aureus for nosocomial infection is nasal carriage among hospital personnel (19). In this study, prevalence of S. aureus isolates from nasal carriage of health personnel and hospital objects (table1) are comparable with other studies done in Gondar, Pakistan and Cameron (17,20,21). The occurrence of S. aureus in hospital objects patients. This may also account for the high incidence of the organism observed in health personnel. Out of 50 isolates of S. aureus from health personnel and objects, 19 had identical

antibiogram pattern with the isolates of patients. This specifies that the objects and/or the health personnel may be the source of S. aureus infection in this study.

Antimicrobial resistance patterns of S. aureus infection in the present study (table4) is comparable with the previous study done in Dessie (22), but the susceptibility of ciprofloxacin and ceftriaxone are fall from the previous study which had such antimicrobial susceptibility patterns as 95.4% and 80% respectively. It may be due to overuse of it as empirical treatment.

S. aureus isolated in this study showed the highest vancomycin sensitivity pattern (table4) which is similar with the previous studies in Kontagora and Suleja Area of Niger State, in Gondar and Nigeria (17,23,24) The highest susceptibility of S. aureus to in our study may be due to its uncommon use or being a new medication. In this study; however, S aureus was highly resistant to penicillin G, amoxicillin and nalidixic acid (table 4). This result is in line with previous studies 25), respectively, but in the case of amoxicillin, our result is completely showed disparity to the study in Bahar-dar (26), which reported S. aureus as 100% susceptible. This difference may be due to inappropriate and incorrect administration of antibacterials as empiric therapies and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organism in the community in the study area. Forty four percent of S. aureus isolates were resistant to oxacillin which is similar to the previous studies in Kontagora and Suleja Area of Niger State and Jimma (23,25).

Multi drug resistance patterns (table 6) of isolates of S. aureus in the current study is higher than the previous studies in Gondar (17) and Dessie (22) but in line with the previous study in Gondar (27). This is probably due to empirical use of broad-spectrum antibacterials, lack of culture and antimicrobial susceptibility tests and non adherence to hospital antimicrobial policy. About 24%, 16%, 6%, and 3% of S. aureus isolates were found to be resistant to three, four, two and one of the tested antibacterials respectively. No one of the isolates was susceptible to all of the tested antibacterials and also none of the S. aureus isolates were pan-resistant (resistant to all the tested antibacterialsl).

VIII.

15 Limitation of the Study

In the present study, the antimicrobial susceptibility pattern was used in an attempt to identify possible cross infection from health personnel and/or hospital objects has a limitation. Since unrelated colony of a single species can undergo evolutionary convergence to the same resistance phenotype under antibacterial selective pressure through mutation and genetic exchange (28), unless confirmed by genomic analysis, no definite conclusions can be drawn with regard to the role of the possible sources of infection.

IX.

16 Conclusion

The present study indicated that S. aureus is still the most common cause of nosocomial infection and hospital objects which were being used by inpatients may be a source of nosocomial S. aureus infections in this hospital. It also demonstrated that health personnel are at risk of the infection and can be a potential source of nosocomial S. aureus infections. In this study MDR was very high and most of the isolates showed high levels of resistance to commonly used antibacterials. However, gentamicin (84%) had high activity against most of the isolates in vitro when compare to other tested antibacterials. Susceptibility rate of S. aureus to vacomycin in this study was 100%.

In the absence of culture and antibacterial susceptibility testing, vancomycin and gentamicin are the best therapeutic options to treat S. aureus infections. In order to confirm S. aureus cross infections among patients, health personnel and objects, further study with the aid of phage typing and other molecular studies should be done.

X.

Figure 1:

3

a) Prevalence of S. aureus infection in inpatients, nasal carriage of health personnel and hospital objects
Of 180 specimens collected, 40(22.2%) were from inpatients, 35(19.4%) from health personnel and 105(58.3%) from hospital objects. From 40 inpatients, 36(90%) had undergone surgery and developed hospital acquired wound infections and the other 4 (10%) were blood samples. A total of 73 S. aureus isolates were identified and of which, 23(31.5%), 14(19.2%), and 36(49.3%) were from inpatients, health personnel and objects respectively(table1)..

Figure 2: Table 3 :

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	Antimicrobial susceptibility patterns			
Antimicrobial agents	Susceptible	Resistance	Intermediate	Total
Oxacillin	41(56.2%)	32 (43.8%)	0(0%)	73(100%)
Vancomycin	73(100%)	0 (0%)	0(0%)	73(100%)
penicillin G	6(8.6%)	66 (90%)	1(1.4%)	73(100%)
Tetracycline	45(62.9%)	28(37.1%)	0(0%)	73(100%)
Sulphamethoxazole	35(47.1%)	33(45.7%)	5(7.1%)	73(100%)
Chloramphenicol	47(62.9%)	25(35.7%)	1(1.4%)	73(100%)
Gentamicin	62(84.3%)	5(7.1%)	6(8.6%)	73(100%)
Ciprofloxacin	45(62.9%)	27(35.7%)	1(1.4%)	73(100%)
Nalidixic acid	1(1.4%)	68(92.9%)	4(5.7%)	73(100%)
Amoxicillin	10(14.3%)	61(82.9%)	2(2.9%)	73(100%)
Ceftriaxone	34(48.6)%	35(47.1%)	4(4.3%)	73(100%)
kanamycin	47(62.9%)	26(37.9%)	0(0%)	73(100%)

Figure 3: Table 4 :

5

Dessie Referral Hospital, May 2013
Study participants and antimicrobial susceptibility patterns

Antimicrobial agents	Inpatients			Health personnel			Objects		
	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%
Oxacillin	14(60.9)	9(39.1)	0(0)	11(78.6)	3(21.4)	0(0)	16(44.4)	20(55.6)	0(0)
Vancomycin	23(100)	0(0)	0(0)	14(100)	0(0)	0(0)	36(100)	0(0)	0(
penicillin G	0(0)	23(100)	0(0)	1(7.1)	13(92.9)	0(0)	5(13.9)	30(83.3)	1(1.4
Tetracycline	16(69.6)	7(30.4)	0(0)	7(50)	7(50)	0(0)	22(61.1)	14(38.9)	0(0)
Sulphamethoxazole	12(52.2)	9(39.1)	2(8.7)	8(57.1)	5(35.7)	1(7.1)	15(41.7)	19(52)	2(
Chloramphenicol	16(69.6)	6(26.1)	1(4.3)	12(85.7)	2(14.3)	0(0)	19(52.8)	17(47.2)	0(0)
Gentamicin	22(95.7)	0(0)	1(4.3)	14(100)	0(0)	0(0)	26(72.2)	5(13.9)	5(
Ciprofloxacin	15(65.2)	8(34.8)	0(0)	10(71)	3(21.4)	1(7.1)	20(55.5)	16(44.5)	0(0)
Nalidixic acid	0(0)	21(91.3)	2(8.7)	0(0)	12(85.7)	2(14.3)	1(2.8)	35(97.2)	0(0)
Amoxicillin	0(0)	23(100)	0(0)	2(14.3)	11(78.6)	1(7.1)	8(22.2)	27(75)	1(
Ceftriaxone	10(43.5)	10(43.5)	3(13)	10(71.4)	3(21.4)	1(7.1)	14(38.9)	22(61.1)	0(0)
kanamycin	18(78.3)	5(21.7)	0(0)	10(71.4)	4(28.6)	0(0)	19(52.8)	17(47.2)	0(0)

S= susceptible R= resistance I= intermediate

d) Multi drug resistance pattern of S. aureus isolates from inpatients, heath personnel and objects
Multi-drug resistance (resistance to ?2 drugs)

Figure 4: Table 5 :

6

May 2013

Figure 5: Table 6 :

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