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Epidemiological Analysis of Clinical Isolates of *Staphylococcus aureus* in Ile-Ife, Nigeria

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Abstract: The isolation, frequency and antibiotic susceptibility pattern of clinical isolates of *Staphylococcus aureus* at the Obafemi Awolowo University Teaching Hospitals, Ile-Ife, was studied. Staphylococcal isolates obtained from the various specimens were collected from the Microbiology Laboratory of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife and also from anterior nares of apparently healthy medical personnel of the hospital. Bacterial identification was based on standard methods which include, colonial morphology, catalase and gram stain reaction. Confirmatory test was by coagulase and DNAse tests. The standard disk agar diffusion method was done employing commercially prepared antibiotic discs (Abtek, Habdiscs) of ampicillin 10 μg, chloramphenicol 10 μg, cloxacillin 5 μg, gentamicin 10 μg, penicillin 1 i.u., streptomycin 10 μg and tetracycline 10 μg. A total of 97 isolates were obtained from 54 male and 32 female patients and 11 healthy medical personnel. A high proportion of isolates (58.8%) were recovered from the middle-aged group. About one-third (28.9%) of all the isolates were obtained from wound samples, which was mostly recovered in patients less than 20 years. The resistance profile is shown as follows: penicillin (100%), ampicillin (62%), tetracycline (45.4%), cloxacillin (47.4%), chloramphenicol (24.7%), streptomycin (23.7%), gentamicin (22.7%) and erythromycin (11.3%). Thirty isolates (30.9%) were resistant to at least three classes of antibiotics. There was no significant difference in resistance to penicillin and chloramphenicol when present data were compared with an earlier report in Ile-Ife. Low rates of resistance to erythromycin were noted in this study and previous investigations conducted in Lagos and Jos, Nigeria. This survey clearly indicates that careful surveillance of multiple resistant *S. aureus* is important in infection control and stringent measures against nosocomial infections associated with antibiotic-resistant *S. aureus* are needed in this health institution. Clinicians should enlighten patients on the consequences of indiscriminate use of penicillin and other anti-microbial agents.

Key words: *Staphylococcus aureus*, antibiotic susceptibility, multiple resistant

INTRODUCTION

Staphylococcus aureus is associated with a variety of clinical infections including septicemia, pneumonia, wound sepsis, septic arthritis, osteomyelitis and post-surgical toxic shock syndrome with substantial rates of morbidity and mortality[1,2]. Its intrinsic virulence, versatility of pathogenic strategies, ability to cause a diverse array of life-threatening infections and its capacity to adapt to different environmental conditions makes it one of the most important human pathogens[3,4]. Bacterial resistance to antibiotics is a serious problem in health institutions: treatment failures extend the length of hospital stay, or demand repeated physician visits; hospital beds are blocked to new patients and productive time is lost[5]. The adaptation of *S. aureus* to the modern hospital environment has been marked by the acquisition of drug resistance genes soon after antibiotic introduction. This has led to the emergence of strains resistant to penicillin in 1944[6], methicillin in the early 1960s[7] and now recently, vancomycin[8,9]. There are few published reports on antimicrobial susceptibility of *S. aureus* in Ile-Ife, Nigeria in spite of the established fact that multidrug-resistant *S. aureus*, especially methicillin-resistant *S. aureus* (MRSA) is an important health problem worldwide. This study characterized and determined the antibiotic susceptibility pattern of *S. aureus* obtained from hospital patients and personnel at a referral hospital in Ile-Ife, Nigeria. Furthermore, data from this study were compared with previous reports to ascertain changes in the susceptibility pattern of this pathogen, in this health institution. Information on drug-resistance pattern could assist clinicians in the selection of empiric antimicrobial therapy in the treatment of *S. aureus* infections.

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MATERIALS AND METHODS

Source and collection of isolates: Staphylococcal isolates from various clinical samples were obtained from the Microbiology Laboratory of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife. In addition, isolates from nasal culture of medical personnel in the health institution were included in this study. The health facility is a major referral center for over a million people living within forty-kilometre radius from Ile-Ife, in South Western Nigeria. The isolates were stab-inoculated onto freshly prepared nutrient agar in sterile cryovials. They were then transferred to the research laboratory, inoculated into sterile nutrient broth and incubated at 37°C overnight. The overnight broth cultures were streaked on Mannitol Salt Agar (MSA) plates and incubated at 37°C for 48 h. Growth and fermentation on MSA were examined and noted. A well-separated single colony from the MSA plate was picked, inoculated onto nutrient broth and sub-cultured on nutrient agar for further characterization. Bacterial identification as staphylococci was based on colony morphology on MSA, positive catalase and gram stain reaction. Isolates were confirmed as *S. aureus* based on positive coagulase and DNAse tests.

Antimicrobial susceptibility testing: Antibiotic susceptibility testing was performed by the disk diffusion assay as described by Bauer *et al.*[10]. The antibiotics included penicillin (1 unit), ampicillin (10 μg), chloramphenicol (10 μg), cloxacillin (5 μg), gentamycin (10 μg), streptomycin (10 μg) and tetracycline (10 μg). Interpretation of the diameter of growth inhibition was done using the AB Biodisk manual. Organisms were adjudged as either sensitive or resistant. *S. aureus* ATCC 25923 was the control strain in every test run.

RESULTS

Isolation of *S. aureus* in the age groups and from various types of specimens: Cultures obtained from the various clinical and nasal samples yielded ninety-seven staphylococcal isolates. They were Gram-positive, mannitol and catalase-positive and confirmed as *S. aureus* by positive coagulase and DNAse tests. Demographic analysis showed that 62 isolates were obtained from males and 35 from females. More than half of the total number of isolates (57 of 97; 58.8%) were recovered from the middle-aged group (20-49 years), followed by patients who were less than 20 years (30 of 97; 30.9%) and 10.3% for the group aged 50 years and above (Fig. 1A). Analysis of the *S. aureus* isolates on the basis of source of specimen showed (Fig. 1b) that 28 (28.9%) were obtained from wound samples; 17 (17.5%) from urine; 11 (11.3%) from anterior nares of healthy hospital personnel; 10 (10.3%) from blood; 9 (9.28%) from vaginal swab; 8 (8.25%) from sputum and 4 (4.1%) from semen. Others include three isolates each from pus aspirate, urethral discharge and eye swab, respectively. Only one isolate was obtained from ear swab. A total of 64.3% of isolates obtained from wound samples were recovered in patients less than 20 years and 88.2% of isolates from urine samples in the middle aged group.

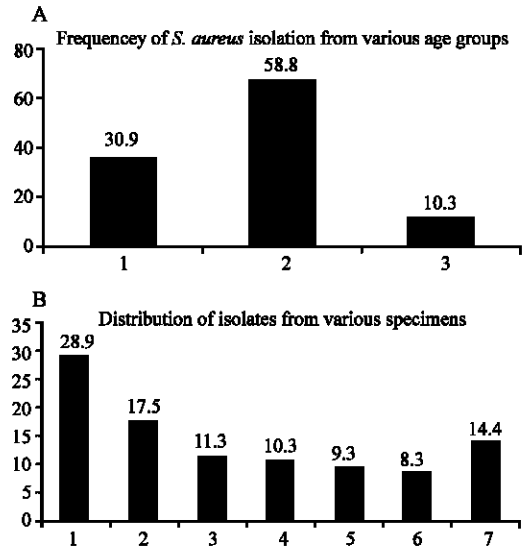

Fig. 1: Frequency of *S. aureus* isolation in various age groups and from different types of specimens. Distribution of *S. aureus* isolated in the age groups (A) and from various specimens (B). Total number of isolate was 97. The isolation rate from each age group and type of specimen are shown as percentages. A: Age Groups 1: <19 years, 2: 20-49 years, 3: >50 years, B: Source of specimen 1: Wound, 2: Urine, 3: Nasal Swab, 4: Blood, 5: Vaginal Swab, 6: Sputum, 7: Others

Antibiotic sensitivity pattern of *S. aureus* isolates: All isolates tested were resistant to penicillin (Table 1). A total of 61.9% were resistant to ampicillin, 47.4% to cloxacillin, 45.4% to tetracycline, 23.7% to streptomycin, 22.7% to gentamycin and 11.3% to erythromycin. Multi-resistant strains (defined as resistance to at least three classes of antibiotics) were found among the isolates. Thirty isolates (30.9%) were resistant to at least three groups of antibiotics while two isolates were resistant to

Table 1: The susceptibility pattern of clinical isolates of *S. aureus* in OAUTHC

Antibiotics	No. tested N = 97	Resistant No./(%)	Sensitive No./(%)
Penicillin	97	97/(100.0)	0/(0.0)
Ampicillin	97	60/(61.9)	37/(38.1)
Cloxacillin	97	46/(47.4)	51/(52.6)
Tetracycline	97	44/(45.4)	53/(54.6)
Chloramphenicol	97	24/(24.7)	73/(75.3)
Streptomycin	97	23/(23.7)	74/(76.3)
Gentamycin	97	22/(22.7)	75/(77.3)
Erythromycin	97	11/(11.3)	86/(89.7)

Table 2: Resistance pattern of multi-resistant strains in relation to sample

	Multi-resistance pattern									
Sample	A	B	C	D	E	F	G	H	I	Sub-total
Sputum	2	-	-	-	1	-	-	-	-	3
Urine	-	-	-	-	-	1	-	-	1	2
Urethral discharge	1	-	-	-	-	-	-	-	-	1
Semen	-	-	-	1	-	-	-	-	-	1
Nasal swab	-	3	-	-	-	-	-	-	-	3
Urine/MSU	3	1	-	-	-	1	-	-	1	6
ECS/HVS/VS	-	-	-	-	1	-	2	-	-	3
Blood	-	-	-	-	-	-	1	1	-	2
Wound swab	-	3	3	-	2	-	-	-	-	8
Eye swab	-	-	1	-	-	-	-	-	-	1
Total	6	7	4	1	4	2	3	1	2	30

A: Pen, St, Tet (PST)/ Pen, Gen, Tet (PGT)
B: Pen, Chl, Tet (PCT)
C: Pen, Chl, St (PCS)/Pen, Chl, Gen (PCG)
D: Pen, Ery, St (PES)
E: Pen, Chl, Ery, St (PCES)
F: Pen, Ery, St, Tet (PEST)
G: Pen, Chl, St, Tet (PCST)
H: Pen, Chl, Ery, Tet (PCET)
I: Pen, Chl, Ery, Gen, Tet (PCEGT)/Pen, Chl, Ery, St, Tet (PCEST)

Pen: Penicillin
St: Streptomycin
Tet: Tetracycline
Gen: Gentamycin
Chl: Chloramphenicol
Ery: Erythromycin

all the classes of antibiotics tested. Resistance to penicillin, streptomycin and or gentamycin and tetracycline was 20% while that for penicillin, chloramphenicol and tetracycline was 23.5%, respectively. Most of the multi-resistant strains were isolated from wound and urine samples (Table 2).

DISCUSSION

Staphylococcus aureus has been a versatile and dangerous pathogen in humans. Nosocomial as well as community-acquired infections caused by this organism have increased steadily, with devastating effects[11]. Specific epidemiological data on the characterization, antibiotic susceptibility and follow up on the spread of staphylococcal infections has therefore been an essential ingredient to guide empiric antibiotic therapy in the ambulatory setting. Data from this study were compared with previous reports to understand the current trend of *S. aureus* resistance to some common antibiotics in the hospital environment.

A total of ninety-seven *S. aureus* isolates, confirmed by slide and tube coagulase and DNAse tests were investigated. More than half of these isolates were recovered from male patients within the middle-aged group (20-49 years). This could be attributed to the active involvement of this category of people in day-to-day activities. Wound samples accounted for the highest number of *S. aureus* isolates recovered in this study (28.9%), which was across all age groups. Wound infection is regarded as the most common nosocomial infection, especially in patients undergoing surgery[12]. It has been associated with prolonged hospital stay, increased trauma care and treatment[13]. This study confirms the report of Ako-Nai *et al.*[14] and Shittu *et al.*[15] that *S. aureus* is the leading etiologic agent of wound infection in this health institution.

The emergence of resistance to antimicrobial agents is a global public health problem, particularly in pathogens causing nosocomial infections[1]. The widespread use of antibiotics by health professionals, unskilled practitioners and laypersons, poor drug quality, unhygienic conditions and inadequate surveillance have contributed to the emergence of antibiotic-resistant microorganisms which is considered to be a major problem in most hospitals[16]. The effect has led to erroneous empirical selection of either ineffective or expensive drugs, prolonged hospitalization and higher mortality. A total of thirty isolates (30.9%) were resistant to at least three classes of antibiotics and most of these strains were isolated from wound and urine samples. Resistance to penicillin, streptomycin and tetracycline and penicillin, chloramphenicol and tetracycline were predominant. The low-level resistance to erythromycin in this study supports the report of Eykyn[17] and studies conducted

Table 3: Comparative analysis of resistance pattern of *S. aureus* from previous and present study

Source of isolates	Reference	Location	No. of isolates	Antibiotics (% resistance) P	T	ST	CHL	MT	ERY	GEN
Nasal	Lamikanra *et al.*[21]	Ile-Ife	167	94.0	31.1	10.2	14.4	0.4	1.2	ND
Clinical	Ako-Nai *et al.*[20]	Ile-Ife	166	99.0	78.0	25.0	19.0	9.0	3.0	2.0
Nasal	Ako-Nai *et al.*[22]	Ile-Ife	37	100.0	84.0	35.0	24.0	19.0	11.0	8.0
Wound	Kolawole and Shittu[23]	Ile-Ife	80	100.0	27.5	100.0	15.0	ND	9.0	6.0
Clinical	Obi *et al.*[18]	Lagos	122	100.0	80.0	60.0	40.0	21.0	15.0	10.0
Clinical	Egah *et al.*[19]	Jos	200	92.5	75.0	ND	ND	ND	17.5	57.5
Clinical	Present study	Ile-Ife	97	100.0	45.4	23.7	24.7	ND	10.3	22.7

P: Penicillin, T: Tetracycline, ST: Streptomycin, CHL: Chloramphenicol, MT: Methicillin, ERY: Erythromycin, GEN: Gentamycin, ND: Not Determined

in Lagos and Jos, Nigeria[18,19]. This could be attributed to the fact that erythromycin is a penicillinase stable, expensive and less abused antibiotic compared with penicillin in Nigeria.

Data on resistance pattern of *S. aureus* in this study and past surveys in Ile-Ife were compared (Table 3). There was no significant difference in the resistance of *S. aureus* to penicillin and chloramphenicol. Low-level resistance to erythromycin was noted. However, percentage resistance of *S. aureus* to streptomycin and tetracycline varied and it appears that the level of resistance to gentamycin is increasing. In order to ascertain if there has been a change in the resistance pattern of *S. aureus* obtained from clinical samples over the years, we compared our data with that of Ako-Nai *et al.*[20], which was conducted in the same health institution. An increase in resistance to erythromycin (3%; 10.3%) and gentamycin (2%; 22.7%) was noted. However, resistance to tetracycline was lower in this study as compared with the 1991 report (78%; 45.4%). Data on susceptibility pattern of *S. aureus* isolates obtained from clinical samples in two studies in Nigeria were compared with our survey. As expected, there was no significant difference in resistance to penicillin. Resistance to tetracycline in this study was the lowest and the resistance level to erythromycin remained relatively unchanged.

The trend in *S. aureus* resistance against first-line antibiotics within our study area has not changed, although it appears that resistance to gentamycin is increasing. High-level resistance to common antibiotics was observed in most of the strains studied. This is an indication that control measures have to be put in place, particularly in the administration of antibiotics in our hospitals. Clinicians should enlighten patients on the consequences of indiscriminate use of penicillin and other anti-microbial agents. Studies that involve more hospitals in Ile-Ife are needed in order to have an understanding of the occurrence and changes in antibiotic-resistance profile of this pathogen, in this study area.

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REFERENCES

1. Centers for Disease Control and Prevention, 1999. National nosocomial infections surveillance system report. Data summary from January 1990-May 1999. Issued June 1999. Am. J. Infect. Control, 27: 520-532.
2. Shopsin, B. and B.N. Kreiswirth, 2001. Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus*. Emerging Infectious Dis., 7: 323-326.
3. Oliveira, D.C., A. Tomasz and H. de Lencastre, 2002. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. Lancet Infectious Dis., 2: 180-189.
4. Lowy, F.D., 2003. Antimicrobial resistance: The example of *Staphylococcus aureus*. J. Clinical Investigation, 111: 1265-1273.
5. Livermore, D.M., 2003. Bacterial resistance: Origins, epidemiology and impact. Clinical Infectious Disease, 36: S11-23.
6. Barber, M. and M. Rozwadowska-Dowzenko, 1948. Infection by penicillin-resistant staphylococci. Lancet, pp: 641-644.
7. Jevons, M.P., 1961. Celbenin-resistant staphylococci. British Medical J., 1: 124-125.
8. Anonymous, 2002. Vancomycin-resistant *Staphylococcus aureus*-pennsylvania. Morbidity and Mortality Weekly Report, 51: 902.
9. Anonymous, 2002. *Staphylococcus aureus* resistant to vancomycin-United States. Morbidity and Mortality Weekly Report, 51: 565-567.
10. Bauer, A.W., W.W. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by standardized single disc method. Am. J. Clinical Pathol., England, 45: 493-496.
11. Lowy, F.D., 1998. *Staphylococcus aureus* infections. New England J. Medicine, 339: 520-532.
12. Dionigi, R., F. Rovera and G. Dionigi *et al.*, 2001. Risk factors in surgery. J Chemother., 13: 6-11.
13. Bowler, P.G., B.I. Duerden and D.G. Armstrong, 2001. Wound microbiology and associated approaches to wound management. Clinical Microbiol. Rev., 14: 244-269.
14. Ako-Nai, A.K., A.B. Lamikanra and A.O. Onipede, 1995. Incidence of pathogenic microorganisms in clinical specimens from hospitals in South-Western Nigeria. East African Medical J., 72: 436-441.

15. Shittu, A.O., D.O. Kolawole and E.A.R. Oyedepo, 2003. Wound infections in two health Institutions in Ile-Ife, Nigeria: Results of a cohort study. Osteomyelitis and Wound Manag., 49: 52-57.
16. World Health Organization, 1999. The Global Threat of Antibiotic Resistance. Publication of the World Health Organisation. Chapter 3.
17. Eykyn, S.J., 1996. Staphylococci. In: Westherall, D.J., J.G.G. Ledingham and D.A. Warrell (Eds.,). Oxford Textbook of Medicine, Oxford: Oxford University Press, pp: 523-533.
18. Obi, C.L., A.E. Iyiegbuniwe, D.K. Olukoya, C. Babalola, E.O. Igumbor and A.A. Okonta, 1996. Antibiograms and plasmids of *Staphylococcus aureus* and coagulase negative staphylococci isolated from different clinical sources. Central African J. Medicine, 42: 258-261.
19. Egah, D.Z., C.S.S. Bello, E.E. Banwat and J.A. Allanana, 1999. Antimicrobial Susceptibility pattern of *Staphylococcus aureus* in Jos, Nigeria. Nigerian J. Medicine, 2: 58-61.
20. Ako-Nai, A.K., A.D. Ogunniyi, A. Lamikanra and S.E.A. Torimiro, 1991. The characterisation of clinical isolates of *Staphylococcus aureus* in Ile-Ife, Nigeria. J. Medical Microbiol., 34: 109-112.
21. Lamikanra, A., B.D. Paul, O.B. Akinwole and M.O. Paul, 1985. Nasal carriage of *Staphylococcus aureus* in a population of healthy Nigerian students. J. Medical Microbiol., 19: 211-216.
22. Ako-Nai, A.K., A.D. Ogunniyi, A. Lamikanra and S.E.A. Torimiro, 1991. A survey of nasal carriage of *Staphylococcus aureus* in Ile-Ife, Nigeria. Ann. Tropical Paediatrics, 11: 41-45.
23. Kolawole, D.O. and A.O. Shittu, 1995. Multi-resistant *Staphylococcus aureus* from septic wounds in Nigeria. Biomed. Lett., 52: 245-252.