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Prevalence and antibiotic susceptibility pattern of *staphylococcus aureus* isolated from various clinical specimens in south east Nigeria

Abstract

The global problem of increasing trend in antimicrobial resistance is particularly pressing in the developing countries, where antibiotic resistant bacteria is often the severe etiologic agent in hospital-acquired infections. This study was aimed to evaluate the frequency and antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from various clinical specimens of out-patients attending Abia State University Teaching Hospital Aba, Nigeria. A total of 424 various clinical specimens comprising urine, pus, high vaginal swab, wound swab, throat swab, blood and stool were collected and cultured on Mannitol salt and MacConkey agar. *S. aureus* was identified based on their conventional cultural characteristics, Gram staining reaction and standard biochemical tests. All the isolates were tested for antimicrobial susceptibility by the disk diffusion technique according to the Clinical and Laboratory Standards Institute guidelines on Muller Hinton Agar. In all, 104 *Staphylococcus aureus* strains were isolated from the 424 clinical specimens (24.5% prevalence rate) with 40 (38.5%) of the isolates being methicillin resistant *S. aureus* (MRSA) and 64(61.5%) being methicillin susceptible *S. aureus* (MSSA) respectively. The highest prevalence was recorded in wound swab 10 (29.4%) while the least was observed in blood specimen 1(6.7%). Generally, the isolates showed high resistance to ampicillin (76.9%), penicillin G (81.7%), Nalidixic acid (72.1%), chloramphenicol (70.1%) and were considerably sensitive to gentamycin (68.3%), ciprofloxacin (61.5%) and cefpodoxime (66.3%). Statistical analysis showed that there was no significant difference on rate of resistance across the various clinical specimens ($P< 0.05$). This study highlighted high prevalence and antibiotic resistance; thus, the need to adopt new strategies in the control of antibiotic resistance cannot be overemphasized.

Keywords: *staphylococcus aureus*, antibiotic resistance, Nigeria

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Abbreviations: MRSA, methicillin resistant *staphylococcus aureus*; MSSA, methicillin susceptible *staphylococcus aureus*; CLSI, clinical laboratory standard institute

Introduction

Staphylococcus aureus has emerged as one of the main important human pathogens, and has over the past decades, been a leading cause of hospital and community-acquired infections.[1] Staphylococcal infections give rise to a wide spectrum of symptoms and diseases in humans. The bacterium is well characterized and known to have a diverse arsenal of virulence factors that causes a prominent inflammatory response.[2,3] This pathogen affects both immune competent and immuno compromised individuals, frequently resulting in high morbidity and with complications, which constitute problem to health care institutions.[4] *S. aureus* has been reported by several studies as the causative agent of wide variety of diseases of supportive infections such as boil, wound infection, pustule, subcutaneous and sub-mucosa abscesses, osteomyelitis, mastitis, impetigo, septicemia, meningitis, bronchopneumonia, food poisoning, a common cause of vomiting and diarrhea, and urinary tract infections. It is also the most common cause of infections in hospitals with high prevalence among newborn babies, surgical patients, malnourished persons, patients with diabetes and chronic diseases.[5–8]

Since the introduction of antimicrobials, bacteria have developed mechanisms for resisting the effects of antibiotics. The emergence of multidrug resistance in Gram-positive bacteria (pneumococci, enterococci and staphylococci) is a particularly important development. Perhaps the pathogen of greatest concern is *S. aureus,* because of its intrinsic virulence, its ability to cause an array of life threatening conditions, and its capacity to adapt to different environmental conditions.[9] *S. aureus* is known to be notorious in the acquisition of resistance to new drugs and continues to defy attempts at medical control. The resistance of *S. aureus* isolates to commonly used antibiotics in Nigeria and other different parts of the world has been widely reported.[10–16] This increased emergence of resistant strains has being attributed to the indiscriminate use of antibiotics in both human and veterinary medicine especially in the developing countries. Since the emergence of *S. aureus* strains with resistance to methicillin shortly after its introduction into clinical medicine,[17] it has established itself as a leading agent for a wide range of infections. Methicillin resistant *Staphylococcus aureus* (MRSA) has become a common problem in hospitals, community and livestock causing a wide range of acquired infections.[18–20] Furthermore, the prevalence of multi-drug methicillin-resistant *S. aureus* (MRSA) with very limited treatment choice is also on the increase.[21–24] Many strains of *S. aureus* carry a wide variety of multi-drug resistant genes on plasmids, which aid the spread of resistance even among different species.[25]

In Nigeria, most symptomatic patients usually indulge in indiscriminate use of antibiotics before consulting the physicians when they could no longer control the symptomatic situations. The

physicians on the other hand usually treat the patients with broad-spectrum antibiotics before microbiological investigations.[26] These widespread indiscriminate use and inappropriate prescription of antibiotics in the treatment of bacterial infections are significant contributing factors to the emergence and spread of bacterial resistance to the commonly used antimicrobial agents.[27] Thus, there is need for continuous and regular antimicrobial resistance surveillance in the country in order to guide empirical therapy and to provide adequate control strategies to combat this public health problem. Therefore, the aim of the current study was designed to evaluate the frequency and antibiotic susceptibility pattern of *S. aureus* isolated from various clinical specimens of outpatients attending Abia State University Teaching Hospital Aba, a tertiary health institution in southeast Nigeria.

Materials and methods

Samples collection, cultivation and identification of *S. aureus*

A total of 424 clinical specimens comprising urine, pus, high vaginal swab, wound swab, throat swab, blood and stool were collected from Abia State university Teaching Hospital Aba and cultured on Mannitol salt and MacConkey agar (Oxoid, England). The specimens were incubated overnight at 37ºC. All sampling procedures were in accordance with guidelines of the National Health Research Ethics Committee, Nigeria (www.nhrec.net). Emergent colonies were confirmed to be *S. aureus* by catalase and coagulase tests.[28]

Antimicrobial susceptibility tests

The modified Kirby-Bauer disc diffusion technique[28] was used to determine the susceptibility of the isolates to the following antibiotics: chloramphenicol 30µg, tetracycline 30µg, erythromycin 5µg, oxacillin 5µg (for detection of MRSA), gentamycin 10µg, ampicillin 10µg, penicillin G 10IU, Nalidixic acid 30µg, ciprofloxacin 5µg, cefpodoxime 30µg and Cefotaxime 30µg (Oxoid, England). The standard suspension of each isolate that matched 0.5 McFarland standard was used to swab the surface of Mueller Hinton agar (Oxoid, England) plate containing antibiotics impregnated discs. The plates were allowed to dry for 15mins and were incubated overnight at 37°C. The results of the antibiotics susceptibility testing were interpreted based on the guidelines of the Clinical Laboratory Standard Institute.[29,30]

Statistical analysis

Comparative resistant rates of *S. aureus* strains from the different clinical specimens were statistically analyzed by T-test and results were considered significant at 95% confidence level.

Results

The results of this study shows that, 104 *Staphylococcus aureus* strains were isolated from the 424 clinical specimens, i.e. 24.5% prevalence rate with 40 (38.5%) of the isolates being methicillin resistant *S. aureus* (MRSA) and 64(61.5%) being methicillin susceptible *S. aureus* (MSSA) respectively. The highest prevalence was recorded in wound swab 10/24 (29.4%) while the least was observed in blood specimen 1/15(6.7%) (Table 1).

The isolates showed high resistance to ampicillin (76.9%), penicillin G (81.7), Nalidixic acid (72.1%), chloramphenicol (70.1%) and were considerably sensitive to gentamycin (68.3%), ciprofloxacin (61.5%) and cefpodoxime (66.3%) (Table 2). Statistical analysis showed that there was no significant difference on rate of resistance across the various clinical specimens ($P<0.05$).

Table 1 The distribution of *S. aureus* isolates among the various clinical specimens.

S. no	Clinical samples	Number sampled	No. positive (%)	MRSA (%)	MSSA (%)
1	Blood	15	1(6.7)	0	1(1000)
2	Ear swab	25	6(24%)	1 (25.00%)	5(83.3%)
3	Throat swab	30	7(23%)	2 (28.6%)	5(71.4%)
4	Wound swab	34	10 (29.4%)	5 (50.00%)	5 (50%)
5	Pus swab	60	13 (21%)	8 (61.5%)	5(38.5%)
6	High Vaginal Swab	75	20(26.7)	4(20)	16(80)
7	Urine	185	47(25.4%)	20 (42.5%)	27(57.4%)
Total		424	104(24.5)	40(38.5)	64(61.5)

MRSA= Methicillin resistant *Staphylococcus aureus*; MSSA=Methicillin susceptible *Staphylococcus aureus*

Table 2 Antibiotic susceptibility pattern of *S. aureus* isolates from various clinical specimens

S. no	Antibiotics	Number of isolates sensitive (%)	Number of isolates resistant (%)
1	Ampicillin	24(23.1)	80(76.9)
2	Penicillin-G	19(18.2)	85(81.7)
3	Oxacillin	36(34.6)	68(65.4)
4	Erythromycin	49(47.1)	55(52.8)
5	Nalidixic acid	29(27.9)	75(72.1)
6	Tetracycline	39(37.5)	65(62.5)

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Table Continued...

S. no	Antibiotics	Number of isolates sensitive (%)	Number of isolates resistant (%)
7	Ciprofloxacin	64(61.5)	40(38.5)
8	Gentamycin	71(68.3)	33(31.7)
9	Chloramphenicol	31(29.8)	73(70.1)
10	Cefpodoxime	69(66.3)	35(33.7)
11	Cefotaxime	60(57.9)	44(42.3)

Discussion

Staphylococcus aureus is a very common cause of infection in hospitals and is most liable to infect newborn babies, surgical patients, old and malnourished persons and patients with diabetes and other chronic diseases.[6] The bacterium is ubiquitous in most environments with remarkable adaptability and versatility, which has equipped it as a commensal as well as a pathogen. It is one of the most infectious agents with high prevalence in various communities and healthcare institutions. In this study, the overall isolation rate was 24.5% among the various clinical specimens examined. This is similar with other studies; 20.8%, 36% and 48% in other parts of Nigeria.[4,31,32] This pattern of prevalence may be related to the level of *S. aureus* infection in the study locality. The high incidence of *S. aureus* observed among the clinical specimens shows the versatility of this organism amongst other bacteria, which makes it the most endemic pathogen in clinical settings. Considering the various clinical specimens examined, the highest incidence of *S. aureus* (29.4%) was observed in wound specimens, a finding consistent with report of Obiazi et al.,[30] and Nwoire et al.[15] However, our findings is in contrast to other results where highest incidence rate was observed in urine specimens 76.4%,[33] high vaginal swab, 42%[31] and nasal swab, 50.0%.[33] The high incidence of *S. aureus* in wound specimens observed in this study could be attributed to poor personal hygiene and exposure of the wounds, which might have made it more prone to contamination and infection. Furthermore, some people in the study area tend to treat their wounds on self-medication or employ services of unqualified or poorly trained quacks before seeking proper medical attention, which could account for the level of colonization by *S. aureus* observed in this study.

Development of resistance to antimicrobial agents by staphylococci is a major concern primarily because they are still frequently associated with hospital and community-acquired infections. The organisms exhibit remarkable versatility in their behavior towards antibiotics, with some strains having become resistant to most commonly used antibiotics.[34] In our results, we observed high level of resistance of *S. aureus* strain to penicillin G (81.7%), ampicillin (76.9%), Nalidixic acid (72.1%), and chloramphenicol (70.1%), which is in accordance with our previous study in Owerri[35] and the reports of Tiwari et al.[36] The high frequency of resistance observed in these antibiotics could be attributed to their use in treatment of diseases in animals and humans. This implies that these antibiotics are no longer be effectively used as empirical therapy for *S. aureus* infections particularly in the study area. The low activity of these antibiotics can also be attributed in part to earlier exposure of the isolates to these drugs, which may have enhanced resistant development. This assertion can further be strengthened by the high level of antibiotic abuse in our locality, arising from self-medication, failure to comply with treatment, antibiotic sale behavior for example, sale of antibiotics without prescription, sale of under dose and substituting brands. On the other hand, we observed that the isolates were considerably sensitive to gentamycin (68.3%), ciprofloxacin (61.5%) and cefpodoxime (66.3%) respectively; this is similar to the reports of other researchers.[31,37] This observed relatively higher level of susceptibility in gentamicin might be due to the route of administration, which is intravenous, thereby making abuse difficult. This shows that these drugs are still effective and could be considered as alternative options in the empirical treatment of *S. aureus* infections in the study area.

Conclusion

In conclusion, the threat posed by staphylococcal infections calls for adequate preventative and control measures to reduce transmission and infection. The most important way to control *S. aureus* infections is by performing regular checkup of antibiotic resistance profile to formulate antibiotic policies and effective infection control practices. Our findings will be useful to identify the challenges of the development of the drug resistance in bacteria with special reference to *S. aureus*. However, the judicious use of antimicrobial agents coupled with the elimination of substandard pharmaceuticals from our drug markets is pivotal to the control of antimicrobial resistance in our environments. Thus, there is need for the development of antimicrobial policy that will guide the prescription, sale, and use of antibiotics through regular surveillance of resistant organisms in our environments.

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Conflict of interest

The author declares no conflict of interest.

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