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IN-VITRO ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* ISOLATES IN UMUAHIA, ABIA STATE, NIGERIA

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ABSTRACT

The antimicrobial susceptibility pattern of *Staphylococcus aureus* in Umuahia was investigated in this study. A total of 113 strains of *S. aureus* consisting of 30 isolates from clinical specimens obtained from 10 medical Laboratories and 83 isolates from nasal swabs of University students in Umuahia, were tested against 10 antimicrobial agents using the disc agar diffusion method. Resistance to penicillin, ampicillin, cloxacillin and tetracycline was 100% among strains isolated from clinical specimens. The clinical isolates also exhibited high rates of resistance to chloramphenicol (97%) and erythromycin (97%). Seven (23%) of these isolates were sensitive to Gentamicin and 6 (20%) to Streptomycin. Likewise, all the 83 (100%) and 82 (98.8%) were resistant to penicillin and cloxacillin, respectively. Isolates from nasal passages appeared to be less resistant to chloramphenicol (54.2%), gentamicin (43.4), streptomycin (31.3%) and tetracycline (51.8%). Ampicillin did not produce any zone of inhibition against 29 (96.7%) isolates from clinical specimens and only slightly inhibited one with zone of inhibition of 8mm. Nineteen of the isolates manifested low to high level of resistance to chloramphenicol with mean zone of inhibition ranging from 15.8 ± 0.7 mm to 9.8 ± 2.0 mm. All the isolates were completely resistant to penicillin and cloxacillin with no zone of inhibition at all. In the case of gentamicin, 5 (16.7%) had intermediate susceptibility (mean zone of inhibition 14 ± 0 mm), 12 (40%) had mean inhibition zone of 9.6 ± 2.9 mm and 6 (20%) were not inhibited. This study shows that *S. aureus* strains isolated from clinical specimens and healthy students in Umuahia are highly resistant to common antibiotics. This may not be unconnected with indiscriminate use of antibiotics and calls for more control and rational use of antibiotics to minimize the rate of development of resistance to other antibiotics.

Key words: *Staphylococcus aureus*, antimicrobial resistance, disc agar diffusion, antibiotic susceptibility

PROFIL DE SENSIBILITE ANTIMICROBIENNE IN-VITRO DE SOUCHES DE *STAPHYLOCOCCUS AUREUS* A UMUAHIA, Etat d'ABIA, NIGERIA

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Résumé

Le profil de sensibilité antimicrobienne de *Staphylococcus aureus* à Umuahia a été recherché dans cette étude. Au total, 113 souches de *S. aureus* composé de 30 souches provenant d'échantillons cliniques obtenus à partir de 10 laboratoires médicaux et 83 souches d'écouvillons nasaux des étudiants universitaires à Umuahia, ont été testées contre 10 agents antimicrobiens par la méthode de diffusion disque sur gélose. La résistance à la pénicilline, l'ampicilline, la cloxacilline et la tétracycline a été de 100% chez les souches isolées d'échantillons cliniques. Les souches cliniques ont également présenté des taux élevés de résistance au chloramphénicol (97%) et à l'érythromycine (97%). Sept (23%) de ces souches étaient sensibles à la gentamicine et 6 (20%) à la streptomycine. De même, tous les 83 (100%) et 82 (98,8%) étaient résistants respectivement à la pénicilline et à la cloxacilline. Les souches de voies nasales semblaient être moins résistantes au chloramphénicol (54,2%), à la gentamicine (43,4), à la streptomycine (31,3%) et à la tétracycline (51,8%). L'ampicilline n'a produit aucune zone d'inhibition contre 29 (96,7%) souches provenant d'échantillons cliniques et seulement légèrement inhibées avec une zone d'inhibition de 8mm. Dix-neuf souches ont manifesté un faible niveau élevé de résistance au chloramphénicol avec une zone d'inhibition moyenne allant de 15,8 ± 0,7 mm à 9,8 ± 2,0mm. Toutes les souches étaient complètement résistantes à la pénicilline et à la cloxacilline sans zone d'inhibition. Dans le cas de la gentamicine, 5 (16,7%) souches avaient une sensibilité intermédiaire (zone d'inhibition moyenne de 14 ± 0 mm), 12 (40%) souches ont eu une zone d'inhibition moyenne de 9,6 ± 2,9 mm et 6 (20%) souches ne ont pas été inhibées. Cette étude montre que les souches de *S.*

aureus isolées à partir d'échantillons cliniques et des étudiants en bonne santé à Umuahia sont très résistantes aux antibiotiques courants. Cela peut ne pas être en rapport avec l'utilisation sans discernement des antibiotiques et des appels pour plus de contrôle et d'utilisation rationnelle des antibiotiques afin de minimiser le taux de développement d'une résistance à d'autres antibiotiques.

Mots clés: *Staphylococcus aureus*, résistance aux antimicrobiens, disque de diffusion sur gélose, sensibilité aux antibiotiques

INTRODUCTION

The reports of high level of antimicrobial drug resistance of *Staphylococcus aureus* in different parts of the world are generating serious public health concerns (1, 2,3). *Staphylococcus aureus* is one of the most successful and adaptable human pathogens that can exist as a commensal on human skin on one hand and become a pathogen capable of causing serious infections in both healthcare facilities and in the community (4). *Staphylococcus aureus* causes a plethora of human infections ranging from minor pus forming skin infections such as boils, styes, pustules, impetigo to wound infections, furuncles, ulcers, burns infections and to serious, sometimes life threatening infections like pleural empyema, pneumonia, meningitis, osteomyelitis and septicaemia(5 , 6). This organism is also frequently associated with surgical wound infections (3).

Antimicrobial agents have been used extensively to combat *S. aureus* infections but the increasing level of resistance of *S. aureus* to many antibiotics is complicating the treatment of serious infections caused by this pathogen (2).The knowledge of the antimicrobial susceptibility profile of this versatile pathogen in a particular area is important as this can contribute to rational choice and use of antimicrobial agents. The objective of this work was to survey the pattern of in-vitro antimicrobial resistance of *S. aureus* isolates to various antimicrobial drugs in Umuahia, Abia State.

MATERIALS AND METHODS

A total of 113 *S. aureus* isolates were tested for antimicrobial drug susceptibility profile. Thirty (30) of the isolates were obtained from ten (10) Medical Laboratories in Umuahia. Seventeen (17) isolates were from Urine samples, 5 from high vaginal swab (HVS) and 8 from wounds. The remaining 83 isolates were from culture of nasal swabs collected from 100 students of the Michael Okpara University of Agriculture, Umudike, between 2010 and 2012.

The primary isolation of the organisms from nasal swabs was done on Mannitol Salt agar,

then sub-cultured on Nutrient agar for biochemical tests. The

30 isolates from Medical Laboratories were re-isolated on Nutrient agar and biochemically characterized like the nasal passage isolates. All the isolates were characterized and identified by Gram staining reaction, catalase and coagulase tests. The antimicrobial susceptibility testing was done using the Bauer-Kirby disc agar diffusion method on Mueller-Hinton agar with commercially available antibiotic sensitivity discs(AbtekBiologicals, Ltd, UK). The procedure of the antimicrobial susceptibility testing was done as described by Ekundayo and Omodamiro (7).

RESULTS

The antimicrobial susceptibility pattern of 113*S.aureus* strainsisolated from clinical specimens and nasal passages in Umuahia was investigated in this study. The pattern of susceptibility of the isolates to ten antimicrobial agents tested is presented in Table 1. All the 30 strains isolated from clinical specimens were resistant to ampicillin, cloxacillin, penicillin and tetracycline. Likewise, all the 83(100%) and 82(98.8%)strains isolated from nasal passages of students in Michael Okpara University of Agriculture, Umudike were resistant to penicillin and cloxacillin, respectively. Seven (23%) of these isolates were sensitive to Gentamicin and 6 (20%) to Streptomycin. Forty seven (56.6% and 57(68.7%) of the isolates from nasal passages were sensitive to Gentamicin and Streptomycin, respectively.

The zone of inhibition produced by the various antimicrobial agents against the *S.aureus* isolated from clinical specimens is presented in Table 2. Ampicillin did not produce any zone of inhibition against 29 (96.7%) isolates and only slightly inhibited one with zone of inhibition of 8mm. Nineteen of the isolates were inhibited by chloramphenicol with mean zone of inhibition ranging from 9.8± 2.0 mm to 15. 8± 0.7 mm. All the isolates were completely resistant to penicillin and cloxacillin with no zone of inhibition at all. In the case of gentamicin, 5

(16.7%) had intermediate susceptibility (mean zone of inhibition 14±0 mm), 12(40%) had mean

inhibition zone of 9.6±2.9 mm and 6(20%) were not inhibited.

TABLE 1: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *S. AUREUS* ISOLATES FROM CLINICAL SPECIMENS AND NASAL PASSAGES IN UMUAHIA

Antimicrobial Agents	Clinical Isolates (n= 30)		Nasal Passage isolates (n= 83)		Total (n=113)	
	N ₀ (%) Sensitive	N ₀ (%) Resistant	N ₀ (%) Sensitive	N ₀ (%) Resistant	N ₀ (%) Sensitive	N ₀ (%) Resistant
Ampicillin	0(0)	30(100)	NT	NT	0(0)	30(100)
Chloramphenicol	1(3)	29(97)	38(45.8)	45(54.2)	39(34.5)	74(65.5)
Cloxacillin	0(0)	30(100)	1(1.2)	82(98.8)	1(0.9)	112(99.1)
Erythromycin	1(3)	29(97)	20(24.1)	63(85.9)	21(18.6)	92(81.4)
Gentamicin	7(23)	23(77)	47(56.6)	36(43.4)	54(47.8)	59(52.2)
Penicillin	0(0)	30(100)	0(0)	83(100)	0(100)	113(100)
Streptomycin	6(20)	24(80)	57(68.7)	26(31.3)	63(55.8)	50(54.2)
Tetracycline	0(0)	30(100)	40(48.2)	43(51.8)	40(35.4)	73(64.6)
Cotrimoxazole	NT	NT	19(22.9)	64(77.1)	19(22.9)*	64(77.1)*
Augmentin	NT	NT	20(24.1)	63(85.9)	20(24.1)*	63(85.9)*

NT= Not done; * Only isolates from nasal passage

TABLE 2: MEAN DIAMETER ZONE OF INHIBITION (MM) AND ANTIMICROBIAL SUSCEPTIBILITY STATUS OF *S. AUREUS* CLINICAL ISOLATES IN UMUAHIA

Antimicrobial agents	Susceptibility status			
	Sensitive	Intermediate	Resistant	Resistant
	N ₀ (\bar{x} DZI± std) ^a	N ₀ (\bar{x} DZI± std)	N ₀ (\bar{x} DZI± std)	N ₀ (%) with no zone of inhibition
Ampicillin 10µg	0(0)	0 (0)	1(8±0)	29(96.7)
Chloramphenicol 30µg	1 (18±0)	9 (15.8±0.7)	10(9.8±2.0)	10(33.3)
Cloxacillin 5µg	0(0)	0 (0)	0 (0)	30 (100)
Erythromycin 5µg	1 (18±0)	1(14±0)	3 (10±2.0)	25(83.3)
Gentamicin 10µg	7(16.1±0.9)	5(14±0)	12 (9.6±2.9)	6(20)
Penicillin 10 IU	0(0)	0(0)	0(0)	30(100)
Streptomycin 10µg	6(18±2.9)	5(12±0)	4(9.5±1.0)	15(50)
Tetracycline 30µg	0(0)	2(17±1.4)	14(9.1±1.7)	14(46.7)

^a \bar{x} DZI± std: Mean diameter of inhibition ± standard deviation; Interpretative reference range: Sensitive DZI 15≥20 mm, Intermediate DZI 13-14 mm, Resistant DZI ≤12 (Cheesbrough, 2002)

DISCUSSION

Staphylococcus aureus has a remarkable ability to develop resistance to antibiotics and has successfully developed resistance mechanisms to each new antibiotic introduced, beginning from the oldest penicillin, to methicillin and other newer antibiotics (8, 9). The strains of *S. aureus* in this study exhibited high level of resistance to multiple antibiotics and this suggests that the strains possess different or multiple resistance mechanisms. Resistance to antimicrobial agents in *S. aureus* has been mediated by genetic elements acquired either by spontaneous chromosomal mutation under drug pressure or acquisition of resistance genes through horizontal transfer from other microorganisms (9,10).

The prevalence of resistance to penicillin, ampicillin, cloxacillin and tetracycline was 100% among strains isolated from clinical specimens. The clinical isolates also exhibited high rates of resistance to chloramphenicol (97%) and erythromycin (97%). This may not be particularly surprising given the unrestricted access to and the indiscriminate use of antimicrobial agents in our study area. Isolates from nasal passages appeared to be less resistant to chloramphenicol (54.2%), gentamicin (43.4), streptomycin (31.3%) and tetracycline (51.8%). High rate of resistance to penicillin and other β -lactam antibiotics has been reported in parts of South-western Nigeria (11, 12). Researchers in other parts of the world have also reported high level of resistance to antibiotics by strains of *S. aureus*(13, 2, 3).

Analysis of the sizes of zone of inhibition produced by different antibiotics against strains of *S. aureus* from clinical specimens shows the degree of resistance to the drugs. Ampicillin did not produce any zone of inhibition against 29 (96.7%) and only slightly inhibited one with zone of inhibition of 8mm. All the isolates were completely resistant to penicillin and cloxacillin with no zone of inhibition at all. Although 29 of the 30 isolates were classified as resistant to chloramphenicol, 9(30%) of the isolates had intermediate susceptibility with mean zone of inhibition of 15.8 ± 0.7 mm, 10(33.3%) had mean inhibition zone of 9.8 ± 2.0 and 10(33.3%) were frankly resistant with no zone of inhibition. In the case of gentamicin, 5 (16.7%) had intermediate susceptibility (mean zone of inhibition 14 ± 0 mm), 12(40%) had mean inhibition zone of 9.6 ± 2.9 mm and 6(20%) were not inhibited. The isolates with zone of inhibition in the category of intermediate susceptibility might have been regarded as sensitive in routine laboratory practice where any reasonable size of inhibition is taken as evidence of susceptibility without the use of interpretative reference range.

In conclusion, this study shows that there is a very high level of resistance to commonly used antibiotics among strains of *S. aureus* in Umuahia. There is need to institute surveillance for drug susceptibility of important pathogen like *S. aureus*. There is a need for greater control and rational use of antibiotics in order to slow down the rate of resistance development and spread of resistant organisms in the community.

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