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Antibiotics Susceptibility Patterns and Plasmid Profile of *Staphylococcus aureus* Isolated from Patients with Wound Infections Attending Four Hospitals in Akure, Ondo State

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Authors' contributions

This work was carried out in collaboration between both authors. Author MKO designed the study and wrote the protocol. Author OO performed the statistical analysis, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Several studies have documented *Staphylococcus aureus* as a leading pathogen implicated in wound infections with a remarkable potential for antibiotics resistance. This study determined the susceptibility patterns of *S. aureus* isolated from wound infections to conventional antibiotics and assessed the plasmid profile of selected multidrug resistant isolates.
Study Design: This study was designed to determine the antibiotics susceptibility patterns of *Staphylococcus aureus* isolates from wound infections.
Place and Duration of Study: This research was carried out in the Department of Microbiology Federal University of Technology Akure, between November 2015 and April 2016.
Methodology: *Staphylococcus aureus* were isolated from wound swab samples of a total of 248 patients (116 males and 132 females within the age range of 5 - 63 years) using standard bacteriological procedures. Susceptibility profile of the isolates to commercially available antibiotics

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was determined using disc diffusion method. Plasmid profiling and curing were carried out for selected multidrug resistant isolates.
Results: This study revealed that males had a lower percentage occurrence (44.04%) of *S. aureus* in wounds, while females had a higher percentage occurrence (55.96%). Many of the isolates displayed multi-drug resistance to many of the conventional antibiotics used. Compared to other conventional antibiotics employed in this study, the isolates displayed the lowest percentage resistance to Vancomycin 3 (2.75%); and highest percentage resistance to Erythromycin 56 (51.38%). Multiple plasmids were discovered in the selected multidrug resistant isolates.
Conclusion: Compared to other antibiotics used in this study, Ofloxacin and Vancomycin showed better efficacy against the tested isolates. However, there is need for development of alternative drugs to increase the treatment options for multidrug resistant *Staphylococcus aureus*.

Keywords: Staphylococcus aureus; wounds; antibiotics resistance; plasmids.

1. INTRODUCTION

Staphylococcus aureus is a widespread, opportunistic pathogen that causes community and hospital acquired infections [1,2]. Several studies have documented *Staphylococcus aureus* as a leading pathogen associated with wound infections [3-10]. Its high pathogenicity is driven by multifactorial and complex mechanisms determined by the ability of the bacteria to express a wide variety of virulence factors with which it colonize and infect the host [11,12]. Colonization of wounds by *Staphylococcus aureus* and rapid proliferation within damaged tissues often lead to disseminated infections [13, 14]. *Staphylococcus aureus* is notorious for its ability to become resistant to antibiotics [15,16]. Plasmids are extrachromosomal DNA molecules that have been shown to carry genes for antibiotics resistance, a single plasmid can carry genes for resistance to multiple antibiotics [17]. Infections due to antibiotics resistant strains of *S. aureus* are associated with increased burden on health resources; and increased morbidity and mortality [18,19,20]. This study aimed at determining the antibiogram of *S. aureus* isolates from wound infections to conventional antibiotics and assessing the plasmid profile of selected multidrug resistant isolates.

2. MATERIALS AND METHODS

2.1 Study Population

The study population consists of a total of 248 patients (116 males and 132 females) within the age range of 5 to 63 years who presented for wound dressing at four hospitals: State Specialist Hospital Akure, Mother and Child Hospital, Police Clinic Akure and Miracle Hospital Akure between November, 2015 and February, 2016. The wound types are accident wounds, post-operative wounds, burns and vagina delivery wounds. The wound sites were categorized as head and neck; upper limb; abdomen; lower limb and vagina.

2.2 Ethical Approval

Ethical approval for the study was obtained from the Ethics and Research Section of the Ondo State Ministry of Health.

2.3 Wound Sample Collection

All the wounds were judged as infected by the presence of purulent material. Before wound cleansing and dressing, the exudates from each wound site was carefully taken by a nurse using sterile cotton-tipped applicators and transferred to the research laboratory for further processing.

2.4 Method of Isolation and Identification of *S. aureus*

The wound swab samples were immediately applied to freshly prepared Mueller Hilton broth and left to stand for one hour, followed by streaking on Mannitol Salt Agar (MSA) plates and incubated aerobically at 37℃ for 24 to 48 hours. Presumptive identification of *Staphylococcus aureus* was based on the characteristic golden yellow colouration on MSA plates, between three to five representative colonies were sub-cultured again on MSA plates. These plates were then incubated aerobically at 37℃ for 24 to 48 hours [21]. After incubation, the cultural and morphological characteristic of distinct, well isolated colonies were studied. These included the shape, size, elevation, edges, opacity, surface and color. Representatives were picked per plate and stock cultures of pure isolates were labeled and stored accordingly at 4℃ for further use. The

presumptive colonies were later Gram stained and other biochemical tests were conducted.

2.5 Biochemical Tests

Preliminary characterization of isolates was based on Gram staining reaction, morphological and cultural characteristics. Furthermore, characterization was carried out using various biochemical tests. These tests include: catalase test, coagulase test, indole production test, citrate utilization test, methyl- red test, voges-proskauer test and sugar fermentation tests as described by [21].

2.6 Antibiotics Susceptibility Testing

Antibiotics susceptibility test of all the isolates was determined by the disk diffusion method as described by [21]. The identified *S. aureus* isolates were tested against 9 commercially available antibiotics: Cotrimoxazole (25 µg), Erythromycin (10 µg), Gentamicin (2 0µg), Augmentin (30 µg), Streptomycin (10 µg), Cloxacilin (5 µg), Ofloxacin (10 µg), Vancomycin (30 µg) and Chloramphenicol (30 µg).

2.7 Quality Control

Staphylococcus aureus ATCC 25923 was used as quality control for antibacterial susceptibility testing as recommended by [22].

2.8 Plasmid Extraction

Overnight growth of the multidrug resistant *S. aureus* broth culture was used for the plasmid isolation. TENS protocol described by [23], was employed in plasmid extraction.

2.9 Curing of Plasmid DNA and Post Curing Sensitivity Testing

This was carried out using the method described by [23]. The bacterial isolates were inoculated into broth and incubated at 37℃ for 18 hours, after incubation, 2 µl of ethidium bromide was added to the broth and this was incubated overnight at 37℃. The test agents were then inoculated onto freshly prepared Mueller Hilton agar plates and antibiotics discs were aseptically placed on the plates, followed by incubation 37℃ for 24 hours. The incubated petri dishes were observed for zones of inhibition and the diameter was measured in millimeter.

3. RESULTS AND DISCUSSION

3.1 Distribution of Wound Samples from Patients Attending Four Hospitals

A total of two hundred and forty eight (248) wound swab samples were collected from patients with wound infections attending: State Specialist Hospital Akure, Mother and Child Hospital, Police Clinic Akure and Miracle Hospital Akure. Based on hospital, the highest number of wound samples 168 (67.74%) were collected from patients attending State Specialist Hospital Akure, while the lowest samples 7 (2.82%) were obtained from patients attending Miracle Hospital Akure; based on age, the highest number of samples 123 (49.60%) were obtained from patients within the age range of 20 – 39, while the lowest 8(3.23%) were obtained from patients less than 10 years of age; based on anatomical site of wound, the highest samples 76 (30.65%) were obtained from lower limbs, while the lowest 9 (3.63%) were obtained from Vaginal; based on type of wound, the highest number of samples 136 (54.84%) were obtained from patients with accident wounds, while the lowest 9 (3.63%) were from patients with Vaginal delivery wounds. The sample distributions based on hospitals, gender and age of patients, anatomical site of wounds, type of wounds and the percentage occurrence of *Staphylococcus aureus* in wounds are presented in Tables 1, 2, 3, 4 and 5 respectively.

The highest proportion of wound samples was obtained from patients within the age group 20 to 39 years. This is in coherence with the work of [7] who obtained the highest wound samples from similar age group. The observed trend could be due to the fact that this is the most physically active age group. The occurrence of more males with accident wounds might be due to the risk taking nature of males both as youths and adults especially in functioning as the breadwinners of families; and occupational predisposition as motor-cycle riders, drivers and farmers. Also, males could be more adventurous and endearing in physical activities thus getting more exposed to the various agents of wounds [8]. The higher number of female patients with more post-operative wounds than males observed in this study could be as a result of a surgical procedure (caesarian section) performed only in females which tends to increase the sample size for female post-operative wounds in this study.

Table 1. Sample distribution based on hospitals

Hospitals	No of samples	Percentage of samples collected (%)
State Specialist Hospital	168	67.74
Mother and Child Hospital	49	19.76
Police Clinic	24	9.68
Miracle Hospital	7	2.82
Total	248	100

Table 2. Sample distribution based on age and gender of patients

Age (years)	Number examined		Total (%)
	Males	Females	
Less than 10	5	3	8 (3.23)
10-19	31	28	59 (23.79)
20-39	52	71	123 (49.60)
40-59	22	21	43 (17.34)
60 and above	6	9	15 (6.04)
Total (%)	116 (46.77)	132 (53.23)	248 (100)

Table 3. Sample distribution based on anatomical sites of wounds

	Number of samples (%)
Head and neck	36 (14.52)
Upper limbs	52 (20.97)
Abdomen	75 (30.24)
Lower limbs	76 (30.65)
Vagina	9 (3.63)
Total	248 (100)

Table 4. Sample distribution based on type of wounds

Wound type	Number of males	Number of females	Total
Accident	80	56	136
Post-operative	26	49	75
Burns	10	18	28
Vaginal delivery	0	9	9
Total (%)	116 (46.77)	132 (53.23)	248 (100)

Table 5. Percentage occurrence of *Staphylococcus aureus* isolated from wounds

Sex	Number of samples	Number of *S. aureus*	Percentage occurrence
Males	116	48	44.04 %
Females	132	61	55.96 %

3.2 Cultural Characteristics and Biochemical Results of *Staphylococcus aureus* Recovered from Wound Swabs

A total of one hundred and nine (109) *Staphylococcus aureus* isolates were recovered from the various wounds. The cultural characteristics of the isolates on Mannitol Salt Agar (MSA) and biochemical results are presented in the Tables 6 and 7.

3.3 Susceptibility Pattern of *S. aureus* Isolated from Wounds to Commercially Available Antibiotics

The isolates exhibited the following resistance pattern to the commercially available antibiotics employed in this study: Ofloxacin 9 (8.25%), Cotrimoxazole 38 (34.86%), Gentamycin 43 (39.45%), Streptomycin 42 (38.53%), Erythromycin 56 (51.38%), Chloramphenicol 54 (49.54%), Augmentin 52 (47.71%), Cloxacillin 31

(28.44%) and Vancomycin 3 (2.75%); the intermediate susceptibility pattern exhibited by the isolates are as follows: Ofloxacin 16 (14.68%), Cotrimoxazole 12 (11.01%), Gentamycin 15 (13.76%), Streptomycin 13 (11..93%), Erythromycin 13 (11.93%), Chloramphenicol 17 (15.60%), Augmentin 0 (0%), Cloxacillin 0 (0%) and Vancomycin 0 (0%); the sensitivity pattern exhibited by the isolates are as follows: Ofloxacin 84 (77.06%), Cotrimoxazole 59 (54.13%), Gentamycin 51 (46.79%), Streptomycin 54 (49.54%), Erythromycin 40 (36.69%), Chloramphenicol 38 (34.86%), Augmentin 57 (52.29%), Cloxacillin 78 (71.56%) and Vancomycin 106 (97.25%). The results are presented in Fig. 1.

Table 6. Cultural characteristics of *Staphylococcus aureus* isolates on mannitol salt agar

Colour	Golden yellow
Shape	Round
Edge	Entire
Opacity	Opaque
Surface	Smooth
Elevation	Raised

Table 7. Biochemical results of *Staphylococcus aureus* isolates from wounds

Biochemical test	Results
Gram reaction and shape	+ cocci
Catalase	+
Slide coagulase	+
Tube coagulase	+
Indole	-
Methyl red	+
Voges proskauer	+
Citrate	+
Glucose	+
Mannitol	+
Trehalose	+
Sucrose	+

Legend: + = Positive, - = Negative

Many of the *S. aureus* isolates demonstrated a high resistance pattern to many of the conventional antibiotics employed in this study. This finding is similar to that of [9] who observed high resistance pattern of the bacterial isolates from wounds to several antibiotics. According to [24], infections caused by multidrug resistant (MDR) organisms are limiting treatment options and compromising effective therapy. Coupled with the fact that many of the conventional antibiotics are affordable and easily accessible, other probable reasons for the observed resistance pattern could be as follows: use of antibiotics without prescription from a qualified physician, over the counter sales of antibiotics, use of antibiotics for conditions that may not clinically indicate their usage, production and sales of sub-standard antibiotics; and patients not following complete treatment course even when the drugs are rightly prescribed. The high sensitivity patterns of the isolates to Ofloxacin and Vancomycin as observed in this study is an indication that these drugs could still be effective for the treatment and management of infections due to *S. aureus*. This result is in coherence with the work of [7] who reported high susceptibility of various pathogens to fluoroquinolones. The observed high susceptibility pattern of the isolates to Vancomycin and Ofloxacin could be as a result of these antibiotics being more expensive than others, as such making them relatively less accessible and consequently less prone to resistance [25].

3.4 Electrophoretic Pattern of Plasmid Profiling of Selected Multidrug Resistant *Staphylococcus aureus* Isolates from Wounds

Plate 1 shows the result of the plasmid profile of selected multidrug resistant *Staphylococcus aureus* isolates from Wounds. Plasmids ranging in size from 95 to 12.5 kb were detected in the selected isolates. Isolates P1, A3 and B2 in lanes 2, 6 and 9 respectively had the highest number of plasmids (4), while isolate V3 in lane 1 had the least number of plasmids (2), the results are presented in Table 8.

Table 8. Plasmid profile of selected multi-drug resistant *S. aureus* isolated from wounds

Lane (isolate code)	Number of plasmid
1 (V3)	2
2 (P1)	4
3 (P2)	3
4 (P11)	3
5 (P15)	3
6 (A3)	4
7 (A4)	3
8 (B1)	3
9 (B2)	4

Legend: A3, A4= S.aureus from Accident wounds; P1, P2, P11, P15 = S.aureus from Post-operative wounds, B1, B2 = S.aureus from burns, V1 = S.aureus from Vaginal delivery wound

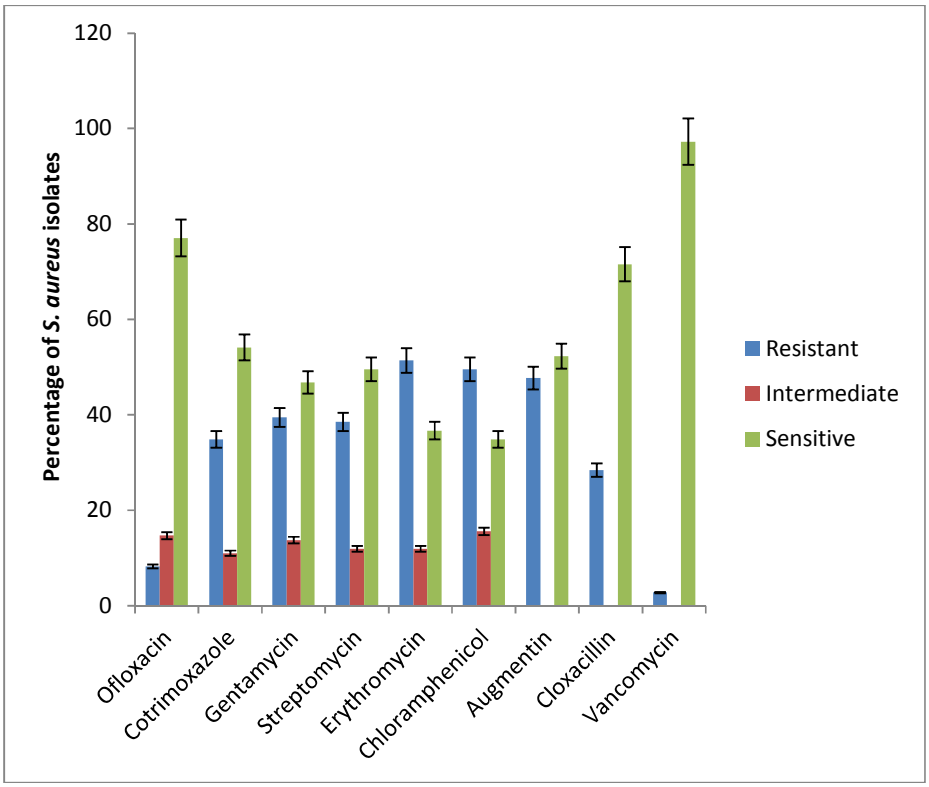

Fig. 1. Susceptibility pattern of *S. aureus* isolated from wounds to conventional antibiotics

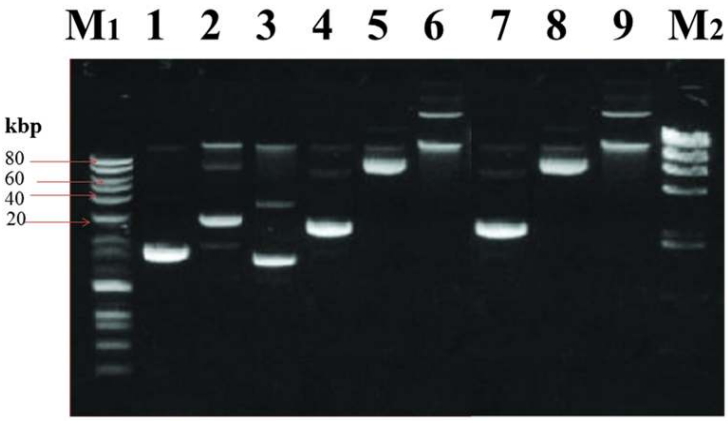

Plate 1. Electrophoretic pattern of plasmid profiling of selected multidrug resistant *S. aureus* isolated from wound infections

M1, M2 = DNA Ladder; kbp = Kilo base pair

The presence of multiple plasmids in the isolates suggests that the observed resistance could be plasmid mediated. This correlates with the work of Akinjogunla and Enabulele (2010) which showed that resistance of *Staphylococcus aureus* isolated from patients with acute otitis media were plasmid mediated antibiotics resistance. Plasmids have been shown to carry genes for antibiotics resistance, a single plasmid can carry genes for resistance to multiple antibiotics (Nirdnoy *et al.,* 2005). The post curing sensitivity testing results revealed that, the resistance to Cotrimoxazole and Chloramphenicol observed in isolates B11 and P7 before curing; and resistance to Gentamycin in isolates V3 and P3 observed before curing were retained, indicating that these resistance were chromosomally mediated.

Table 9. Post curing of multidrug resistant *S. aureus* isolates from wounds

Result	OFX	COT	CN	S	E	CH	AUG	CLX	VAN	IC
Pre	S	R	S	R	I	S	R	R	S	V3
Post	S	S	R	S	S	S	R	S	S	
Pre	R	S	R	R	I	R	R	R	S	P1
Post	S	S	S	S	S	S	S	S	S	
Pre	R	S	R	R	I	R	R	R	S	P3
Post	S	S	R	S	S	S	S	S	S	
Pre	S	R	R	I	R	I	R	R	R	P7
Post	S	R	S	S	S	R	S	S	S	
Pre	S	S	S	R	I	R	R	R	S	P15
Post	S	S	S	S	S	S	S	S	S	
Pre	I	S	R	R	R	R	R	R	S	A4
Post	S	S	S	S	S	S	S	S	S	
Pre	S	S	R	R	R	R	R	R	S	A5
Post	S	S	S	S	S	S	S	S	S	
Pre	R	R	R	S	I	R	R	R	S	B2
Post	S	S	S	S	S	S	S	S	S	
Pre	S	R	R	R	R	R	R	S	S	B11
Post	S	R	S	S	S	R	S	S	S	

Legend: R = resistant, I = intermediate, S = sensitive, Pre = before curing, Post = after curing

4. CONCLUSION

Compared to other antibiotics used in this study, Ofloxacin and Vancomycin showed better efficacy against the tested isolates. However, there is need for development of alternative drugs to increase the treatment options for multidrug resistant *Staphylococcus aureus*.

CONSENT

Both authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images.

ETHICAL APPROVAL

Both authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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