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Original Research Article

METHICILLIN-RESISTANCE IN *Staphylococcus aureus* ISOLATES FROM PATIENTS ADMITTED AT SPECIALIST HOSPITAL OKITIPUPA, ONDO STATE, WESTERN NIGERIA.

ABSTRACT

Aims: To determine the rate of methicillin resistance and antibiotic resistance patterns of *Staphylococcus aureus* isolated from patients hospitalized at State Specialist Hospital, Okitipupa, Ondo State, Nigeria.

Study design: Prospective cross sectional.

Place and Duration of Study: Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa, between March and June 2015.

Methodology: Forty three (43) samples were obtained from nasal cavity, skin, wound, or umbilical cord of 20 patients aged 4 days to 80 years. Isolates were identified by cultural characteristics on Mannitol Salt Agar, Nutrient agar and biochemical tests. Antibiotic sensitivity testing was performed by the disk diffusion method (Kirby-Bauer).

Results: Seventeen *S. aureus* isolates were identified, as well as coagulase-negative staphylococcus (n=21). Isolation of *S. aureus* was not age- or sex-related. Percentage resistances of the isolates to the antibiotics were to oxacillin (100%), cloxacillin (88.2%), amoxicillin-clavulanic acid (augmentin) (76.5%), cefuroxime (70.6%), erythromycin (70.6%), ceftazidine (64.7%), gentamicin (47.1%,) ofloxacin (41.2%), ceftriaxone (23.5%). Ofloxacin received the highest sensitivity (52.9%) from the isolates, suggesting that fluoroquinolones may be the drugs of choice in the treatment of infections caused by *S. aureus*.

Conclusion: The study revealed multiple drug resistances in *S. aureus* isolates isolated from hospitalized patients. Oxacillin resistance, used as an indicator of methicillin resistance was 100%. The high rates of resistance to the β-lactams – Oxacillin, cloxacillin and the combined amoxicillin-clavulanic acid, highlights the decreasing importance of these drugs in antimicrobial therapy for *S. aureus* infections.

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Keywords: *Staphylococcus aureus, MRSA, oxacillin, multiple-drug-resistance, Nigeria*

1. Introduction

Staphylococcus aureus is a gram-positive, facultative anaerobic, unencapsulated cocci which occur in clusters. The bacterium occurs as a commensal on the human anterior nares and on the skin (and less commonly in other locations) of the human body[1][2]. It is a normal inhabitant of the healthy lower reproductive tract of women [3][4]. *S. aureus* colonizes approximately 20% to 30% of the human population [5], and is reported to be found in 25% to 27% of health workers [6]. The presence of *S. aureus* does not always indicate infection. *S. aureus* can survive from hours to weeks, or even months, on dry environmental surfaces, depending on strain [7].

Though the bacterium occurs as normal flora, it can infect tissues when the skin or mucosal barriers have been breached. This can lead to many different types of infections ranging from furuncles and carbuncles to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis [8][9]. Anyone can develop a *S. aureus* infection, although certain groups of people are at greater risk, including newborn infants, breastfeeding women, and people with chronic conditions such as diabetes, cancer, vascular disease, and lung disease. Injection drug users, those with skin injuries or disorders, intravenous catheters, surgical incisions, and those with a weakened immune system. *S. aureus* is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections [10]. Each year, some 500,000 patients in United States' hospitals contract a staphylococcal infection [11].

The treatment of choice for *S. aureus* infection is penicillin, a β- lactam. Penicillin is bactericidal by inhibiting the formation of peptidoglycan cross-linkages that provide the rigidity and strength in a bacterial cell wall. In most countries, however, penicillin resistance is extremely common. Members of the genus *Staphylococcus* produce penicillinase (a β- lactamase), which inactivate penicillins, making them naturally resistant to these antibiotics [12]. Following the emergence of penicillin resistance, penicillinase-resistant β-lactam antibiotics such as oxacillin or flucloxacillin were developed and used as first-line therapy. However resistance to penicillinase-resistant β-lactams soon emerged in many bacterial strains and has become a global health problem [13].

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Methicillin-resistant S. aureus *(MRSA) is one of a number of greatly feared strains of* S. aureus *which have become resistant to most β-lactam antibiotics. For this reason, vancomycin, a glycopeptide antibiotic, is commonly used to combat MRSA. Studies reveal that the gene encoding resistance to methicillin also encodes resistance to other penicillinase-resistant β-lactams, resulting in cross-resistance [14]. Methicillin resistance is also an indicator of multiple resistance to other antibiotics [15][8].*

MRSA strains are most often found associated with institutions such as hospitals, but are becoming increasingly prevalent in community-acquired infections [16]. The mortality rate of MRSA is reported to be on the high side. Reports from a recent study highlighted the mortality of two patients out of four due to treatment failure caused by MRSA resistant to both vancomycin and daptomycin, a first-line therapy for the treatment of severe MRSA infections [17][9]. Methicillin-resistant *Staphylococcus aureus* blood stream infection is a major healthcare burden in some healthcare settings, and is associated with 10%-20% mortality [18].

Strategies for monitoring antibiotic susceptibility patterns should include periodic isolation and testing of isolates for antibiotic susceptibility in all localities, using a state-owned hospital as a sampling center for a cross sectional study. Results of cross sectional studies usually provide a good overview of situations in the entire community.

The aims of the present study were to determine the rate of methicillin resistance and antibiotic resistance patterns of *Staphylococcus aureus* isolated from patients hospitalized at State Specialist Hospital, Okitipupa, Ondo State, Western Nigeria. No previous study of this nature has been carried out in this locality, and so we provide needed data on the situation of resistance in this part of the world.

2. Material and Methods

2.1 Sample collection, Handling, Culture and Identification

Samples were collected from State Specialist Hospital, Okitipupa from March to April 2015. Sterile cotton swabs moistened in sterile distilled water were used to collect samples from the skin, nostrils, wound exudates or umbilical cord excision sites of hospitalized patients. The swabs sticks were inserted into their tubes and transported immediately into the Microbiology laboratory for analysis. Prior to sample

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collection, permission was obtained from the Hospital Authorities through a written application. The patients also had to give their consent before samples were collected from them.

All swab samples were directly inoculated onto Nutrient agar and Mannitol salt agar (MSA) plates. The plates were incubated at 37°C for 24 h, and then examined for colony characteristics. Colonies that were white, round, smooth, substantial and opaque on Nutrient agar, and colonies producing a golden-yellow pigment on MSA were presumptively considered *S. aureus* and subsequently sub-cultured on nutrient agar to obtain pure, discreet colonies. Pure colonies that yielded Gram positive clustered cocci, and reacted positively to coagulase and catalase tests were identified as *S. aureus* [19][20].

2.2 Antimicrobial susceptibility testing

The isolates were screened using the disc diffusion method [21]. Gram positive antibiotic discs (Rapidflex) included Ceftazidime 30 µg, Cefuroxime 30µg Gentamicin 10µg Ceftriazone 30µg Erythromycin 30µg Cloxicillin 5µg Ofloxacin 5µg and Augmentin (Amoxicillin/Clavulanic acid) 30µg. Oxacillin (1µg) obtained from Oxoid Ltd Britain was also screened.

Antimicrobial susceptibility tests were performed for all isolates according to the criteria of the clinical and laboratory standards institute (CLSI) [22]. Bacterial suspensions were prepared, adjusted to the 0.5 McFarland Standards, and inoculated onto Mueller-Hinton agar (Oxoid) by surface swabbing. Using sterile forceps, the antibiotic-containing discs were placed aseptically on the inoculated plates and left on the table for 1 hour for proper diffusion to occur. The plates were subsequently incubated in an inverted position, at 35°C for 16-18 hours and thereafter examined for clear zones of inhibition. Inhibition zone diameters (IZD) around each antibiotic disk (if any), were measured using a transparent ruler, and recorded in millimeters (mm). A standardized table was used to determine if the bacterium was “Resistant”, “Intermediate” or “Sensitive”.

3. Results

3.1 Isolation of *S. aureus*

Twenty (20) patients of both sexes aged 4 days to 80 years were sampled. A total of 43 samples were collected from one or more body parts of each patient and analyzed, and seventeen (17) *S. aureus* isolates were recovered. The skin samples yielded 8 (47.06%) of the *S. aureus* isolates, nasal samples

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yielded an equal number of *S. aureus* isolates: 8 (47.06%), while a traumatic wound yielded 1 (5.88%) *S. aureus* isolate. Isolation of *S. aureus* was not found to be sex- or age-related as an equal number of *S. aureus* was isolated from male and female patients, while *S. aureus* was isolated from all the age groups. Also isolated were coagulase-negative staphylococcus (n=21), *Streptococcus species* (n=3), and unidentified rod-shaped bacteria (n=2). (Table 1). The numbers and distribution of isolates in each type of sample are shown in Figure 1.

Table 1: Identification scheme for *S. aureus* isolates

Sample code	Source of sample	Gram reaction	Mannitol salt agar	Catalase	Coagulase	Isolate	Age of patient	Sex of patient
A_1	Traumatic wound	+ clustered cocci	Golden yellow	+	+	*S. aureus*	35	F
A_2	Skin	- rods	No pigment	-	-	Unidentified	,,	,,
B_1	Skin	+ clustered cocci	Golden yellow	+	+	*S. aureus*	23	F
B_2	Nares	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,
C_1	Traumatic wound	+ clustered cocci	No pigment	+	-	C-nSt	27	F
C_2	Nares	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,
D_1	Skin	+ clustered cocci	No pigment	+	-	C-nSt	35	F
D_2	Nares	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
E_1	Skin	+ chain cocci	No pigment	-	-	*Strept.* sp.	30	F
E_2	Nares	+ chain cocci	No pigment	-	-	*Strept.* sp.	,,	,,
F_1	Skin	- rods	No pigment	-	-	Unidentified	80	M
F_2	Nares	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
G_1	Umbilical cord	+ chain cocci	No pigment	-	-	*Strept.* sp.	4 days	M
G_2	Skin	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,
H_1	Umbilical cord	+ clustered cocci	No pigment	+	-	C-nSt	4 days	M
H_2	Skin	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,
I_1	Surgical wound	+ clustered cocci	No pigment	+	-	C-nSt	22	F
I_2	Skin	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
I_3	Nares	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,
K_1	Skin	+ clustered cocci	No pigment	+	-	C-nSt	33	F
K_2	Nares	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
K_3	Surgical wound	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
L_1	Surgical wound	+ clustered cocci	No pigment	+	-	C-nSt	30	F
L_2	Skin	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
L_3	Nares	+ clustered cocci	No pigment`	+	-	C-nSt	,,	,,
M_1	Skin	+ clustered cocci	No pigment	+	-	C-nSt	20	M
M_2	Nares	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
N_1	Skin	+ clustered cocci	Golden yellow	+	+	*S. aureus*	52	M
N_2	Nares	+ clustered cocci	No pigment	+	-	C-nst	,,	,,
O_1	Skin	+ clustered cocci	Golden yellow	+	+	*S. aureus*	26	M
O_2	Nares	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,
P_1	Skin	+ clustered cocci	Golden yellow	+	+	*S. aureus*	78	M
P_2	Nares	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	..
Q_1	Skin	+ clustered cocci	Golden yellow	+	+	*S. aureus*	35	M
Q_2	Nares	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,
R_1	Skin	+ clustered cocci	No pigment	+	-	C-nSt	25	F
R_2	Nares	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
S_1	Skin	+ clustered cocci	No pigment	+	-	C-nSt	40	M
S_2	Nares	+ clustered cocci	No pigment	+	-	C-nSt	,,	,,
T_1	Skin	+ clustered cocci	No pigment	+	-	C-nSt	35	F
T_2	Nares	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,
V_1	Skin	+ clustered cocci	Golden yellow	+	+	*S. aureus*	39	F
V_2	Nares	+ clustered cocci	Golden yellow	+	+	*S. aureus*	,,	,,

C-n-St- coagulase-negative *Staphylococcus*, *Strep* sp- *Streptococcus* species

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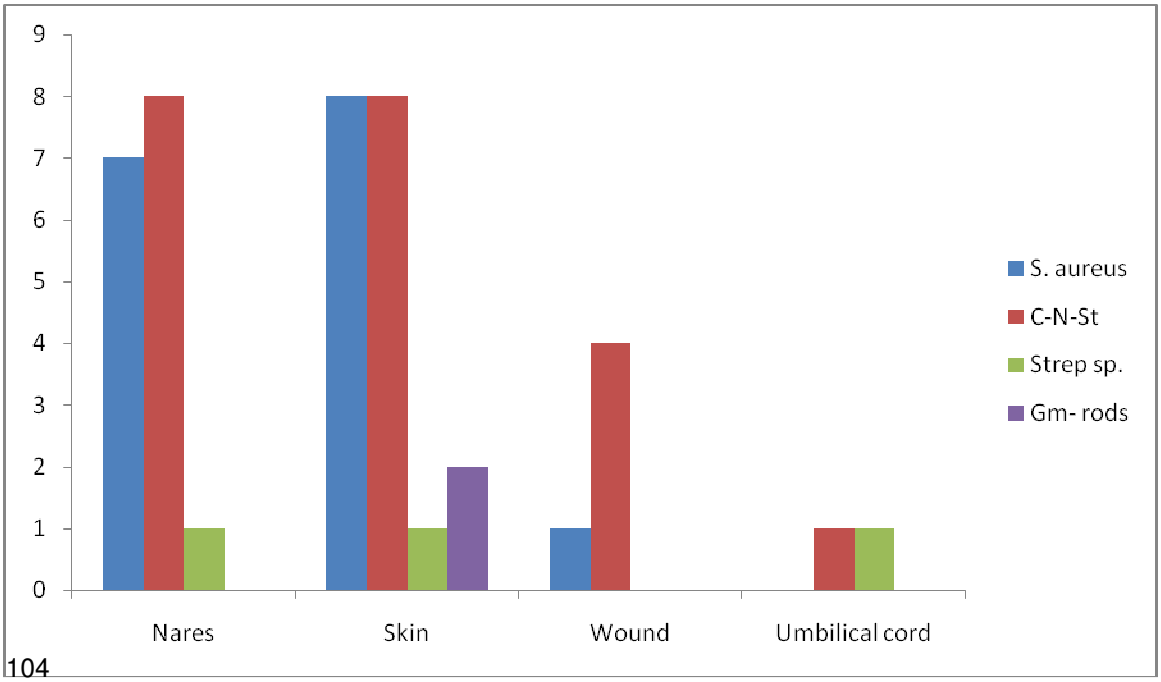

Figure 1: Numbers of different bacteria isolated from the samples

3.2 Antibiotic susceptibility pattern of *S. aureus* isolates

The seventeen (17) *S. aureus* isolates showed varying degrees of resistance to the antibiotics. The percentage resistances to the antibiotics in decreasing order were to oxacillin (100%), cloxacillin (88.2%), amoxicillin-clavulanic acid (augmentin) (76.5%), cefuroxime (70.6%), erythromycin (70.6%), ceftazidine (64.7%), gentamicin (47.1%,) ofloxacin (41.2%), and ceftriaxone (23.5%). Overall, the isolates showed an average resistance of 64.7%, sensitivity 22.9% and intermediate 12.4%. *S. aureus* strain N_1 was resistant to all nine antibiotics tested (100%), while A_1, P_2 and T_2 were resistant to eight (88.9%). The highest degree of resistance was to oxacillin (100%), while the lowest degree of resistance was to ceftriaxone (23.5%), followed by ofloxacin (41.2%). Ofloxacin received the highest sensitivity (52.9%) from the isolates, suggesting that fluoroquinolones may be the drugs of choice in the treatment of infections caused by *S. aureus*. This finding is supported by reports of previous studies [23] (Table 2). The percentage resistances of all the *S. aureus* isolates are represented by a line chart (Figure 2).

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Table 2: Zones of Inhibition (mm) produced by antibiotics against *S. aureus*

S. aureus	CAZ (30µG)	CRX (30µG)	GEN (10µG)	CTR (30µG)	ERY (30µG)	CXC (5µG)	OFL (5µG)	AUG (30µG)	OX (5µG)	S (%)	I (%)	R (%)
A_1	0	0	0	28	0	0	0	0	10	11.1	-	88.9
B_1	17	0	22	17	0	10	30	0	8	22.2	22.2	55.6
B_2	15	0	19	14	0	0	25	0	8	22.2	22.2	66.7
C_2	11	0	14	15	0	0	29	16	0	11.1	22.2	66.7
G_2	15	0	0	15	0	8	25	0	0	11.1	22.2	66.7
H_2	16	0	0	15	0	8	25	0	0	11.1	22.2	66.7
I_3	13	0	16	15	0	0	27	16	0	22.2	11.1	66.7
N_1	0	0	11	0	0	0	0	0	0	-	-	100
O_1	≥30	≥30	≥30	≥30	22	22	24	≥30	14	88.9	-	11.1
O_2	0	0	30	20	22	0	20	16	12	33.3	11.1	55.6
P_1	22	10	≥30	≥30	≥30	0	15	0	0	44.4	11.1	44.4
P_2	0	0	26	0	0	0	0	0	0	11.1	-	88.9
Q_1	0	20	13	16	0	13	0	24	0	22.2	11.1	66.7
Q_2	0	23	14	18	22	15	0	30	0	33.3	33.3	33.3
T_2	0	0	10	11	0	0	23	16	0	11.1	-	88.9
V_1	0	17	10	12	17	10	0	21	0	22.2	11.1	66.7
V_2	0	18	0	14	0	0	0	0	0	11.1	11.1	77.8
S (%)	10.8	23.5	41.2	17.7	29.4	5.9	52.9	23.5	-			
I (%)	23.5	5.9	11.8	58.8	-	5.9	5.9	-	-			
R (%)	64.7	70.6	47.1	23.5	70.6	88.2	41.2	76.5	100			

Key: CAZ- ceftazidine, CRX- cefuroxine, GEN- gentamicin, CTR- ceftriazone, ERY- erythromycin, CXC- cloxacillin, OFL- ofloxacin, AUG- amoxicillin, OX- oxacillin. , S- susceptible, I- intermediate, R- resistant.

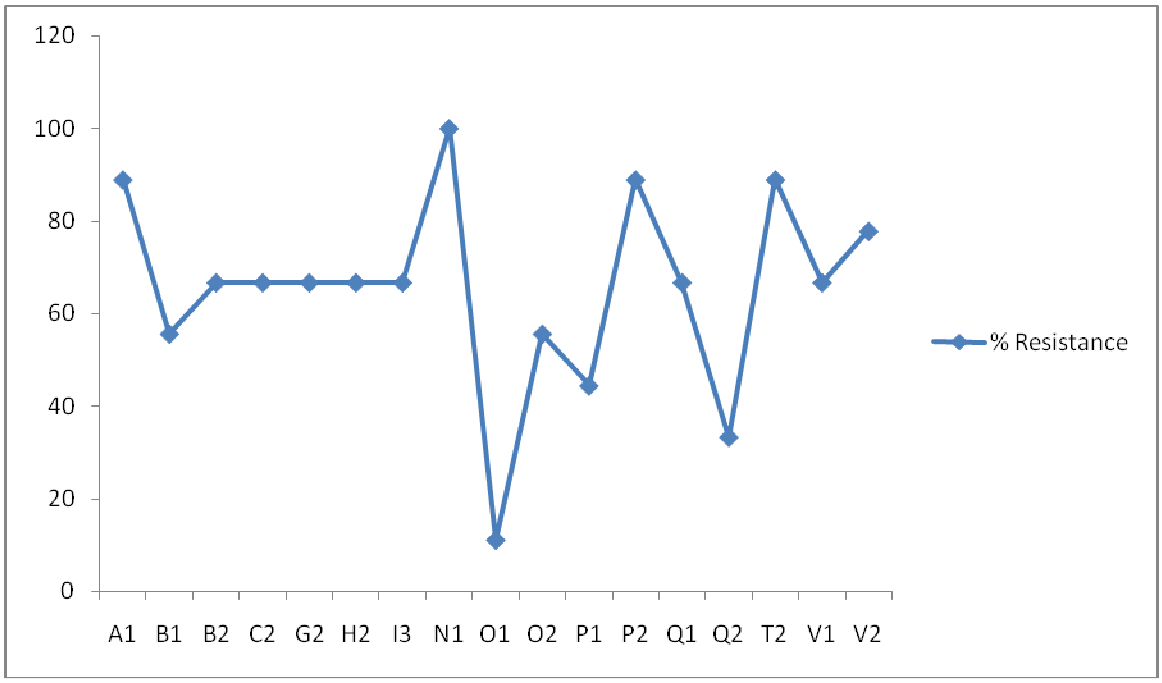

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Figure 2: Percentage resistances of *S. aureus* isolates A_1 –V_2 depicting varying degrees of multiple drug-resistance. *S. aureus* strain N_1 was resistant to all nine antibiotics in the study.

4. **Discussion**

Isolation of *S. aureus* in the present study was not found to be sex- or age-related as an equal number of *S. aureus* was isolated from male and female patients, while *S. aureus* was isolated from all the age groups. Isolation of *S. aureus* from the skin of 4 day old infants and not from their umbilical cord wounds implies that infants as young as 4 days can be colonized by *S. aureus.*

Results from the present study agree with reports of previous workers who found high rates of methicillin reistance in *S. aureus*. Oxacillin resistance, used as an indicator of methicillin resistance was found to be 100% in this study, in line with the global phenomenon of methicillin resistant *S. aureus* (MRSA) [24][25][26]. Findings in this study also agree with reports that MRSA is intrinsically cross-resistant to virtually all β-lactam antibiotics [14]. However, the 100% oxacillin resistance found in this study is alarming, and much higher than that reported from another part of Nigeria where phenotypic resistance to methicillin and oxacillin were 32.7% and 40.3% respectively [15].

The author found high rates of MRSA and multiple drug resistant *S. aureus* on the body and clinical samples of hospitalized patients. This is in consonance with reports that resistant microbes abound in the hospital environments because of antibiotic treatment regimes administered to hospital patients. In hospitals, inappropriate use of antimicrobial agents tends to create a selective pressure that promotes the emergence of resistant strains and predisposes patients to colonization with such organisms. The carriage of *S. aureus* on the bodies, especially hands of 25%-27% of health workers reported by previous workers [6] is an important factor in transferring resistant microbes to patients hospitalized in the wards.

Due to the frequent disease outbreaks and high rates of mortality attributed to MRSA, the dominance of MRSA and other antibiotic resistant pathogens in the hospital environment is a phenomenon that must be approached in a more proactive manner. More emphasis should be placed on proper and regular hand washing by health workers, sterilization/disinfection of instruments, materials and surfaces, as well as maintenance of sanitary conditions in the wards. All these will greatly reduce the occurrence and transfer

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of resistant microbes in the hospitals. Regular washing of bed clothes with soaps and disinfectants, and use of sterile hand gloves when handling patients will ensure that antibiotic resistant microbes do not survive and are not transferred from patient to patient.

Development of alternative ways to treat or control bacterial infections is an issue that should attract more interest from the government and other funding bodies. Research on plant-based drugs should receive more funding since plants have shown a lot of promise as sources of alternative treatments for antibiotic resistant infections [27]. Vaccine development is another area that should not be undermined in the search for solution for antimicrobial resistance in bacterial pathogens. More research effort is needed in the area of DNA vaccines, which is reported to be effective, with no adverse effects in experimental animals [28][29].

5. Conclusion

The high rates of resistance to the β-lactams – Oxacillin, cloxacillin and the combined amoxicillin-clavulanic acid- highlights the decreasing importance of these drugs in antimicrobial therapy for *S. aureus* infections. Antibiotic resistance is highest where a majority of the population is poor and ignorant of appropriate use of antibiotics as is the case with the study area in this investigation. The authorities in the health sector should mount public enlightenment programs to educate the masses on the dangers of abusing antimicrobials, as well as the appropriate ways to use them. Development of and strict adherence to sound antibiotic policies in the health sector will help slow the emergence of drug resistance.

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