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Antimicrobial Susceptibility Pattern of Staphylococcus aureus Isolates from Orthopaedic Patients in Abuth, Zaria

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Abstract

As *Staphylococcus aureus* remain the predominant microbial flora of the human respiratory tract and skin; it also account for the most human integumental infections and life-threatening systemic diseases especially in **orthopaedic** surgical site infections (SSIs). This study evaluates the antibiotics susceptibility pattern of *Staphylococcus aureus* isolates from orthopaedic patients to various antimicrobial agents used in the treatment of surgical sites infection in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. A total of 100 clinical swab samples of surgical sites were collected from orthopaedic patients in ABUTH, Zaria, Nigeria out of which 39 were identified as *Staphylococcus aureus* using API STAPH identification kit. Disc agar diffusion method was used for the antibiotics susceptibility test while nitrocefin microplate assay was used to test for beta lactamase production. Our findings showed that 97.4% of the isolates were susceptible to both vancomycin and gentamicin followed by ciprofloxacin (94.9%) and pefloxacin (84.6%). The isolates were highly resistant to ampicilin (94.9%), ceftriaxone (79.5%), cefoxitin (64.1%) and amoxicillin-clavulanic acid (59%) which is beta-lactam antibiotics. Further evaluation showed that 64% of the isolates produced beta-lactamase, while 36% do not. We conclude that the Staphylococcus aureus isolates from orthopaedic patients in ABUTH, Zaria were highly resistant to beta lactam antibiotics used in this study.

Key words:

Staphylococcus aureus; Orthopaedic patients; Antibiotic susceptibility; ABUTH; Zaria

Introduction

Staphylococcus aureus is commonly carried on the skin or in the nose of healthy individuals. It is an important pathogen in human infections causing illness ranging from minor skin infections and abscesses to life - threatening diseases such as pneumonia, meningitis, endocarditis, toxic shock syndrome and septicaemia which may be rapidly fatal [1]. Bacterial resistance to antibiotics has been recognized since the first drugs were introduced for clinical use. Penicillin was first introduced in 1941, when less than 1% of Staphylococcus aureus strains were resistant to its action. By 1947, 38% of hospital strains had acquired resistance and currently over 90% of Staphylococcus aureus isolates are resistant to penicillin. Increasing resistance to antibiotics is a consequence of selective pressure [2]. In orthopaedics, S. aureus has been implicated in surgical site infection, painful infection of joint fluid known as septic or infective arthritis, post-operative infection, implant devices, infection following trauma, chronic osteomyelitis subsequent to an open fracture, meningitis following skull fracture. This study was aimed at determining the antibiotic susceptibility pattern of the S. aureus isolates from orthopaedic patients in a tertiary hospital in North-western Nigeria.

Methodology

Sample collection

One hundred clinical samples were collected aseptically from the wound, skin and bed of orthopaedic patients in Ahmadu Bello University Teaching Hospital Zaria, Nigeria over a period of 5 months. Ethical approval and patients' consent were obtained.

Identification of *S. aureus* isolates

API STAPH identification kit (bioMerieux, Inc, Durham, USA) was used to identify the S. aureus isolates. The procedures were carried out according to the manufacturer's instructions.

Antibiotic susceptibility test

Disk diffusion tests was performed for each of the isolates previously identified as *S. aureu* follow the method recommended by the Clinical Laboratory Standard Institute [3]. List of antibiotics used are: Cefoxitin 30 µg, Ceftriaxone 30 µg, Vancomycin 30 µg, Ampicillin 10 µg, Gentamicin 10 µg, Pefloxacin 5 µg, Ciprofloxacin 5 µg, Amoxicillin-clavulanic acid 30 µg, Erythromycin 15 µg and Clindamycin 2 µg (Oxoid Ltd. Basingstoke, London).

Test for β-lactamase production (Nitrocefin test)

Enzyme extracts of the S. aureus isolates were prepared as described by Caddick [4] with modification. Microplate Nitrocefin assay was carried out as follows: 1 mg lyophilized Nitrocefin powder (Oxoid, UK) was reconstituted in 1.9 ml of 0.1 M phosphate buffer, pH 7

supplied by the manufacturer. The reconstituted nitrocefin was further diluted 1 in 10 with PBS to give 50 µg/ml solution. The disrupted cell preparations were used immediately by dispensing 50 µL of preparation into separate wells of a 96 well plate. 50 µL of diluted nitrocefin solution was added into each of the wells and incubated at 37°C for 10 minutes. In the presence of β-lactamase, the chromogenic nitrocefin substrate changes colour from yellow to pink/red.

Determination of multiple antibiotics resistance (MAR) index

The Multiple Antibiotic Resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the organisms is resistant to by the total number of antibiotics tested [5-7].

Results

A total number of 100 samples were collected and the distribution of S. aureus from the sample sites is presented in Table 1.

Source	No of sample	*S. aureus* No (%)
Wound	22	6(27.3)
Skin	43	16(37.2)
Bed	35	17(48.6)
Total	100	39(39.0)

Table 1: Distribution of *S. aureus* isolates.

Antibiotic Susceptibility Pattern of isolates

A high percentage (97.4%) of isolates were susceptible to both vancomycin and gentamicin followed by ciprofloxacin (94.9%) and pefloxacin (84.6%). The level of resistance of the S. aureus isolates to antibiotics is as follows: ampicilin (94.9%), ceftriaxone (79.5%), cefoxitin (64.1%) and amoxicillin - clavulanic acid (59%) Table 2.

Antibiotics	Sensitive	Intermediate	Resistant
No of sample (%) n=39			
Ampicillin 10 µg	2 (5.1)	0	37 (94.9)
Ceftriaxone 30 µg	8(20.5)	13 (33.3)	18 (46.2)
Cefoxitn 30 µg	14 (35.9)	0	25 (64.1)
Amoxicillin-clavulanic acid 30 µg	16 (41.0)	0	23(59.0)
Clindamycin 2 µg	12(30.8)	13 (33.3)	14 (35.9)
Erythromycin 15 µg	19 (48.7)	10 (25.6)	10 (25.6)
Pefloxacin 5 µg	33 (84.6)	0	6 (15.4)
Vancomycin 30 µg	38 (97.4)	0	1 (2.6)
Ciprofloxacin 5 µg	37 (94.9)	2 (5.1)	0
Gentamicin 10 µg	38 (97.4)	0	1 (2.6)

Table 2: Percentage susceptibility of *S. aureus* isolates.

Beta lactamase Test and their Resistant Pattern to Antibiotic

The result of the beta lactamase test is shown in Figure 1.

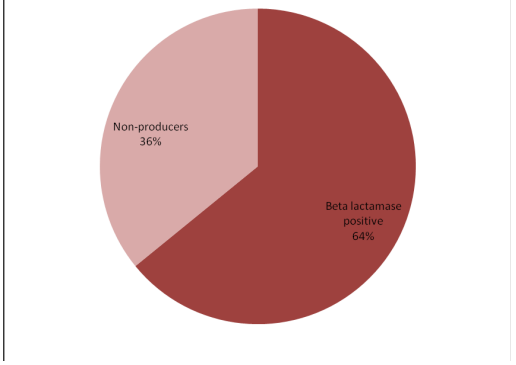

Figure 1: Beta lactamase production by *S. aureus* isolates.

Resistant Pattern of beta lactamase Producing *S. aureus*

The resistant patterns of beta lactamase producing *S. aureus* isolates to antibiotics are presented in Figure 2. There it was observed that these beta lactamase producing isolates were generally resistant to ampicillin (96%), ceftriaxone (88%), cefoxitin (76%) and amoxicillin - clavulanate (68%) which are beta lactam antibiotics.

Multiple Antibiotic Resistant (MAR) Index

MAR	S. aureus isolates (n= 39)
0.1	4 (10.3)
0.2	3 (7.7)
0.25	2 (5.1)
0.3	4 (10.3)
0.4	5 (12.8)
0.5	9 (23.1)
0.6	8 (20.5)
0.7	2 (5.1)
0.8	1 (2.6)
0.9	1 (2.6)

Table 3: Result of multiple antibiotic resistant (MAR) index for *S. aureus* solates antibiotics resistance pattern of *S. Aureus.*

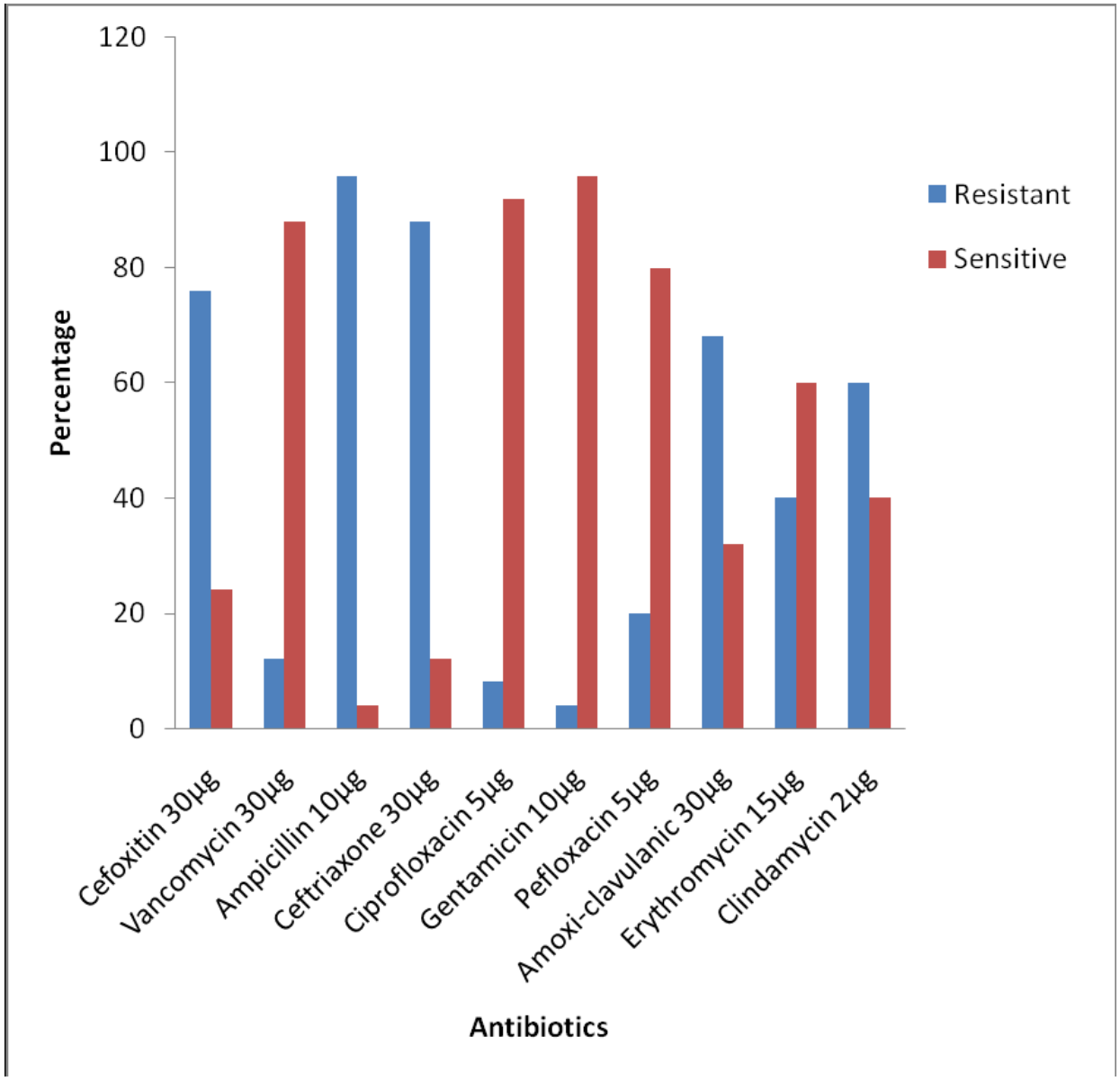

Figure 2: Resistance pattern of beta - lactamase producing *S. aureus* to antibiotics.

The result of the MAR index of the S. aureus showed that 32(82.1%) of the resistant *S. aureus* isolates had MAR index greater than 0.2, details are shown Table 3.

This result showed that 50% (19) of the isolated Staph. aureus were multidrug resistant (MDR) and 28% (11) were extensively drug resistant (XDR). The isolates were observed to show a concurrent pattern of resistance to Cephalosporine, Macrolide and Betalactame/ Betalactame inhibitors (21%) while 10% (4) showed resistance to Cephalosporine, Betalactame/Betalactame inhibitors and Fluoroquinolones.

Discussion

S. aureus is known to be one of the causes of nosocomial infection [8-10], the isolation of *S. aureus* from the patients' beddings in this study is in support of this. The majority of nosocomial infection is caused by the patient's own endogenous microbial flora present upon admission to the hospital [11]. The 97.4% susceptibility to gentamicin observed in this study is consistent with previous reports from Africa [12,13] Gentamicin is an example of aminoglycoside whose mode of action is to inhibit protein synthesis by inhibiting 30 S ribosome of the organism [14]. High susceptibity to vancomycin was observed in this study. Vancomycin is a glycopeptide which inhibits peptidoglycan synthesis in the bacteria, it is a known drug of choice in the treatment of methicillin resistant *S. aureus* [15]

Ciprofloxacin is a quilonone which acts by inhibition of enzymes involved in DNA replication and function, the 94.9% susceptibility of *S. aureus* observed in this study is in conformity with previous reports [16-18]. The little level of resistance to ciprofloxacin observed in this study might be connected with the increasing rate of availability of different cheap brands of generic ciprofloxacin in the market which might have probably led to the misuse of it.

The *S. aureus* isolates in this study were generally resistant to penicillins as was shown by the resistance pattern of the beta lactamase producing *S. aureus* isolates in Figure 2. Beta lactamase hydrolyses the amide bond of the β-lactam ring resulting in an inactive compound. Some researchers found penicillins to have the highest rate of resistance to clinical samples especially *S. aureus*. [19,20]. Penicillins inhibit bacterial cell wall synthesis [21], *S. aureus* develop resistance to penicillins by the production of beta lactamases and by permmeabilty barrier of the cell surface [22].

The high percentage of the *S. aureus* having MAR index greater than 0.2 (Tables 3) suggests that the isolates originated from a high risk source of contamination where antibiotics are often used [6,7]. It also indicates that a large proportion of the bacterial isolates have been exposed to several antibiotics. The high incidence of multi drug resistance observed in this study (Table 4) could be attributed to a combination of microbial characteristics such as selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug resistant organisms [23]. Other reasons could be due to increase in irrational consumption rate of antibiotics, transmission of resistant isolates between people, self-medication, non-compliance with medication and sales of substandard drug.

S/N	Isolates	Antibiotics Resistant Pattern	CART	LR
1	W4	AMP	BT	NIL
2	W7a	FOX, AMP, CRO, ERY, DA, AMC	CEP, BT, MAC, LIN	MDR
3	W7b	FOX, AMP, CRO, AMC	CEP, BT	XDR
4	W20	FOX, AMP, CRO, AMC	CEP, BT	XDR
5	W39	FOX, AMP, CRO, VA	CEP, BT, MAC, GL	MDR
6	W51	FOX, AMP, DA, AMC	CEP, BT, LIN	MDR
7	S1	DA	LIN	NIL
8	S2	FOX, AMP, AMC, ERY, DA	CEP, BT, MAC, LIN	MDR
9	S8	AMP	BT	NIL
10	S12	FOX, AMP, CRO, AMC, VA	CEP, BT, GL	MDR
11	S20	FOX, AMP, CRO, AMC	CEP, BT	XDR
12	S23	AMP, ERY, DA	BT, MAC, LIN	MDR
13	S24	FOX, AMP, CRO, AMC, ERY, DA	CEP, BT, MAC, LIN	MDR
14	S25	AMP, DA	BT, LIN	XDR
15	S27	FOX, AMP, CRO, AMC, VA	CEP, BT, GL	MDR
16	S41	FOX, AMP, CRO, AMC	CEP, BT	XDR
17	S46	FOX, AMP, CRO, AMC, VA, PER	CEP, BT, GL, FLU	MDR
18	S47	DA	LIN	NIL
19	S51	FOX, AMP, AMC	CEP, BT	XDR
20	S55	FOX, AMP, PER, AMC	CEP, BT, FLU	MDR
21	S58	AMP	BT	NIL
22	S72	AMP, DA	CEP, BT, MAC, LIN	MDR
23	B1	AMP, CRO, ERY, DA	CEP, BT, MAC, LIN	MDR
24	B7	AMP	BT	NIL
25	B8	AMP	BT	NIL
26	B13	FOX, AMP, CRO, AMC, VA	CEP, BT, GL	MDR

27	B16	FOX, AMP, AMC, ERY	CEP, BT, MAC	MDR
28	B20	FOX, AMP, CRO	CEP, BT	XDR
29	B26	FOX, AMP, CRO, AMC	CEP, BT	XDR
30	B35	FOX, AMP, CRO, AMC	CEP, BT	XDR
31	B47	FOX, AMP, CRO, AMC	CEP, BT	XDR
32	B49	FOX, AMP, AMC	CEP, BT	XDR
33	B50	AMP, ERY, DA	BT, MAC, LIN	MDR
34	B55	FOX, AMP, AMC, PEF	CEP, BT, FLU	MDR
35	B60	AMP, ERY, DA	BT, MAC, LIN	MDR
36	B62	AMP	BT	NIL
37	B69	FOX, AMP, CRO, AMC, ERY, DA	CEP, BT, MAC, LIN	MDR
38	B77	FOX, AMP, CRO, PEF	CEP, BT, FLU	MDR

Key: Lincosamides (LIN), Cephalosporine (CEP), Macrolide (MAC), Betalactame/ Betalactame Inhibitors (BT), Glycopeptide (GL), Fluoroquinolone (FLU), Class of antibiotics resistant to (CART), Level of resistance (LR), Cefoxitin (FOX) Vancomycin (VA), Ampicillin (AMP), Ceftriaxone (CRO), Ciprofloxacin (CIP), Gentamicin (CN), Pefloxacin (PEF), Erythromycin (ERY), Amoxicillin-clavulanic acid (AMC), Clindamycin (DA)

Table 4: Antibiotic resistance pattern of the isolated *S. aureus.*

Conclusion

High susceptibility S. aureus isolates to gentamicin and ciprofloxacin was observed in this study, this is an indication that these antibiotics can be used for empirical treatment of infections from orthopaedic patients in this hospital since vancomycin is not readily available in this community. The S. aureus isolates were highly resistant to beta lactam antibiotics. The abuse of beta-lactam antibiotics and other classes of antibiotics in our community should therefore be controlled.

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