ANTIBIOTICS RESISTANCE PATTERN OF *Staphylococcus aureus* ISOLATES FROM ORTHOPAEDIC PATIENTS IN AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, ZARIA, NIGERIA

Obajuluwa A. F[1] (Corresponding author), Onaolapo J. A[1], Olayinka B. O[1], Adeshina G.O[1] & Igwe J. C[2]
[1]*Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.*
[2] National Biotechnology Development Agency, Abuja, **NIGERIA**

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 resistance pattern of *Staphylococcus aureu*s 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 the isolates were highly resistant to ampicilin (94.9%), ceftriaxone (79.5%), cefoxitin (64.1%) and amoxicillin-clavulanic acid (59%) which are 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.

Keywords: *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 resistance pattern of the *S. aureus* isolates from orthopaedic patients in a tertiary hospital in North-western Nigeria.

METHODOLOGY

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. 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 30µg, Ceftriaxone 30µg, Vancomycin 30µg, Ampicillin 10µg, Gentamicin 10µg, Pefloxacin 5µg, Ciprofloxacin 5µg, Amoxicillin-clavulanic acid 30µg, Erythromycin 15µg and Clindamycin 2µg (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: 1mg lyophilized Nitrocefin powder (Oxoid, UK) was reconstituted in 1.9ml of 0.1M phosphate buffer, pH7 supplied by the manufacturer. The reconstituted nitrocefin was further diluted 1 in 10 with PBS to give 50µg/ml solution. The disrupted cell preparations were used immediately by dispensing 50µL of preparation into separate wells of a 96 well plate. 50µL of diluted nitrocefin solution was added into each of the wells and incubated at 37^0C 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 out of which 39 isolates were confirmed to be *S. aureus.*

Resistant Pattern of beta lactamase Producing *S. aureus*

The result of the beta lactamase production showed that 64% of the *S.aureus* isolates produced beta lactamase while 36% did not. The resistant patterns of beta lactamase producing S.aureus isolates to antibiotics are presented in Figure 1. 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.

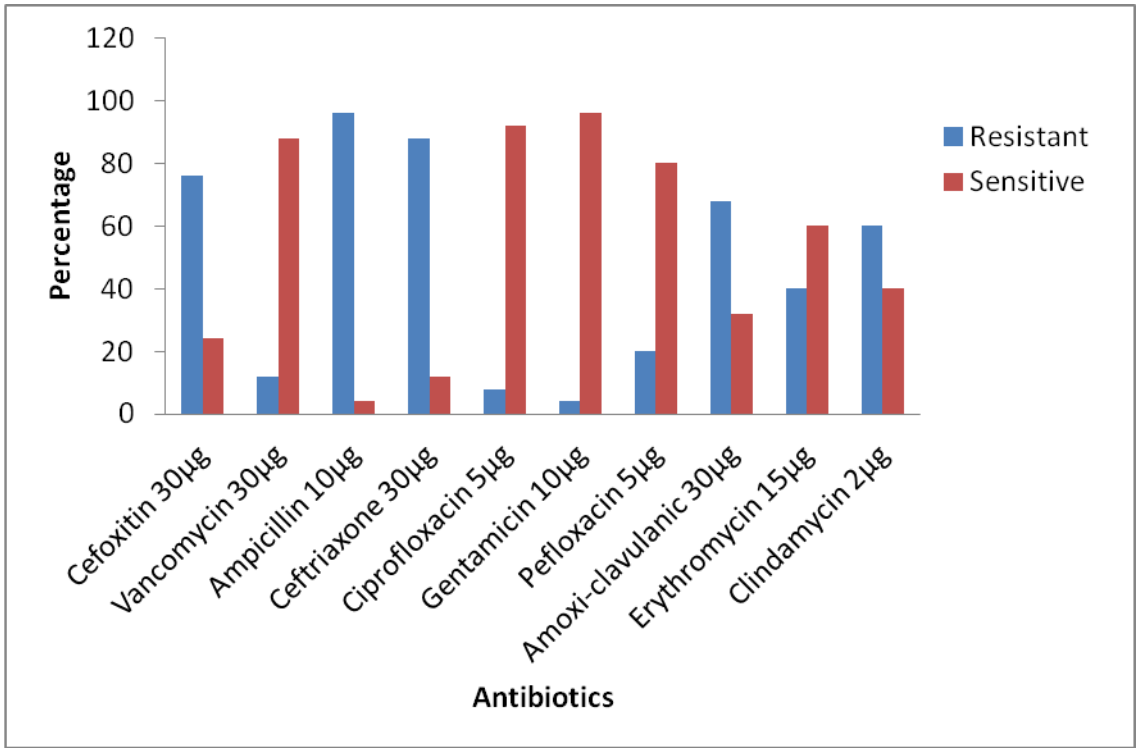

Figure 1: Resistance pattern of beta - lactamase producing *S. aureus* to antibiotics
Multiple Antibiotic Resistant (MAR) Index

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 1.

Table 1: Result of Multiple Antibiotic Resistant (MAR) index for *S. aureus* isolates

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)**

Antibiotics Resistance Pattern of *S. aureus*

This result as presented in Table 2 showed that 50% (19) of the isolated *S. 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% (8)) while 10% (4) showed resistance to Cephalosporine, Betalactame/ Betalactame inhibitors and Fluoroquinolones.

Table 2: Antibiotic Resistance Pattern of the Isolated *Staph. aureus*

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),Multi drug resistance (MDR) Cefoxitin (FOX) Vancomycin (VA), Ampicillin (AMP), Ceftriaxone (CRO), Ciprofloxacin (CIP), Gentamicin (CN), Pefloxacin (PEF), Erythromycin (ERY), Amoxicillin-clavulanic acid (AMC), Clindamycin (DA).

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 *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 1. 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.* [12,13]. Penicillins inhibit bacterial cell wall synthesis [14], *S. aureus* develop resistance to penicillins by the production of beta lactamases and by permmeabilty barrier of the cell surface [15].

The high percentage of the *S. aureus* having MAR index greater than 0.2 (Tables 1) 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 2) 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 [16]. 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. An isolate is said to be multidrug resistant if it is resistant to at least one agent in three or more antimicrobial categories [17-19].

CONCLUSION

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.

REFERENCES

1. Holmes A., Ganner M., McGuanes, Pitt T.L, Cookson B.D., Kearns A.M. (2005). *Staphlococcus aureus* isolates carrying Panton- Valentine leucocidin genes in England and Wales: frequency. Characterization, and association with clinical disease. *Journal of Clinical Microbiology* 43 (5): 2384-2390.
2. Power E.G.M. (1998). Bacteria Resistance to antibiotics. In: Hugo W.B. and Russel A.D. (6th ed.) *Pharmaceutical Microbiology*, London, Edinburgh, Blackwell Scientific Publications; 181-197.
3. Clinical and Laboratory Standards Institute (2007). Performance standards for antimicrobial susceptibility testing; 17th informational supplement.CLSI M100-S17. CLSI, Wayne, PA
4. Caddick J.M. (2005). Molecular typing of hospital-acquired, community acquired and multi-drug resistant methicillin –resistant *Staphylococcus aureus*. PhD Thesis Aston University, Birmingham.

5. Krumperman, P.H. (1983). Multiple antibiotic indexing *Escherichia coli* to identifying risk sources of faecal contamination of foods. *Applied and Environmental Microbiology* 46: 165- 170.
6. Paul, S. Bezbarauh, R.L. Roy,M.K. and Ghosh, A.C. (1997).Multiple antibiotic resistance (MAR) index and its reversion in *Pseudomonas aeruginosa. Letters in Applied Microbiology*. 24: 169- 171.
7. Christopher A.J, Hora S and Ali Z (2013). Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly-India. *Annals of Tropical Medicine and Public Health.* 6(3): 285-289.
8. Narezkina A., Edelstein I, Dekhnich A, Stratchounski L, Pimkin M and Palagin I (2006). Prevalence of methicillin – resistant *Staphylococcus aureus* in different regions of Russia: results of multicenter study. 12th European Congress of Microbiology and Infectious Diseases.
9. Keshni N., Ilisapeci N., Sharan R., Kerri V., Stephen M.G. and Karen B.(2014). A descriptive study of nosocomial infections in an adult intensive care unit in Fiji-2011-12. Journal of Tropical Medicine vol 2014
10. Sugata D., Soumi D., Neeraj S and Avijit H. (2015).Nosocomial infection in the intensive care unit: Incidence, risk factors, outcome and associated pathogens in a public tertiary teaching hospital of Eastern India; Indian J. Crit. Care Med; 19(1):14-20.
11. Arif M.A, Shahid A.A., Shazia A. and Irfan M. (2007). Nosocomial infections due to methicillin – resistant *Staphylococcus aureus* in hospital patients, *Pak J Med Sci*., 23 (4):593 – 596.
12. Emmanuel O.N., Magaji S.N. (2011). Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria; Pan Afr Med J. 2011; 8: 4. PMCID: PMC3201603
13. Cohen ML: Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science* 1992, 257:1050-1055.
14. Russell A.D. (2004). Types of Antibiotics and Synthetic Antimicrobial Agents in Denyer S.P., Hodges N.A. Gorman S.P. (eds) Hugo and Russell's *Pharmaceutical Microbiology* 7th edition Blackwell Science Ltd.152-178.
15. Pichichero M.E (2005). "A review of evidence supporting the American Academy of Pediatrics recommendation for prescribing cephalosporin antibiotics for penicillin-allergic patients". *Pediatrics* 115 (4): 1048–1057.
16. Orozova, P., Chikova, V., Kolarova, V., Nenova, R., Konovska, M. and Najdenski, H. (2008). Antibiotic resistance of potentially pathogenic *Aeromonas* strains. *Trakia Journal of Sciences*, 6(1): 71-78.
17. Nikaido H. (2009). Multidrug Resistance in Bacteria. *Annu Rev of Biochem;* 78:119-146.
18. Magiorakos et al (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*; 18:268–281.
19. World Health Organization.(2014). Antimicribial Resistance Fact sheet N°194 Updated April 2014.