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Multiple Antibiotic Resistance Pattern among Staphylococcus Aureus Strains Isolated from South/Western Nigeria Academic Teaching Hospital and the Environs…Which Way Out?

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Abstract: Widespread use of antibiotics has been responsible for the development of numerous problems including the emergence of multi-drug resistance bacteria and increased number of hospital acquired infections with increase health care costs. Eight hundred and fifty samples of different cultures were taken from clinical and non- clinical sources. The clinical sources were the routine specimens of wound swabs, urine, stool, blood and sputum from the Department of Microbiology and Parasitology laboratory of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile–Ife. The non-clinical samples were obtained from the nasal cavity of apparently healthy food handlers at restaurants in Obafemi Awolowo University campus and food vendors in Ile–Ife central market. Samples were cultured on mannitol salt agar and incubated at 37^{o}C for 24-48 hours. *Staphylococcus aureus* were isolated and identified based on mannitol fermentation, Gram's reaction, positive results for catalase, coagulase and DNAse tests. Susceptibility of the isolates to eight different antibiotics was tested using the disk diffusion technique. Inducible resistance of clindamycin by erythromycin was performed on the isolates. Four hundred and five (405) *S. aureus* isolates were identified from 770 presumptive staphylococci based on positive results for coagulase and DNase tests. These comprised 56.8% clinical and 43.2% non-clinical isolates. All the urine isolates were resistant to penicillin. All other isolates from both sources were resistant to penicillin at variance higher levels. Among the clinical isolates, resistance to chloramphenicol was the highest (91%), followed by ciprofloxacin (70%) and gentamicin (69%). Among the non-clinical isolates, 62% from the food handlers and cell phones were resistant to cefoxitin. The prevalence of methicillin resistant *Staphylococcus aureus* was 18%. One hundred and one (101) multiple antibiotic resistance patterns comprising 58 and 43 were observed among clinical and non-clinical isolates, respectively. Majority of the Clinical isolates constituted 4.8%, MRSA. 27.4% MSSA, 45.2% MDR/MRSA, 22.6% MDR/MSSA and Non- Clinical were 10.3% MRSA, 23.4% MSSA, 52.6% MDR/MRSA and MDR/MSSA types.
The study concluded that *S. aureus* was implicated in a wide variety of infections and the prevalence of multiple antibiotic resistance types were high in the study area.

Introduction

A number of investigations have indicated that *S. aureus* is the main aetiological agent of many infections in Nigeria (AkoNai *et al*, 1999; Anah *et al.*, 2008; Odetoyin *et al.*, 2008; Adeleke and Asani, 2009; Bekibele *et al.*, 2009; Onipede *et al.*, 2009). However, many studies, identification and antibiotic susceptibility testing of *S. aureus* isolates have been based on phenotypic methods and few data exist on the characterization of *S. aureus* using molecular methods (Adesida *et al.*, 2005; Shittu and Lin, 2006; Okon *et al.*, 2009).

The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria (Mathew *et al.*, 2007). Also, unhealthy methods practices in most pharmaceutical manufacturing industries contribute

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towards the likelihood of creating antibiotic resistance strains (Tacconelli *et al.*,2008). The risk for colonization increases if the superbugs are not sensitive to the antibiotics used and hence high tissue penetration as well as broad spectrum activity against bacteria will occur. In the case of increased rate of MRSA infections, susceptibility are seen with glycopeptides, cephalosporins and especially quinolones (Tacconelli *et al., 2008*). Community Acquired- Methicillin Resistance *Staphylococcus aureus* (CA-MRSA) has now emerged as an epidemic that is responsible for rapidly progressive fatal diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis (Hiramatsu *et al.*, 2007).

Methicillin Resistance *S. aureus* is the most frequently identified antimicrobial drug resistant pathogen in hospitals. The epidemiology of infections caused by MRSA is rapidly changing. Outbreaks of community associated infection (CA)-MRSA have been reported in correctional facilities, among athletic teams, among military recruits, in newborn nurseries, and among men who engage in homosexual activities. CA-MRSA infections now appear to be endemic in many urban regions and cause most CA-*S. aureus* infections (Hiramatsu *et al.*, 2007).

Sub optimum antibiotic concentrations in critically ill people increase the frequency of antibiotics resistant organisms due to indiscriminate exposure to the drug (Thomas *et al.*, 1998). While taking antibiotic doses less than those recommended may increase the rates of resistance, shortening the course of antibiotics may actually decrease rates of resistance (Li *et al.*, 2007). Poor hand hygiene by hospital staff has been associated with the spread of resistant organisms (Girou *et al.*, 2006) and an increase in hand washing compliance results in decreased rates of the organisms (Swobarda *et al.*, 2004). There are three known mechanisms of fluoroquinolones resistance. Some type of efflux pumps can act to decrease intracellular quinolones concentration. In Gram–negative bacteria, plasmid – mediated resistance genes produce proteins that can bind to DNA gyrase, protecting it from the action of quinolones. Finally, mutations at key sites in DNA gyrase or Topoisomerase 1V can decrease their binding affinity to quinolones, decreasing the drug's effectiveness (Li *et al.*, 2009). Research has shown that the bacterial protein LexA may play a key role in the acquisition of bacterial mutations giving resistance to quinolones and rifampicin (Cirz *et al.*, 2005).

This study investigated resistance patterns among clinical and non-clinical isolate of *S. aureus* obtained from Ile –Ife, Nigeria with possible recommendation for way out.

Materials and Methods

Source of bacterial isolates

Staphylococcus aureus isolates were recovered from both clinical and non-clinical specimens. The clinical sources were from the routine specimens of wound swabs, urine, stool, and sputum samples of the patients submitted to the Microbiology laboratory of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), (Urban Centre), Ile – Ife.

The non-clinical isolates were recovered as nasal swabs from food handlers at the Obafemi Awolowo University (OAU) campus restaurants, marketers at the Ile-Ife central market and also from fomites within the hospital, which comprised of doctors stethoscopes, and cell phones from the community dwellers and the Health care workers.

Samples collection

Samples were collected between the period of October 2007 and November 2009. Eight hundred and fifty (850) swab samples from clinical and non-clinical sources at Obafemi Awolowo University Teaching Hospital Complex; OAU Campus community and Ile-Ife environs were obtained. Sterile cotton–tipped applicators (Sterilin, England) appropriately moistened with sterile distilled water were used for swabbing sample surfaces.

Samples from both hospital (hospitalized patients who were on different types of antibiotic treatment) and the community with different sexes, age ranges and of different diagnostic infections histories ranging from diabetic ulcers, cancers (breast cancer, prostrate carcinoma) obstructive uropathy, (e.g benign prostrate hypertrophy (BPH)) septicaemia, urinary tract infection, burnt injuries, gastroenteritis, pelvic inflammatory diseases, sexually transmitted infections, pneumonia and many other clinical diagnosis cases were considered.

The non-clinical samples were obtained from the nostrils of apparently healthy community food handlers, also from cell phones and stethoscopes. The swabs after collection were taken to the laboratory immediately for bacteriological analysis.

Microbiological analysis

Isolation of *S. aureus* was done by standard procedure in which the samples were inoculated on freshly prepared mannitol salt agar plates (Oxoid, Basingstoke, Hampshire, England) and incubated at 37^{o}C for 24h. Golden yellow colonies on Mannitol Salt agar (MSA) after the incubation period were taken presumptively for *S. aureu*

Phenotypic and biochemical identification of the isolates

The isolates were Gram stained as described by

Olutiola *et al.* (1991). Biochemical identification of the isolates was carried out using standard methods The following biochemical tests were carried out on the isolates namely catalase, tube coagulase test, and the DNase test using DNase agar based (Oxoid Ltd., Basingstoke, Hampshire, England). The confirmed isolates were stored as stock culture on nutrient agar slants and kept at about 4°C until further use.

Gram's reaction Test

Gram staining technique is a differential staining procedure that separate bacteria into two classes i.e Gram positive and Gram negative. A smear of an 18-24 hour old cuture on nutrient agar was prepared on a clean microscopic slides. The smear was then heat-fixed by passing the slide through a bursen burner flame. The smear was flooded with crystal violet and allowed to react for 1 minute after which the stained was poured of and the smear rinsed under gentle running tap water. Thereafter, the slide was flooded with Gram's iodine solution (a mordant) and then rinsed off under gentle running tap water. The smear was later decolorized with 95% ethanol, rinsed under gentle running tap water, and counterstained with safranin for about 30 seconds. The slide was then washed under gentle running tap, allowed to air dried and then examined under the oil immersion objective of the light compound microscope (Leica Gallen 111; Leica Inc, NY, USA). Gram positive staphylococci appeared as round clustered and purple in color.

Catalase test

This test was used to differentiate between staphylococci (catalase positive) and streptococci (catalase negative). Catalase positive bacteria are capable of producing an enzyme known as catalase which breaks down hydrogen peroxide to water and two drops of 3% hydrogen peroxide was placed on a clean grease-free slide. Colonies of the isolate similar to Gram positive cocci in clusters was emulsified in the drop. Rapid effervescence of gas was recorded as positive. A control slide containing only drop of hydrogen peroxide showed no gas bubbles.

Coagulase test

The isolates were inoculated into 1ml of nutrient broth in test tubes and incubated overnight at 37˚C. One milliliter of fresh human plasma was added into the tubes previously incubated with the isolates and further incubated at 37˚C and were examined at intervals for 4 hours. Formation of clot up to 4hr at 37 ˚C indicates positive coagulation (Olutiola *et al.*, 1991). ATCC25923 serves as control strain.

DNase test

This is a confirmatory test for *S. aureus* based on its ability to produce DNase enzyme that can degrade nucleic acids. Thirty nine grams of the agar was suspended in 1 litre of distilled water and dissolved completely by boiling. The content was sterilized by autoclaving at 121ºC for 15 minutes. The plates were inoculated by spotting the bacterial culture onto the surface of the agar so that a thick plaque of growth became evident after 18 hours incubation. The plates were flooded with 1N HCl and allowed to stand for 2 minutes. The clear zones around the colonies were taken as positive result for the growth of *S. aureus.* *Staphylococcus aureus* ATCC25923 and *S. epidermidis* served as positive and negative control.

Standardization of Inoculum

The inoculum used for this test was prepared from stock of each isolate. The isolate on slant was sub - cultured onto freshly prepared nutrient agar plate incubated at 37 ^{0}C for 18 hours. Subsequently, a colony of the isolate was picked with a sterile wire loop and transferred into 4ml of nutrient broth in a MacCartney bottle and incubated at 37 ^{0}C for 18 hours and growth of the same turbidity compared to 0.05M Barium Sulphate ($BaSO_4$) opacity standard used (CLSI, 2009).

Inoculation of plating medium and antibiotic testing.

The susceptibility of the isolates to 8 selected antibiotics was carried out using the disc agar diffusion method according to National Committee for Clinical Laboratory Standards (now Clinical Laboratory Standard Institute guidelines (CLSI, 2009).

Freshly prepared Mueller – Hinton Agar (MHA) plates were flooded with 2ml suspension of the test organisms in nutrient broth 10^6cfu/ml. *Staphylococcus aureus* ATCC 25923 was used as the control strain in every test run. The excess inoculums were drained up and culture plates were then left to stand for a few minutes. The antibiotics (Mast Diagnostic UK) employed included the follow: penicillin (10 µg), oxacillin (1 µg), cefoxitin (30 µg), gentamicin (10 µg), erythromycin (15 µg), Clindamycin (2 µg), tetracycline (30 µg), chloramphenicol (30 µg), and ciprofloxacin (5 µg). Discs of the respective antibiotics were aseptically placed on the surface of the pre - inoculated agar plates using sterile forceps.

Thorough contact of the discs with the agar was ensured by pressing the discs firmly but carefully on the plates with sterile forceps. There after culture plates were allowed to stand for 1hour to allow the antibiotics to diffuse into the agar medium and subsequently incubated at 37 ^{0}C for 24 hour.

Reading and Interpretation of results

Antibiotic susceptibility was indicated by the zone of inhibition around each disc. The diameter of the zone of inhibition produced by each antibiotic disc was measured with a transparent caliberated ruler in

millimeter and the antibiotic susceptibility to the diameter of zone of inhibition were classified as resistant or susceptible, or Intermediate resistant based on the standard interpretative chart by CLSI standard (CLSI, 2009)

Clindamycin Induction Test

Detection of inducible resistance of clindamycin by erythromycin (D-test) was performed on the *S. aureus* isolates as described by (Fiebelkorn *et al.*, 2003) in which erythromycin and clindamycin were placed 15mm apart. A truncated or blunted clindamycin zone of inhibition (D –shaped) indicated inducible resistance. Constitutive resistance was recognized using clindamycin zone diameter of ≤ 14mm (Fiebelkorn *et al.*, 2003). Multi–resistance was defined as resistance to penicillin along with at least three classes of antibiotics.

Molecular detection of the *nuc and mec A* genes by PCR

Phenotypically identified *S. aureus* isolates (only selected strains not all isolates) were confirmed as *S. aureus* by the detection of the *nuc* gene using the polymerase chain reaction (PCR). In addition, the prescence of the *mec A* gene was determined to confirm the isolate as MRSA. Primers (*nuc*-1) 5'AGTTCTGCAGTACCGGATTTGG-3' (*nuc*-2) 5'-AAAATCGATGGTTGGC -3' and (*mec A*1) 5' – CTC AGG TAC TGC TAT CCA CC; (*mec-A*2) 5' – CTC TTG GTA TAT CTT CAC C -3' which amplified a 280bp and 449bp segments of the *nuc* and *mec* A genes respectively were employed.

Each PCR assay was made up of the following : 25 µl of mastermix (Sigma) containing 1.5 units of Taq DNA polymerase, 10 Mm Tris – HCl , 50 mM KCl, 1.5 Mm $MgCl_2$, 0.001% w/v gelatin and 0.2Mm dNTPs, 1µl (20 pmol) of the forward and reverse primers and 5 µl of template DNA. The thermocycler was programmed with the following parameters: predenaturation at 95°C for 5 minutes, 30 cycles of denaturation at 95°C for 30 seconds , extension at 72 °C for 1 minute and final extension at 72 °C for 5 mins. The PCR conditions were utilized for the detection of the *nuc A,* and *mec A* genes.

PCR products were detected by gel electrophoresis using 1.5% (w/v) agarose. The agarose gel was run in 1XTBE 0.089M Tris 0.089 M Boric acid, 0.002M EDTA disodium buffer) (pH 8.3) for 2 hrs at 80 V. Thereafter, the gels were stained with ethidium bromide and visualized under UV light.

Statistical Analysis of Data

Stastistical analysis methods to determine frequency distribution, mean, harmonic mean, standard deviation, analysis of variance using T- test correlation was employe

Results

***Staphylococccus aureus* isolates**

A total of 405 *S. aureus* isolates were obtained from the 721 staphylococci recovered from 850 samples collected from Hospital and Community sources. The hospital isolates comprised of 230 (57%) and 175 (43%), community. Hospital isolates were sourced as follows; wounds (58) ; stools (47) ; urine (58) ; sputum (37) and blood (30), (Table 1).

The highest rate of isolation of *S. aureus* isolates from clinical sources was from wounds (14.3%) and stools samples (14.3%), while cell phones 15% and food handlers (15%) constituted the highest among non – clinical *S. aureus* isolates. Overall, the prevalence of *S. aureus* isolates recovered from hospital sources are of statistical different (T = 0.141).

Table 1: Distribution of *S. aureus* isolates in the sample sources.

Hospital Sources.	**Nos of *S. aureus* isolated**
Urine n=100	58 (14.3%)
Wound n=100	58 (14.3%)
Stool n=100	47 (11.6%)
Sputum n=100	37 (9.1%)
Blood n=70	30 (7.4%)
TOTAL	**230**
MEAN	**11.4% ± 7.8**
Community Sources	
Cell phone n= 100	62 (15.3%)
Stethoscopes n=51	51 (12.6%)
Food handlers**.** n=100	62 (15.3%)
Total	**175**
Overall isolated total 405	**14.333 ± 1.333**

Inducible Clindamycin by erythromycin

The results of the inducible clindamycin by

erythromycin test showed that 7(1.7%) of the 405 *S. aureus* isolates from clinical and non-clinical were positive showing a characteristics D- shape.

Antibiotic Susceptibility Profile of the Isolates

Table 2 shows the antibiotic resistance types of the 405 clinical and non-clinical *S. aureus* isolates tested against eight different antibiotics. Urine isolates were 100% resistant to penicillin while other isolates from both sources were at various higher degree resistant ranging from 73- 90% to penicillin. Among the 230 clinical *S. aureus* isolates, 91% of the urine isolates were resistant to chloramphenicol followed by 70 and 74% showing resistance each to ciprofloxacin and gentamicin respectively. Meanwhile, all the urine isolates were 59 % sensitive to cephocitin.

Sixty three (63%) of the wound isolates were resistant to cephocitin followed by chloramphenicol (78%) and erythromycin (34%%).However, 64% were sensitive to ciprofloxacin and 40% clindamicin, Among the sputum *S. aureus* isolates, 76 and (64%) were resistant to chloramphenicol and cephocitin respectively while 67 and 50% of blood isolates showed resistant to gentamicin and chloramphenicol. From the 175 non-clinical *S. aureus* isolates, all the isolates were 76% resistant to penicillin. Meanwhile, (47%) of the food handlers isolates were resistant to ciprofloxacin. Among the cell phone isolates, (76%) were sensitive to chloramphenicol and 53% were equally resistant to oxacillin and (24%) to gentamicin. Meanwhile, 88% of the stethoscope *S. aureus* isolates were resistant to penicillin.

Table 2: Occurrence of antibiotic resistance type

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Urine (58)	100	48	17	41	70	69	74	91
Wound (58)	87	34	12	63	36	58	60	75
Sputum (37)	86	46	8	64	35	51	59	76
Blood (30)	73	37	43	57	33	53	67	50
Stool (47)	76	23	45	56	35	37	91	15
Food handlers Nasal cavity (68)	90	28	4	62	47	46	24	35
Cell phones (62)	90	42	53	50	29	58	24	29
Stethoscope (51)	88	25	12	53	35	47	47	31

KEY

1.	Penicillin	**4.**	Cephocitin	**7.**	Gentamycin
2.	Eythromycin	**5.**	Ciprofloxacin	**8.**	Chloramphenicol
3.	Oxacillin	**6.**	Clindamycin		

Table 3 Multiple Antibiotic Resistance pattern of urine isolates, n=57

Number of antibiotics	**MAR pattern**	**% number of organisms**	**%total organisms**
2	P,C	2 (3.46)	2 (3.46%).
3	P, E, C	4 (6.90)	
	P, GN,C	1 (1.72)	
	P, CIP, C	1 (1.72)	6 (10.52%)
4	P , DA, GN,C	2 (3.51)	
	P, DA, E C	1 (1.72)	
	P, DA, CIP, C	4 (6.90)	7 (12.30%)
5	P, CIP, E, GN C,	2 (3.51)	
	P, DA, E, CIP, C	1 (1.72)	
	P, DA, E GEN, C	1 (1.72)	

			4(7.01%)
6	P, DA, E,CIP,GN,C	37 (64.9)	
			37 (64.9%)

Multiple antibiotic resistant pattern of the stool isolates

Table 3 shows the multiple antibiotics resistance pattern of the stool isolates. Twelve different MAR patterns were observed among the isolates. The multiple antibiotic resistance pattern was in multiple of 2 to 6 classes of antibiotics.

Six (12.77%) of the total stool isolates showed 2 patterns in combination of 2 different classes of antibiotic in which 5 (10.60%) showed MAR pattern P, GN and 1 (2.13%) DA, P types. Eleven (23.40%) of the total isolates also showed 3 patterns in combination of 3 classes of antibiotics in which 1 (2.13%) has an MAR pattern P CIP, GN, ; P, DA, GN, and P E, GN each.

Meanwhile, 16 (34.04%) and 8 (17.02%) of the total isolates displayed 4 and 2 pattern types to 4 and 5 different classes of antibiotics respectively.

TABLE 3: Multiple Antibiotics Resistance Pattern of the Stool Isolates

Number of Antibiotics	MAR pattern	%Number of organisms	%Total organisms
2	P, GN	5 (10.6%)	
	P, DA,	1 (2.13)	
			6 (12.77%)
3	P,CIP,GN	1 (2.13)	
	P, DA, GN	3 (6.38)	11 (23.40%)
	P, E, GN	7 (14.90)	
4.	P, CIP, E, GN	10 (21.28)	
	P, DA, E, GN	3 (6.38)	
	P, CIP, E, C	2 (4.26)	
	P, DA, GN, C	1 (2.13)	
			16 (34.04%)
5	P, DA, E,CIP, GN	7 (14.89)	
	P, CIP , E, GN C	1 (2.13)	8 (17.02%)
6	P, DA, E, CIP,GN, C	6 (12.77)	6 (12.77%)

KEY

Penicillin (P)
Oxacillin (OX)
Cefoxitin (FOX)
Gentamycin (CN)
Erythromycin (E)
Clindamycin (DA)
Chloramphenicol (C)
Ciprofloxacin (CIP)

Multiple antibiotic resistance pattern of food handlers isolates.

Table 4. Shows multiple antibiotic resistance pattern of the food handlers isolates. Ten different MAR patterns were observed among the isolates. The multiple antibiotic resistance patterns were in multiple of 2 to 6 different classes of antibiotics. Six (10.71%) of the 56 isolates showed 2 MAR patterns in combination of two antibiotics P.C , and P, E types constituting 3(5.36%) each.

Eight (14.29%) of the total isolates showed 2 MAR patterns in combination of 4 different class of antibiotics in which 4 (7.14%) each expressed different MAR types P, DA, E, C and P, DA, E,CIP. Meanwhile,16 (28.6%) of the total isolates in combination of 5 different antibiotics showed 3 MAR patterns types P, DA, E CIP, C ; P,E, CIP, GN, C ; P, DA, E,GN, C which constituted 13 (23.21%), 2 (3.57%) and 1 (1.79%) respectively. Furthermore, 14 (25.00%) of the total isolates also showed one MAR pattern P, DA, E, CIP, GN, C in combination of 6 different classes of antibiotic used.

TABLE 4: Multiple Antibiotics Resistance Pattern of the Food Handlers Isolates n=27

Number of antibiotics	MAR pattern	Number Of Organisms	%Total organisms
2.	P,C	3 (5.36)	
	P, CIP	3 (5.36)	
			6 (10.71%)
3.	P, E,CIP	6 (10.71)	
	P, E C	6 (10.71)	
			12 (21.43%)
4.	P, DA, E, C	4 (7.14)	
	P, DA, E, CIP	4 (7.14)	
			8 (14.29%)
5.	P, DA, E ,CIP, C	13 (23.21)	
	P, CIP, E, GN,C	2 (3.57)	
	P, DA, E GN, C	1 (1.79)	
			16 (28.60%)
6	P, DA, E, CIP,GN,C	14(25.00)	14(25.00%)

KEY

Penicillin (P)
Oxacillin (OX)
Cefoxitin (FOX)
Gentamycin (CN)
Erythromycin (E)
Clindamycin (DA)
Chloramphenicol (C)
Ciprofloxacin (CIP)

Multiple antibiotic resistance pattern of the wound isolates

Table 5, shows the multiple antibiotic resistance (MAR) pattern of the wound isolates. Thirty two (10) different (MAR) patterns were observed among the isolates. The multiple antibiotic resistance patterns were in multiple of 2 to 6 different classes of antibiotics. One (2.00%) of the isolates showed one single pattern in combination of two antibiotics of type DA, P. pattern. Twelve (24.00%) of the total wound isolates showed 3 different patterns in combination of 3 different classes of antibiotics in which 3 (6.00%) showed the MAR patterns of P, DA, GN while 1 (2.00%) and 8(16.00) showed MAR pattern of P, CIP, C ; P, E, C respectively. Meanwhile 4 (8.0%) showed MAR type P, E, CIP,GN, C and 29(58.0%) of the isolates showed single MAR pattern in combination of 6 different classes of antibiotics (P, DA, E, CIP, GN, C).

Number of antibiotics	MAR pattern	number of organisms	%total organisms
2	P, DA,	1 (2.0)	
			1 (2.00%)
3	P, DA, GN	3 (6.00)	
	P, CIP, C	1 (2.0)	
	P, E, C	8 (16.0)	
			12 (24.00%)
4	P, DA, E GN	1 (2.00)	
	P, DA, CIP, C	1 (2,00)	
	P, E, GN, C	1 (2.00)	
	P, CIP, E ,C	1 (2.00)	4 (8.00%)
5	P,E, CIP, GN, C	4 (8.0)	4 (8.00)
6	P, DA, E, CIP, CN, C	29 (58.00)	29(58.00%)

TABLE 5: Multiple Antibiotics Resistance Pattern of the Wound Isolates

Multiple Antibiotic resistance patterns of the Blood Isolates

Table 6, shows the multiple antibiotic resistannce pattern of blood *S. aureus* isolates. Fourteen different antibiotics resistant patterns were observed among the isolates. The MAR patterns were in multiple of 2 to 6 different classes of antibiotic. Three (10.71%) of the isolates showed 3 MAR patterns in combination of 2 different classes of antibiotics in which 1 (3.57%) shows MAR pattern of CIP, GN and DA, P, each while 2 (7.14%) showed type DA, GN. Meanwhile, 2 (7.14%) also showed MAR pattern types E,CIP, GN and P, E C. However, 2 (7.14%) of the total isolates showed 2 MAR patterns in combination of 3 antibiotics in which 1(3.57%) each were showed by MAR pattern types E, CIP, GN and P, E, C each.

Eight (28.57%) of the total isolates displayed 5 MAR in combination of 4 different classes of antibiotics with pattern DA, E, CIP,GN ; P, E, CIP, GN ; P, DA, E, GN, P,E, GN, C and E, CIP, GN, C.

Table 6: Multiple Antibiotic Resistance pattern of blood isolates Antibiotic

MAR Parttern	Number of organisms		% total number
2	CIP , GN	1 (3.57)	
	DA, GN	2 (7.1)	4 (14.29%)
	P DA,	1 (3.57)	
3.	E, CIP,GN	1 (3.57)	2 (7.14%)
	P, E, C	1 (3.57)	
4.	DA, E, CIP, GN	1(3,57)	
	P, E, CIP,GN	3 (10.71)	
	P, DA, E, GN	1(3.57)	
	P, E,GN,C	2 (7.14)	
	E, CIP, GN, C	1 (3.57)	8 (28.57%)
5	P, DA, E,CIP,GN	2 (7.14)	
	P, CIP E, GN, C	1(3.57)	4 (14.29%)
	P, DA, CIP, GN, C	1 (3.57)	
6	P, DA, E,CIP, GN,C	10 (35.71)	10 (35.71%)

Penicillin (P) Clindamycin (DA) Gentamicin CN

Oxacillin (OX) Chloramphenicol (C) Erythromicin (E)

Cefoxitin (FOX) Ciprofloxacin (CIP)

Multiple Antibiotic resistance pattern of sputum *S. aureus* Isolates

Table 7, shows the multiple antibiotic resistance pattern of sputum *S. aureus* isolates. Eight (11) different resistant patterns were observed among the isolates; the MAR pattern were in multiple of 2 to 6 different classes of antibiotics. One (2.70%) showed 1 MAR pattern in combination of 2 antibiotics DA,P. While 2(5.41%) also showed 2 MAR patterns in combination of 3 different antibiotics in which 1 (2.70%) showed P, E GN and P, DA, GN respectively.

Meanwhile, 14(37.84%) and 2(5.41%) showed 6 and 1 MAR patterns in combination of 4 and 5 antibiotics, respectively. Eighteen (48.65%) of the total isolates displayed MAR pattern of P, DA, E, CIP, GN, C in combination of 6 different classes of antibiotic used.

Table 7: Multiple Antibiotic Resistance Pattern of *S. aureus* from Sputum

Isolate number	MAR Pattern	% organisms	Total % isolated
2	P, DA,	1 (2.70)	1 (2.70%)
3	P, E, GN	1 (2.70)	
	P, DA, GN	1 (2.50)	2 (5.41%)
4	P, DA E,GN,	3 (8.10)	
	P, DA, E, C	5 (13.51)	14 (37.84%)
	P, E, GN C	3 (8.10)	
	P, DA, CIP, C	1 (2.70)	
	P, CIP, E, C	1(2.70)	
	P, DA, E, GN	1 (2.70)	
6	P, E, CIP, GN, C	2 (5.41)	2 (5.41%)
7	P, DA, E, CIP,GN,C	1 (12.5)	18 (48.65%)

Key:

Penicillin (P)	Clindamycin (DA)
Oxacillin (OX)	Chloramphenicol (C)
Cefoxitin (FOX)	Ciprofloxacin (CIP)
Gentamycin (CN)	Erythromicin (E)

Multiple Antibiotic Resistance Patterns of Stethoscope Isolates

Table 8, shows the multiple antibiotic resistant pattern of stethoscopes *S. aureus* isolates. Fourteen different multiple antibiotic resistant patterns were observed among the isolates, The MAR pattern were in multiple of 2 to 6 different classes of antibiotics Six (13.04%) of the 46 isolates shows 3 MAR pattern in combination of 2 antibiotics in which 4 (8.70%) showed types P, E; P, C, and P, D, A.

However, 10 (21.74%) of the total isolates showed 4 MAR pattern in combination of 3 different antibiotics in which 3 (6.52%) showed MAR pattern P, E, C, P . 5 (10.37%) displayed MAR pattern P, E, C, while 1 (2.17%) is from P, DA, GN and 2 (4.35%) showed P, E, CIP pattern.

Meanwhile, 6 (13.04%) and 12 (26.09%) showed MAR patter of 4 and 2 in combination of 4 and 5 antibiotics respectively. Moreover, 12 (26.09%) showed 1 MAR pattern P, DA, E, CIP, GN, C.

Table 8: **Multiple Antibiotic Resistant Patterns obtained from the Stethoscope**

Isolate	MAR Patterns	Number of organisms	%total number of no
2.	P, E	4 (8.70)	
	P, C	1 (2.17)	
	P, DA,	1 (2.17)	6 (11.76)
3.	P, E, CIP	2 (4.35)	
	P, E, C	5 (10.87)	
	P, DA, GN	1 (2.17)	
	P, CIP, E,	2 (4.35)	10 (19.61)
4	P, DA, E, C	3 (6.52)	
	P, CIP, E, C	1 (2.17)	
	P, DA E CIP	1 (2.17)	
	P, E, GN, C	1 (2,17)	6 (11.76)
5.	P, CIP, E, GN, C	2 (4.35)	
	P, DA, E CIP, C	10 (21.74)	12 (23.53)
6.	P, DA, E, CIP, GN, C	12 (26.09)	12 (23.53)

Multiple Antibiotic Resistance Patern of Cellphone Isolates

Table 4.21 shows the multiple antibiotic resistance pattern of cell phone *S. aureus* isolates. Nineteen different multiple resistant patterns were observed among the isolates. The MAR pattern was in multiple of 2 to 6 different classes of antibiotics. 2 (3.57%) of the 19 isolates showed 2 MAR patterns in combination of 2 different classes of antibiotics with patterns types P, DA, and P, GN.

Meanwhile, 10 (17.86%) and 9 (16.07%) of the total cell phone *S,aureus* isolates showed 5 and 7 MAR pattern in combination of 3 and 4 antibiotics in which 12 (3.57%) showed MAR pattern P, E, GN and 4 (7.14%) showed MAR patterns; P, E, C; P, E, CIP; P, E, GN, C, DA and P, CIP, E, while 1 (1.79%) P, DA, GN; P, DA, CIP, C; P, DA, GN, C; P, DA, E, C each.

Seventeen (30.36%) displayed 3 MAR types P, E, FOX CN, C in combination of 4 antibiotics. However, 18 (32.14%) showed MAR types P, DA, E, CIP, GN, C in combination of 6 different classes of antibiotic.

Table 9: **Multiple Antibiotic Resistant Patterns obtained from the cell phone.**

Isolate	MAR Patterns	Number of Organisms	%total no of organisms
2	P, DA,	1 (1.79)	
	P,GN	1 (1.79%)	2 (3.57%)
3	P,E GN	2 (3.57)	
	P, E C	4 7.14)	
	P, DA, GN	1 (1.79)	10 (17.86%)
	E, CIP, GN	1 (1.79)	
	P, CIP, E,	2 (3.57)	
4	P,E, GN,C	1 (5.0)	
	P, DA, CIP, C	1 (5.0)	
	P, E,CIP,C	1 (5.0)	
	P, DA GN, C	3 (15.00)	9 (16.07%)
	P. DA E, CIP	1 (5.00)	
	P, DA E, C	1 (5.00)	
	P, E GN C	1 (1.79)	
5	P, CIP, E, GN,C	3 5.36)	
	P, DA E, GN C	2 (3.57)	
	P, DA, E,CIP,GN, C	6 (10.71)	
	P, DA, E, CIP, C	6 (5.00)	17 (30.36%)
6	P, DA, E, CIP, GN, C	8 (32.14)	18 (32.14%)

Occurrence of Methicilin Resistant *S. aureus* and MSS

Table 10 shows the occurrence of methicillin and Multi-drug resistant types. Clinical isolates constituted 4.8% MRSA, 27.4% MSSA, 45.2% MDR/MRSA and 22.6% MDR/MSSA while non – clinical *S. aureus* isolates constituted 10.3%MRSA, 23.4%MSSA, 52.6% MDR/MRSA and 13.7% MDR/MSSA

	n	MRSA	MSSA	MDR/MRSA	MDR/MSSA
Wound	58	6	14	27	11
Stool	47	1	22	18	6
Urine	58	2	15	22	19
Sputum	37	0	6	23	8
Blood	30	2	6	14	8
TOTAL	**230**	**4.8%**	**27.4%**	**45.2%**	**22.6%**
Food handlers	62	9	20	29	4
Cell Phones	62	2	11	39	10
Stethoscope	51	7	10	24	10
TOTAL	**175**	**10.3%**	**23.4%**	**52.6%**	**13.7%**

Table 10: Occurrence of Methicillin and Multi-Drug Resistant (%) Type

Discussion and conclusion

In this finding, different clinical diagnostic cases were studied for possible associated *S. aureus* infections viz; urine, wound, sputum stools and blood. Nasal samples of healthy individuals among food handlers in the community were considered for the non- clinical. The susceptibility of the *S. aureus* isolates of clinical and non- clinical sources to the antibiotics as observed in the study area, indicated that chloramphenicol, clindamycin and ciprofloxacin could be useful as the major potent antibiotics in treatment of staphylococcal infections.

Occurrence of methicillin resistant among the *S. aureus* isolates recovered from both clinical and non-clinical sources ranging from 4.8 and 10.3% respectively indicated that MRSA were among the major common nosocomial pathogens in the study areas. The result was low compared to the National Nosocomial Infectious Surveillance NNIS System (USA), which reported 61% prevalence of MRSA among hospitalized patients in 2004 in the United States.

Widespread use of antibiotics has been responsible for the development of numerous problems including the emergence of multi drug resistance bacteria and increased number of hospital and community acquired infections with increased health care cost (Synder *et al*., 2000). Rising to the challenge posed by infectious diseases, which are emerging as a global health concern, over 1.4 million people worldwide are suffering from hospital acquired infections (Synder *et al*., 200). Reports on antibiotic resistance have grown to be increasingly common and pathogens that are resistant to almost all antibiotics have been reported (Locksely *et al*., 1982). However in this study, it is worth noting that highest level of resistance was observed in Penicillin both in the clinical setting and among the community food handlers . Penicillin are known to exert their antimicrobial effect by inhibition of the synthesis of peptidoglycan, which is a heteropolymeric component of the cell wall, that provides a rigid mechanical stability by virtue of its highly cross-linked lattice wall structure (Rayner, 2003) which result in loss of bacterial cell rigidity and subsequent rupture or lysis of the bacterial cells which the antibiotics could not achieve with *S. aureus* strains in this study.

When bacteria become resistant to first- line antimicrobials, treatment has to be switched to second or third line drugs which are sometime more toxic and expensive (WHO, 2002). The emergency of new strains of *S. aureus* has always been reported after the introduction of new classes of antimicrobial agents which has been a stumbling block for effective management of staphylococcal infections and enhanced development of multiple resistant strains (WHO, 2002).

Resistant strains of *S. aureus* are devastating human pathogens and according to the data published by the US Centres for Disease Control and Prevention (CDC) as part of their national nosocomial infection surveillance system, well over half of all intensive care unit isolates from documented infections are caused by MRSA . The level of antibiotic resistance in a given community or hospital can be predicted by these important measures such as: the proportion of resistant organisms introduced from outside the population, the extensive use of antimicrobial agents and the proportion that spreads from one person to another (Wenzel and Edmond, 2000; Edmond, 2000). In this study, the inducible clindamycin resistance phenotypes were observed in seven clinical MRSA isolates. Laboratories are encouraged to carry out the D-test routinely and conduct investigations on inducible – clindamycin resistant strains in order to prevent misuse of clindamycin and emergence of clindamycin resistant strains of *S.aureus.*

Inducible resistance is not detected by routine antimicrobial susceptibility tests (Drinovie *et al*., 2001) except with the D-zone test as recommended by CLSI (CLSI., 2005). It involves the placement of an erythromycin disk in close proximity (15-18mm) to a disk containing clindamycin, during antibiotic susceptibility testing, using the disk diffusion method. A truncated or blunted clindamycin zone of inhibition (D.shape) observed in this study indicated inducible resistance as reported by (Pal *et al*., 2010).

Accurate identification and detection of inducible clindamycin resistant strains of *S. aureus* is important to prevent misidentifying resistant srains as susceptible which could lead to misuse of clindamycin, emergence of clindamycin resistant strains and possible clinical treatment failure according to the reports of some workers (Ajantha *et al*., 2008 ; Sedighi *et al*., 2009).

Conclusion

The study has established that *S. aureus* is implicated in a wide variety of infections and the prevalence of multiple antibiotic resistance is high in the study area. There was excellent correlation between antibiotic susceptibility testing (resistance to cefoxitin).

First line antibiotics namely penicillin has no significant usefulness either in controlling the proliferation of the pathogens or the infection caused by multiple antibiotic- resistant *S. aureus*. In addition, cefocitin and gentamicin are not fully potent enough as anti- staphylococcal agents in the study

area.

Recommendation

On the basis of the findings of this study the following are recommended:

Overcoming the spread of antibiotic resistant organisms will therefore require a concerted effort of both medical personnel and the public. The co-ordinate effort to educate clinicians and discourage non- professional prescription in the out - patient setting, campaign against self- medication, legislature for drug sales and advertising may contribute to rationale antibiotic use. Judicious use of antibiotics in hospital may enable physicians to evaluate their therapeutic rationale. Continuous epidemiological surveillance of antibiotic resistance patterns among clinical and non-clinical isolates and the development and adherence to effective therapeutic schedules in Ile – Ife may reverse the increasing pace of development of antibiotic – resistance. In essence, the infection control researchers must rapidly uncover which resistance mechanisms are involved so that appropriate measures (alternative drug regimens) isolation precautions, etc can be instituted. Effective hospital, community and personal hygiene control programs with suitable qualified personnel should be put in place. The effective use of genuine and standard antiseptics and biocides with notable broad – spectrum activity, and sterilization procedures is necessary in the prevention and spread of antimicrobial resistant pathogens

Furthermore, all hospital fomites such as stethoscopes should be well sterilized regularly and routinely with mild alcohol each and after used, the cellphones commonly used among community dwellers as well as health care workers in their facilities should be wiped routinely with alcohol base solvent at each transfer from community- hospital and hospital /community vize versa. Nurses, doctors, laboratory personnel etc should play a major role by maintaining a high standard of hygiene. However, since patients in the wards could possibly be the source and / or vectors in spreading of antibiotic resistant *S. aureus*, patients with different infections diagnoses should not be kept in the same room or ward to prevent cross infection. Visitors and relations coming from outside into the wards should be made to always observe simple rules of hygiene to guard against hospital/ community transfer of infections.

Further research could be carried out such as spa typing, Multilocus sequence typing (MLST) for effective determination of *S. aureus* clones circulating in both the hospital and the community. D- test should be included in the antibiotic susceptibility testing in clinical microbiology laboratories in Nigeria to prevent misuse of clindamycin as well as clindamycin resistant strains.

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