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The Prevalence and Plasmid DNA Mediated Antibiotic Resistance of Isolates of Staphylococcus *aureus* From Recurrent Furunculosis in University College Hospital, Ibadan Nigeria.

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Abstract: *The purpose of this study was to determine the prevalence of recurrent furunculosis in terms of age, gender and genetic basis from infected volunteers in university college hospital Ibadan Nigeria. A total of 102 isolates of Staphylococcus aureus from various anatomical pathological skin lesion were obtained from four hundred biochemically characterized samples. Antibiotic susceptibility profiles and minimum inhibitory concentration of the isolates was determine by Kirby Bauer and broth dilution method. β-lactamase potential of the isolates was determined by iodometric cell suspension methods and plasmid profiles by the use of lystostaphin for lyzing the cell wall.*
The percentage gender distributions were 46.0% females and 54.0% males. The isolates exhibited the lowest resistance of 11.75% to amoxicillin-clavulanic acid and 55.88% as the highest resistance to tetracycline. Thirty of the isolates possessed β-lactamase in varying degrees out of which 29 were plasmid-borne and 7.0 had multiple plasmid DNA of 2-4 copies, ranging between 2.20 and 23.10 kb. The resistance elicited by the strains evident in the Minimum Inhibitory Concentrations of the selected antibiotics and the associated R-plasmid encoded β-lactamase recorded accounted for the recurrent furunculosis which was found to vary among gender and age groups.
Keywords: *Antibiotic resistance, Plasmid DNA, Recurrent furuncle, Staphylococcus aureus.*

I. Introduction

Furunculosis, a cosmopolitan pyodermal infection of human skin caused by *Staphylococcus aureus* is recurrent among most infected individuals It is characterized by a honey crusted 'cropped' latent boil with potential to recur in a susceptible host. *Staphylococcus aureus* is a common colonizer of the skin, with a remarkable ability to hydrolyse β-lactam antibiotics, degrade skin lipid barrier and spread within the skin loci .
Staphylococcus aureus is the major pathogen of the genus *Staphylococcus*. It may be found as commensal frequently on the skin of carriers without any infection. It is however an important pathogen which can be responsible for a great variety of pyogenic infections in man and animals. It is the causative agent of many suppurative processes ranging from localized abscesses which can occur anywhere in the body to fatal septicemia and pneumonia. *Staphylococcal* furunculosis infection in a susceptible host, 'crops' and appear as healed, maintains a life cycle and then recurs as several boils weeks or month later.
The condition can be very distressing and there may be need to exclude diabetes and other conditions through blood tests. It is not often due to the immune status of the host but rather to the continuous presence of the bacterium *Staphylococcus aureus* on the skin. Plasmid mediated resistance to antimicrobial agents among pathogenic bacteria constitutes a major clinical and economic problem world-wide and *Staphylococcus aureus* is known to be notorious in its acquisition of resistance to antibiotics.
Consequently, antibiotic resistance plasmids has been the subject of extensive genetic and biochemical studies. Plasmid profiling is used to establish the genetics and spread of antibiotic resistance. The high level multidrug resistance elicited by the strains of *Staphylococcus aureus* isolated was established to be due to the associated transferable R-plasmid encoded β-lactamase which can be bedrock for the recurrent nature of furunculosis recorded in this study.

II. Materials And Methods

Sample Collection:
A total of 400 of 'cropped boils' producing 102 isolates of *Staphylococcus aureus* were collected from consented human volunteers, with recurrent episodes of furunculosis consisting of 40 hospital recorded and 100 unreported furunculosis from different age groups and gender. The specimens were transported in 0.1% bacteriological peptone water and processed within 24 hours of collection
Bacteriology:

Every specimen was processed for the isolation and identification of *Staphylococcus aureus* by means of relevant selective plating on mannitol salt agar and biochemical characterizations for the determination of catalase, coagulase, Deoxyribonuclease, haemolytic and fermentative properties of the isolates.

β-lactamase Detection
Pure Nutrient agar culture of every isolate of *Staph. aureus* obtained overnight at 37^0C, was harvested and homogenized in phosphate buffered penicillin G. The bacterial suspension measuring $x10^7$ cells/ml. on McFarland turbidity standards was tested for β-lactamase production by the cell suspension iodometric method .

In-vitro antibiotic susceptibility testing:
All the identified *Staphylococcus aureus* isolates were subjected to *in-vitro* antibiotic susceptibility test using Chigbu and Ezeronye inhibition zone diameter as modified by Clinical Laboratory standard (CLSI) with the following antibiotics amoxicillin(25 µg/ml), cotrimoxazole(25 µg/ml), gentamicin (10 µg/ml), chroramphenicol(30 µg/ml), augmentin(30 µg/ml), erythromycin(5 µg/ml), tetracycline(10 µg/ml)(Oxoid product).
The zones of growth inhibition were recorded and the isolates classified resistant or sensitive. *Staphylococcus aureus ATCC29213* was used as a control. Five antibiotics: augmentin, cefotaxime, ceftriaxone, penicillin G and cloxacillin were then tested for their minimum inhibitory concentrations against the β-lactamase strains of the *Staph. aureus* isolates. Graded decreasing double-fold concentrations of each antibiotic were prepared in nutrient broth and to each dilution was added 0.lml of a 10^{-2} diluted culture of individual strain, including of *Staphylococcus aureus ATCC29213* as control strain. All were incubated at 37^0C for 24hrs followed by examination to determine the M.I.C. of each of the antibiotics .

Plasmid DNA profiling.
Plasmid DNA was isolated as described by Kado and Liu (1981) modified by use of lystostaphin for lyzing the cell wall. The lysate were kept in ice for 30min and centrifuged for 5 min, phenol(1:1) treatment was followed with the clear supernatant. Plasmid DNA were precipitated with equal volume of chilled isopropyl alcohol and DNA pellet dissolved in 100 µl of TE buffer (diethyl ether). A 0.8% agarose gel was used to resolve DNA fragment and it was prepared by combining 0.8 g agarose in ten times concentration of Tris acetate ethylene diamine tetraacetate (10ml10xTAE) buffer and 90ml distilled water in a 250ml beaker flask and heating in electrothermal heater for 2 min until the agarose is dissolved. 2.5ml of ethidium bromide (5.0mg/ml) was added to the dissolved agarose solution by swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and the casting combs were inserted. It was then allowed to gel for 30 min . The casting comb was then carefully removed after the gel had completely solidified. Electrophoresis buffer was the added to the reservoir until the buffer just covered the agarose gel. 0.5µl of gel tracking dye (bromophenol blue) was added to 20µl of each sample with gentle mixing. 20µl of the sample was then loaded on to the wells of the gel , the mini horizontal electrophoresis gel set- up was then covered and the electrodes connected . Electrophoresis was carried out at 100-120mA for 1.hr.At the completion of the electrophoresis, the gel was removed from the buffer and gel was viewed under a long wave UV- light box. The band pattern of the DNA fragments were then photographed with a Polaroid camera and documented using an electrophoresis gel documentation system. The molecular sizes of each plasmid were determined by comparison with plasmid of known mass.

III. Results.

Of the 102 isolates of *Staphylococcus aureus* obtained, 20 (19.6%), was obtained from the ear infections, 17(16.67%), from the nose and 12 (11.76%),from the armpit while breast and lips maintained 2(1.96%) equally as elicited in (Table 1).an indication of the ability of *Staph aureus* to cause infection in almost every part of the anatomical loci.
The percentage gender distribution ratio was 56% males to 46% females. The ratios of the unreported furunculosis cases was found to be higher than the hospital reported in males(53:3) and in female (37:9) as elicited in(Table 2) and the prevalence of the infection was found to be highest within the age range 11-50years in males and 11 – 70 years in females.
In the antibiogram study, the percentage resistance of the isolates to the antibiotics used was recorded to be the highest for tetracycline (55.88%) and (11.75%) lowest for amoxicillin-clavulanic acid in this study.
The MIC"s of the 5 selected antibiotics varied as follows: amoxicillin-clavulanic acid (3.95-250 µg/ml) cloxacillin (31.25-250 µg/ml), cefotaxime(15.63-250 µg/ml), ceftriaxone(31.25-250 µg/ml) penicillin G (62.5-250 µg/ml), when p-value of 0.05 was considered statistically significant(Table 3).

Of the 102 isolates of *Staphylococcus aureus*, 29 isolates were plasmid encoded β-lactamase positive. Plasmid was extracted from 30 isolates including one isolate without β-lactamase. Multiple plasmids were detected in seven strains with molecular weight ranging from 2.02 - 23.13kb (Fig 1 and 2).

Table 1. **PATHOLOGICAL DISTRIBUTION OF THE ISOLATES OF *STAPHYLOCOCCUS AUREUS* FROM LESIONS OF FURUNCULOSIS.**

Clinical source	Nos of isolates	% of total collection
Head	5	4.90
Ear	20	19.60
Nose	17	16.67
Lip	2	1.96
Neck	5	4.90
Armpit	12	11.76
Buttock	6	5.88
Forearm	4	3.92
Breast	2	1.96
Thigh	5	4.90
Elbow	4	3.92
Eyelid	6	5.88
Cheek	5	4.90
Chin	5	4.90
Knee	4	3.92
Total	**102**	**100%**

Table 2 AGE AND GENDER DISTRIBUTION OF THE ISOLATES

Age (Yrs)	Male		Female		
	NHR	HR	NHR	HR	
1-10	5	1	2	1	
11-20	10	0	6	1	
21-30	10	0	6	0	
31-40	10	0	6	0	
41-50	10	0	6	0	
51-60	2	0	5	1	
61-70	2	0	4	2	
71-80	1	1	1	2	
81-90	1	1	1	1	
91-100	2	0	0	2	
Total	**53**	**3**	**37**	**9**	**= 102**

NHR: Non Hospital Reported HR: Hospital Reported.

Table 3.

MINIMUM INHIBITORY CONCENTRATION OF THE β LACTAMASE BORNE *STAPHYLOCOCCUS AUREUS*

STRAIN NUMBER		MIC in (µg/ml)					
	Pathological source	Amx-clv	Clx	Cet	Ceft	Pen G	Lactams
Sa01	Breast	250	62.5	62.5	125	125	+
Sa02	Thigh	7.8	250	250	250	62..5	+
Sa03	Ear	250	250	31.25	125	62.5	+
Sa04	Neck	250	125	250	250	125	+
Sa05	Elbow	3.95	31.25	62.5	62.5	62.5	+
Sa06	Buttock	3.95	250	125	62.5	62.5	+
Sa07	Ear	250	125	250	125	62.5	+
Sa08	Ear	15.63	62.5	62.5	62.5	125	+
Sa10	Neck	15.63	125	62.5	62.5	125	+
Sa11	Eye	250	250	125	62.5	62.5	+
Sa13	Cheek	7.8	125	15.63	31.25	62.5	+
Sa16	Eye	15.63	62.5	62.5	31.25	62.5	+
Sa18	Ear	1.93	250	62.5	125	125	+
Sa23	Nose	7.8	250	31.25	31.25	125	+
Sa24	Elbow	15.63	250	15.63	62.5	62.5	+
Sa25	Chin	3.95	125	125	62.5	250	+
Sa33	Lip	62.5	250	15.63	250	125	+
Sa40	Ear	15.63	62.5	62.5	62.5	125	+
Sa45	Head	31.63	250	125	125	62.5	+
Sa46	Head	3.95	250	31.25	125	62.5	+
Sa50	Nose	15.63	125	15.63	125	62.5	+
Sa51	Elbow	15.63	125	31.25	62.5	62.5	+
Sa52	Buttock	31.25	250	125	31.25	62.5	+
Sa53	Elbow	250	125	31.25	62.5	62.5	+
Sa62	Armpit	125	125	125	31.25	125	+

Sa63	Head	62.5	125	125	125	62.5	+
Sa64	Lip	125	250	250	250	125	+
Sa91	Armpit	125	62.5	62.5	125	125	+
Sa94	Ear	125	31.25	250	250	125	-
Sa97	Neck	31.25	250	31.25	31.25	62.5	+

Key: Sa: Staphylococcus aureus ,Amx-clv: Amoxicillin-clavulanic acid, Clx: Cloxacillin, Cet: Cefotaxime, Cef: Ceftriaxone, Pen G:Penicillin G.

Figure 1.0 Plasmid analysis of resistant strains of *Staphylococcus aureus.*

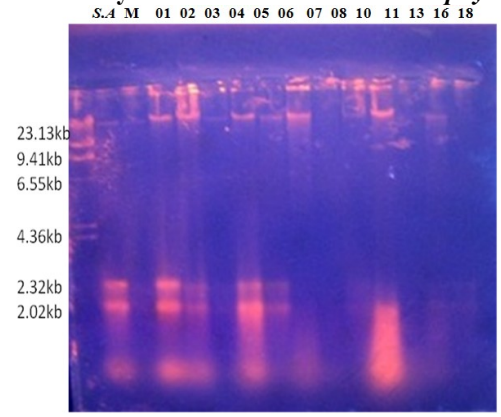


Figure 2 .0

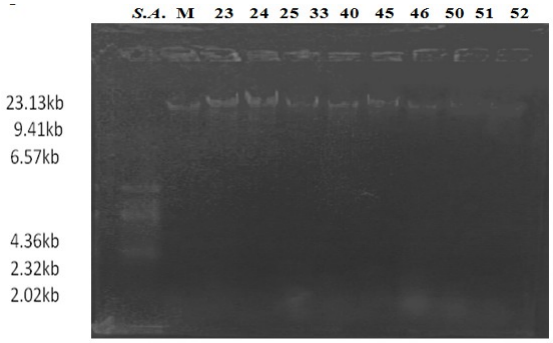
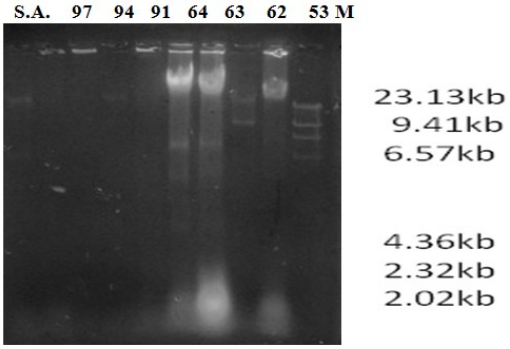


Fig 2 (contd).



Key: Numeric numbers represent *Staphylococcus aureus* strains
Kb: Kilobase(Molecular Weight determination).
M: Molecular Weight markers C1&C2: *S.albus(as control).*

IV. Discussion and Conclusion.

In the pathological distribution of recurrent furunculosis in this study, the highest occurrence was found in the ear anatomical locus (19.6%) and every other anatomical locales of the body has its varied percentage, an indication of the ability of *Staph aureus* to cause infection in almost every part of the body, thus agreeing with the widely acclaimed status of *Staphylococcus aureus* as a 'bug' of medical importance. The biochemical data recorded establish the characteristics of the organism, *Staphylococcus aureus* which agreed with the findings of Harold (2000). The positive reactions of the test strains to such biochemical tests such as mannitol fermentation, gelatin liquefaction, hemolytic ability and DNase activity have been suggested by some workers as indications of potential pathogenicity.

This sharp contrast in the samples collected from the two settings(non-hospital reported and hospital reported) could be attributed to the preference of the populace to indulge in self management of furunculosis until the infection becomes life threatening before seeking medical attention as well as the low literacy level in the community and perceived exorbitant hospital bill that might be unaffordable by the patients as unraveled from the anonymous questionnaires.

The values of MIC recorded for the 5 selected antibiotics generally showed resistance by the isolates of *Staph aureus* to these antibiotics including interestingly the extended spectrum β- lactams cefotaxime and cefuroxime . Among other functions, ,R-plasmids are known to confer information to synthesize antibiotic degrading enzymes as evident in twenty nine out of 30 isolates processed for plasmid encoded β –lactamase enzymes. The preponderance of this enzyme corroborates the view of Olukoya (1995) on the threat to chemotherapeutic application of the β-lactam antibiotics due to the alarming world-wide increase of bacterial resistance to β-lactam antibiotics.

However, the general prevalence of furunculosis could be a reflection of lack of social infrastructure in the environment associated with the individual cultural and hygiene practice which are considered to be amongst the epidemiological factors aiding the spread and control of an infection. It has been recognized that people with hyperacious sebaceous secretion due to their genetic make-up habor more lipid materials on their body than those without. Hence the predisposition of the former to attract microbes-laden particles into their body that would give rise to development of boil.

It could also reflect on puberty symptoms as well as some profession that are associated with frequent contact with oil. Recurrent furuculosis was found to be prevalent in males within the age group 11-50 years and 11-70years was recorded in females. Plasmid profiles have been reported to be useful in tracing the epidemiology of antibiotic resistance. Resistance was observed in isolates with various molecular size plasmid encoding β-lactamase as well as strain *Sa 94* that lack this enzyme.

This could be attributed to the pathological sources of the isolates. All the antibiotic resistance isolates selected for this study were plasmid encoded. Spread of recurrent furunculosis with respect to age and gender, has been identified in this study and the associated isolation of plasmid DNA as a major cause of the recurrent furunculosis while self therapeutic management of antibiotics could also not be overlooked in the episodes of recurrent furunculosis. Large-scale studies are necessary to unravel and further explore the etiologic agents of recurrent furunculosis. Also, randomized controlled clinical trials are essential to examine the feasibility and cost-benefit ratios of various types of antibiotic chemoprophylaxis in the prevention of recurrent furunculosis in the high risk patients.

References

[1]. Abdul, H.K.and Dipak, K.P. Antibiotic susceptibility and R-plasmid mediated drug resistance in Staphylococcus aureus. Medical Journal of Islamic World Academy of Science .2005; **15**:31-34.

[2]. Adeleke, O.E. and Odelola H.I. Plasmid profiles of multidrug resistant local strains of Staphylococcus aureus. Afri.J.Med sci 1997; **(18):** 119-121

[3]. Asheshov, E.H. The genetics of penicillinase production in *Staphylococcus aureus* strain PS80. J Gen Microbiol. 1969; **59**(3):289–301.

[4]. Atkinson, B.A. and Lorian, V. Antimicrobial agent susceptibility patterns of bacterial in hospital from 1971-1982. Clin. Microbiol. 1984; **(20**):791-5

[5]. Bauer, A., Kirby, W., Sherns,W. et.al., Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol, 1966; **45**:493-496.

[6]. Binswanger, I.A. Kral, A.H, Bluthenal, R.N., et. al., High prevalence of abscesses Biol. 2000; **18**: 261-269.

[7]. Bimboin, H.C, Doly,J. A rapid alkaline procedure for screening recombinant plasmid DNA. Nucleic Acid Res. 1979; **7**:1513-1523

[8]. Bobrowski, M.M. Bacterial β-1actamases and their significance in resistance to penicillin and cephalosporins. Postepy Med. Doshiadczalnej. 1974; **28**(4): 587.

[9]. Catlin, B.W.Iodometric detection of Hemophilus influenzae ß-lactamase. Rapid presumptive test for ampicillin and cephalosporin resistance. Antimicrob Agen Chemother .1975; **7**:265-70.

[10]. Cheesbrough, M. District Laboratory practice in Tropical Countries. Part 2. Cambridge University press 2002; 544-52.

[11]. Chigbu CO, Ezeronye OU. Antibiotic resistant Staphylococcus aureus in Abia state of Nigeria. Afr J Biotechnol. 2003; **2**(10): 374

[12]. Clinical and Laboratory Standards Institute(2011): antimicrobial susceptibility testing standards,

[13]. M02-A10 and M07-A8.

[14]. Datta, N.,Hedges R.W., Shaw,E.J.,Skyes,R.B.,Richman, M.J.Propertties of R-factor fro Pseudomonas aeruginosa. J Bacteriol. .1971; **108**:1244-1249.

[15]. Davis, S.L, Perri, M.B. and Donabedian, S.M. Epidemiology and outcomes of community- associated methicillin-resistant Staphylococcus aureus infection J. Clin. Microbiol. 2007;**1**20: 1222-1224

[16]. Detta, N., Hadges, R.W and Shaw, E.J. Properties of an R-factor from Pseudomonas aeruginosa. Am. J. bacterial. 1971; **108**:1244-1249.

[17]. Fact sheet: boils and impetigo. NSW Public Health Bull. 2003;**14** (1-2):29

[18]. Gira, A.K., Reisenauer, A.H .and Hammock, L. et al.,. Furunculosis and familial deficiency of mannose-binding lectin. Eur J Clin Invest. 2005;**35**:531-32.

[19]. Gold, H.S, Moellering, R.C. Jr. Antimicrobial-drug resistance. N. Engl. J. med . 1999; **335**:1445-47.

[20]. Harold, J.B. Microbiological applications: Laboratory Manual in General Microbiology.2007; **pg**.43-57.

[21]. Indalo, A.A. Antibiotic sale behaviour in Nairobi: a contributing factor to antimicrobial drug resistance. East Afr. Med. J. 1997; **74**:171-173.

[22]. Jacobson, K..L, Cohen, S.H., and Inciardi, J.F.The relationship between antecedent antibiotic use and resistance to extended-spectrum cephalosporins in groupI β-lactamase-producing organism. Clin Infect Dis .1995; **21**:1107. Jevanand, H.R,, Ragavan, P.U.M and Gunapathi, R.S. Study of R-factors among multi- drug resistant Salmonella typhi. Indian J. Med. Microbiol. 1997; **15**:37-39.
[23]. Kado, C.I. and Liu, S.T. Rapid procedure for procedure and isolation of large and small plasmids. J. Bacteriol. 1981; **145:**1365-1373.
[24]. Kenneth Todar. Todars online Testbook of Bacteriology Reviews.2008:Pp1-5
[25]. Maibach HI, Aly R. Bacterial infection of the skin. In: Dermatology. Edited by Moschella SL, Hurley HJ. 3rd edn. Volume1. W.B.Saunders Company.1992 P.724-25
[26]. Meyers, J.A., Sanchez, D., Elewell' O., Falkow, S. Simple agarose gel-electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. bacterial. 1976; **127**:1529-1537.
[27]. Olukoya, D.K, Asielue, J.O, and Olasupo. Plasmid profiles and antibiotic patterns of Staphlococcus aureus isolate from Nigeria. Afr. J Med Sci. 1995; **24**: 135-139
[28]. Takahaashi, S and Nagano, Y. Rapid procedure for isolation of plasmid DNA and The Micrococcaceae. J. Med. Microbiol. 1984; **5**: 267.
[29]. Zimakoff, J, Rosdahl, V.T, Petersen, W. and Scheibel, J.Recurrent Staphylococcal furunculosis in families. Scand J. Infect Dis.1988; **20**:403.