MICROBIOLOGICAL ASSAY OF SOME SELECTED BRANDS OF AMPICLOX FROM OVER-THE-COUNTERS RETAIL OUTLETS ON *STAPHYLOCOCCUS AUREUS* OBTAINED FROM CASES OF FURUNCULOSIS.

ABSTRACT

Furuncles is a pyodermal infection which manifest as multiple boil on different parts of the body. It occurs most often in the dense dermal connective tissues when *Staphylococcus aureus* invades via hair follicles or sebaceous ducts and set up an acute inflammatory swelling. It spreads locally in the dermis, and necrosis of a patch of skin at the centre of the lesion results from toxic action. A total of forty (40) pus samples were collected from consented volunteers of varied age and gender of which twenty (20) *Staphylococcus aureus* were isolated and confirmed by Gram staining and other conventional biochemical tests which include; catalase, haemolysis, gelatin hydrolysis, DNase, sugar fermentation, citrate, coagulase, and oxidase tests. The gender ratio were recorded to be 21 (52.5%) males and 19 (47.5%) females. The antimicrobial assay by agar well diffusion of the three (3) brands of ampiclox; Vitaclox 500mg capsule (Sagar Vitaceutical Nigeria Ltd.), Vanclox 500mg capsule (Evans Therapeutic Ltd.) and Beecham Ampiclox 500mg capsules (GlaxoSmithKline plc.) on the isolates (*Staphylococcus aureus*) were determined. The isolates of *Staphylococcus aureus* investigated exhibited 100% resistant to all the 3 different brands of ampiclox exposed. Some of the resistant isolates selected for plasmid DNA analysis exhibited plasmid bands of varied kilobases that ranged from 2.32kb to 23.13kb The alarming resistance observed in this current study is suggestive of abuse or misuse of this frequently prescribed ampiclox that are easily accessible over the counters.

Keywords: Microbiological assay, Ampiclox, furuncles, *Staphylococcus aureus,* Over-the-counters.

INTRODUCTION

Furunculosis is a prevalent skin infection which in most cases is unrelated to any blood disorder. It occurs most often in the dense dermal connective tissues when *Staphylococcus aureus* invades via hair follicles or sebaceous ducts and set up an acute inflammatory swelling. It spreads locally in the dermis, and necrosis of a patch of skin at the centre of the lesion results from toxic action. In some instances, particularly in individuals with impaired resistance to infection, in

cases of untreated diabetics, the infection may spread extensively in the dermal and underlying soft tissue of the neck , giving rise to a carbuncle consisting of a complex loculated abscess, or several abscesses, with multiple discharging sinuses(Atanaskova and Tomecki,2010)

Furuncles typically begin as red, tender lumps, quickly filling with pus, growing larger and more painful until they rupture and drain. While some furuncles may disappear within a few days, most take about two weeks to heal. Furuncles can appear anywhere on the skin but are commonly found on the face, neck, armpits, buttocks or thighs and hair bearing areas of the body where sweat or friction is prevalent. Anyone can develop furunculosis, but individuals with ,diabetes, a suppressed immune system, acne are at greater risk(El-Gilany and Farthy, 2009)

Staphylococcus aureus was discovered in 1880 by Sir Alexander Ogston in pus from a surgical abscess (Guo *et al*., 2020). Since it's discovery, it has been known to be a significant pathogenic organism in human and mammals, associated with a wide range of diseases, including minor skin infections and severe cases of bacteraemia and necrotizing pneumonia. For many years, the available treatment choices were restricted to the topical use of carbolic acid and the mortality rate among patients infected with *Staphylococcus aureus* was greater than 80% (Decker et.al, 1986).

Staphylococcus aureus normally colonize the anterior nares and can remain dormant for an extended period of time. In the presence of predisposing factors such as prolonged hospitalization, immune suppression, surgical procedures, use of invasive medical devices and chronic metabolic diseases, the organism can be introduced into the skin from its carrier site, leading to the development of localized skin infections. These localized infections can then spread causing various diseases like carbuncles, cellulitis, or impetigo (Batlett *et al*., 2017).

Ampiclox is an antibiotic combination consisting of ampicillin and cloxacillin. They are both synthesized from natural penicillins and serve as an improved modification over the parent penicillins, in terms of resistance to the actions of gastric acid and beta-lactamase enzyme, thereby increasing their spectrum of antimicrobial efficacy (Adeleke *et al*., 2010).

Ampicillin is a broad spectrum antibiotic that belongs to the amino-penicillin class which are third generation penicillins, that have similar structural characteristics to the natural penicillins. Like the natural penicillins, they are also susceptible to the action of beta-lactamase enzyme, however they possess an amino group that makes them more hydrophilic, thereby improving oral absorption by facilitating the ease of crossing lipo-polysaccharide layers. Cloxacillin on the other hand, is a narrow spectrum antibiotic that belongs to the isoxazolyl class. They have limited antimicrobial activity, however the presence of an R chain makes them non-susceptible to attack by beta-lactamase enzyme. Cloxacillin is mostly active against Gram positive cocci, especially beta-lactamase producing strains of staphylococci. It is also effective against sensitive strains of *Neisseria gonorrhoeae*, *Neisseria meningitidis* and gram positive anaerobes. The emergence of resistance to ampicillin by certain bacteria such as *Staphylococcus aureus* due to the production of beta-lactamases has limited its effectiveness, which serves as the rationale for the combination of cloxacillin with ampicillin in ampiclox(Adeleke etal.,,2010).

Ampiclox is available in strengths of 250mg and 500mg in both oral and parenteral forms. The most commonly reported adverse effects of ampiclox are seizures, enterocolitis, agranulocytosis, gastrointestinal disturbances, hepatotoxicity and nephrotoxicity (Bush, 2010).

Ampiclox is one of the most commonly prescribed antibiotics for treatment of various microbial infections. It is therefore essential to investigate the efficacy of different brands of ampiclox available since prevalence in ampiclox prescription has been observed to favour certain brands that are easily available on counters(Adeleke *et al*., 2010). This study is aimed at assaying some brands of ampiclox on *Staphylococcus aureus* obtained from cases of furunculosis.

MATERIALS AND METHODS

SOURCES OF THE SAMPLES

A total of three(3) different brands of ampicox : Vitaclox 500mg capsule (Sagar Vitaceutical Nigeria Ltd.), Vanclox 500mg capsule (Evans Therapeutic Ltd.) and Beecham Ampiclox 500mg capsules (GlaxoSmithKline plc.) far below their expiry date were purchased from local pharmacy shops in Ibadan and designated with the codes; SVNL, ETL, GSK for easy understanding.

COLLECTION OF SAMPLES

A total of forty (40) pus samples were collected from consented volunteers identified with cases of furunculosis. They were of varied age, sex and loci of infection. The samples were collected with cotton-wool swab moistened with sterile normal saline, and were processed immediately in the department of pharmaceutical microbiology laboratory.

BACTERIOLOGY

The samples were cultured on mannitol salt agar medium and were incubated at 37^{o}C for 24hrs. Distinct colonies obtained were Gram stained and processed for biochemical tests which include; catalase, Coagulase, DNase, haemolytic hydrolytic and other conventional relevant tests for the identification and isolation of *Staphylococcus aureus*.

MICROBIOLOGICAL ASSAY

Agar Cup Diffusion method:

Microbiological assay of the brands of ampiclox was carried out using a modified agar cup diffusion plate method. Discrete colonies with similar appearance were aseptically transferred and emulsified in 3-4mL of sterile nutrient broth in test tubes. The tubes were incubated for 4-6 hours to obtain a uniform turbidity which were adjusted to 0.5 McFarland standards by adding more sterile broth. Müeller Hinton Agar medium were inoculated by swabbing and excess bacteria load were reduced by squeezing the stick against the wall of the tube above the suspension.A stock concentration of 1 µg/mL was prepared for each brand of ampiclox using sterile distilled water. The preparations were serially diluted to 0.5 µg/mL and 0.25µg/mL respectively. A sterile cork borer(6mm) was used to make wells on the surface of the agar plates The different concentration of ampiclox was then impregnated aseptically with the aid of sterile micropipette tips into the well bored at equidistant in the petriplates. The plates were incubated aerobically at 37^{o}C for 24hours. Zones of growth of inhibition were thereafter observed and the diameter of each zone of inhibition were measured and recorded in millimetre (mm) .The results were then interpreted according to the CLSI standard interpretative chart. *Staphylococcus aureus* ATCC 29213 was used as a standard control strain.

PLASMID DNA EXTRACTION

Exactly 1.5mL of the bacteria broth culture initially vortex mixed was transferred into sterile eppendorf tube. This preparation was centrifuged at 13,000rpm for 120seconds before discarding

the resultant supernatants by gentle decanting leaving a little broth. This was vortex mixed again at high speed until pellet is completely dissolved in the broth after which 300µl of tens was added. This mixture was inverted until it became slimly, 150 µl of 3.0M sodium acetate (Ph 5.2) was added and vortex mixed for about 10 seconds. Then centrifuged at 13,000 rpm for 5 minutes and the supernatant transferred into another sterile eppendorf tube. This supernatant was added to 900µl of ice cold absolute ethanol, vortex mixed and centrifuged at 13.000rpm for 10 minutes. The supernatant from here was discarded leaving white pellet subnatant . 1000µl of ice cold 70% ethanol was added to the pellet followed by centrifugation at 13,000 rpm for 5 minutes, this was repeated before drying the pellet in air. The dried pellet was suspended in 40µl of TE buffer.

AGAROSE GEL ELECTROPHORESIS

The dissolved pellet (10µl) mixed with loading buffer (1µl) was loaded on 0.8% agarose in TBE (x 1) buffer, stained with 0.1% ethidium bromide alongside Hind III marker. This was run at 100V for 1hours 30minutes after which the gel was viewed with photo gel documentation system.

RESULTS

The age and gender distribution of the samples was in ratio 21 males to 19 females as shown in Table 1 below.

Table 1: AGE AND GENDER DISTRIBUTIONS OF THE SAMPLES FROM CONSENTED VOLUNTEERS

Sample	Age	Gender

S01	13	Male
S02	22	Male
S03	44	Female
S04	58	Female
S05	31	Male
S06	47	Female
S07	52	Male
S08	40	Female
S09	19	Female
S10	60	Male
S11	45	Male
S12	49	Female
S13	37	Male
S14	23	Male
S15	56	Female
S16	34	Male
S17	49	Male
S18	57	Female
S19	21	Male
S20	42	Male
S21	55	Female
S22	14	Male
S23	37	Female
S24	41	Female
S25	30	Male
S26	48	Male
S27	38	Female
S28	23	Male
S29	50	Female
S30	28	Female
S31	60	Female
S32	22	Male
S33	35	Female
S34	44	Male
S35	18	Male
S36	27	Female
S37	34	Male
S38	25	Female
S39	51	Male
S40	53	Female

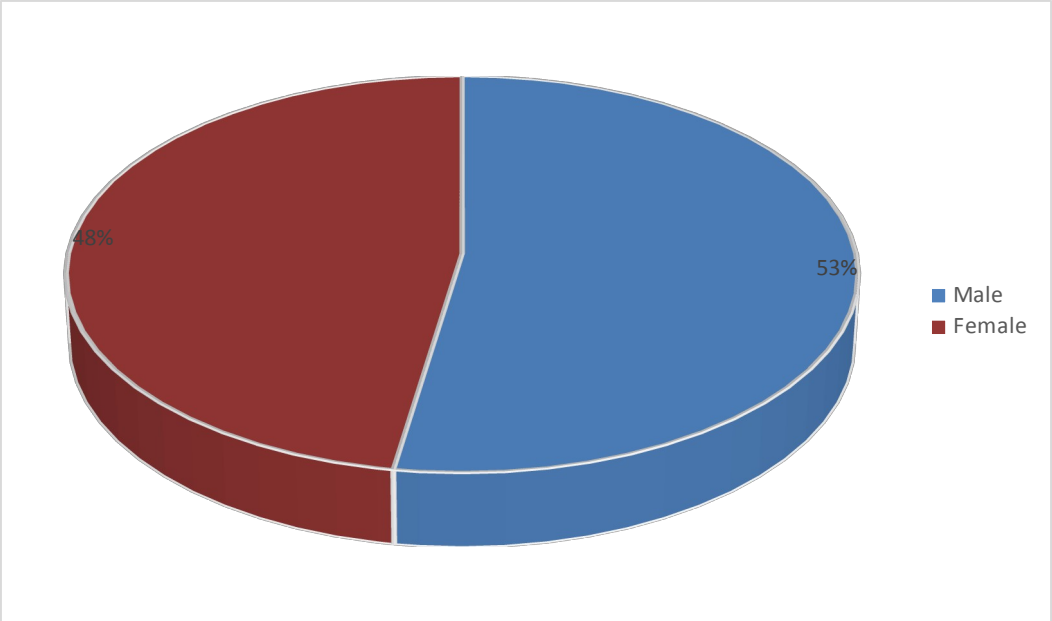

Figure 1: Percentage Gender Distribution

The antimicrobial activity of the twenty (20) isolates of (*Staphylococcus aureus)* were determined using the agar cup plate diffusion technique and the diameter of the zone of growth inhibition in mm obtained was interpreted according to the Clinical Laboratory Standards Institute (CLSI,2020) as elicited in Table 2 below.

Table 2: Antimicrobial activity of different Brands of Ampiclox on *Staphylococcus* aureus from cases of furunculosis.

	BRAND NAMES OF AMPICLOX AND THEIR CONCENTRATION(µg/mL)								
Isolate codes	**SVNL**			**EVL**			**GSK**		
	1.0	**0.5**	**0.25**	**1.0**	**0.5**	**0.25**	**1.0**	**0.5**	**0.25**
Sa 01	16	14	8	18	14	12	14	12	10

Sa05	16	8	10	14	18	12	14	10	8
Sa08	18	16	14	16	14	10	16	12	8
Sa09	18	14	12	18	16	12	16	14	12
Sa11	16	12	10	18	12	14	14	12	10
Sa12	12	10	8	14	12	8	12	10	8
Sa15	14	12	10	14	10	8	12	10	8
Sa16	18	16	14	16	12	10	16	12	8
Sa20	20	18	12	20	16	12	18	12	10
Sa21	20	16	12	20	18	16	20	18	14
Sa22	18	14	12	18	16	10	14	12	10
Sa24	14	12	8	14	12	10	14	12	8
Sa25	16	14	12	16	14	12	16	12	10
Sa28	16	14	10	16	14	12	14	12	8
Sa30	12	10	8	14	12	8	12	10	8
Sa33	16	12	10	14	12	10	14	12	8
Sa34	18	16	12	16	14	12	14	12	10
Sa37	14	12	8	12	10	8	12	10	8
Sa39	16	12	8	14	12	10	14	10	8
Sa40	16	14	12	16	14	12	16	12	10
ATCC 29213	24	24	23	25	23	24	26	30	28

Key: Numeric – Zone of inhibition in mm. **Sa --** *Staphylococcus aureus,* **SVNL -** Sagar Vitaceutical Nigeria Ltd **EVL -** Evans Therapeutic Ltd **GSK -** GlaxoSmithKline plc**.**

CLSI BREAKPOINT CHART (2020)

Ampicillin-clavulanic acid	**Susceptible**	**Intermediate**	**Resistant**
S.aureus	≥22	-	≤21

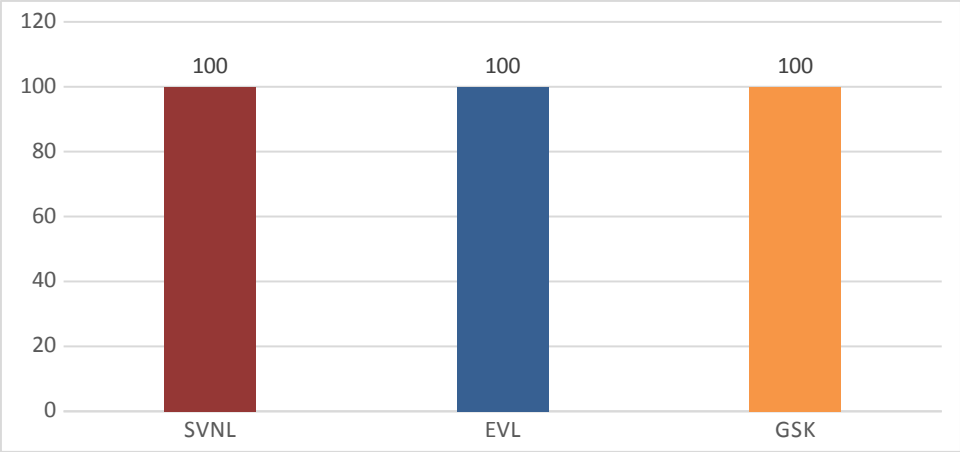

Figure 2: Percentage of the isolates of *Staphylococcus aureus* resistant to the different brands of ampiclox investigated. **SVNL -** Sagar Vitaceutical Nigeria Ltd, **EVL -** Evans Therapeutic Ltd, **GSK -** GlaxoSmithKline plc.

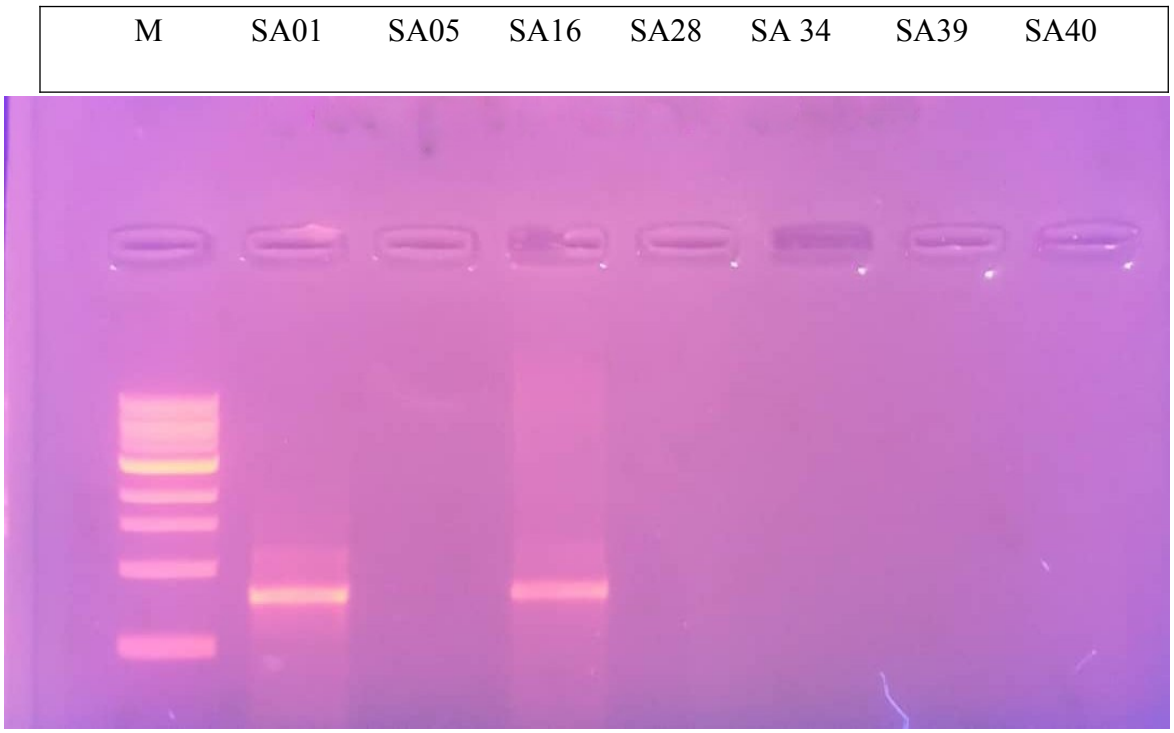

Figure 3: Electrophoresis gel profiles of plasmid DNA some selected *Staphylococcus aureus* from cases of furunculosis**.**

DISCUSSION

Antimicrobial chemotherapy is essential in the treatment of infections caused by micro-organisms. However increase in the development of resistance of microorganisms to the available antibiotics, have become a global health concern (Hoeger, 2004). It has therefore become necessary to ascertain the potency and actual concentration of active ingredients in the available antibiotic preparations as a means of combating the problems of resistance. The potency of an antibiotic can be measured using several methods including microbiological assays, immunological assays, radio-immuno assays and automated chemical assays. However, microbiological assay of antibiotics presents a convenient method of determining the potency of antibiotic and is also useful in the determination of resistance and susceptibility of pathogenic microbes to antibiotics (Hoeger, 2004).

The percentage gender prevalence of *Staphylococcus aureus* isolates from consented volunteers in this study was in ratio 52.5% male to 47.5% female, Though in close proximity which could be due to sample size, the relatively higher percentage from male could be attributed to frequent a behavior observed more commonly in men than in women which include frequent sharing of bathing soap, shaving blades, differences in immune status and inadequate attention to skin care in comparison to female that place much premium to skin care. This agrees with the findings of Seidenfeld and Martin (1983), on *Staphylococcus aureus* infection in a high school football team. . The results from the microbiological assay carried out interpreted according to clinical and laboratory standards institute (CLSI) standards, indicated that the local isolates were 100% resistant to the three brands of ampiclox used, though the control strain was sensitive to all the concentration of the antibiotic prepared. This could be attributed to the abuse or unguided use of this frequently prescribed antibiotics, and patient's non-adherence to antibiotic therapy regimen which corroborates the study of Stuart,(2002*)* on factors impacting on the problem of antibiotic.

Some of isolates selected for plasmid DNA profiling elicited plasmid band of notable kilobases while some didn't exhibited plasmid. The plasmid DNA obtained from could be attributed to the resistance while the resistance factors in those without plasmid DNA could be chromosomal (Malachowa and DeLeo, 2010). An alarming episode of resistance of *Staphylococcus aureus* to various brands of antibiotics used in the study, could serves an indices of resistant phenomenon in isolates of *Staphylococcus aureus* studied. And this may lead to therapeutic failure and economic loss. Therefore enlightenment awareness and surveillance should be put in place to sensitize the public of the implication of resistant threat in defiance to the guided use of conventional antibiotics.

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