

## BRANDS OF AMPICLOX AGAINST CLINICAL STRAINS OF STAPHYLOCOCCUS AUREUS

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RUNNING TITLE: AMPICLOX BRANDS AGAINST STAPHYLOCOCCUS AUREUS

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### Abstract

Proliferation of different brands of antibiotics including ampiclox (a notable penicillinase inactivator) was considered a relevant factor in the antibiotic resistance of *Staphylococcus aureus*. Consequently the antibiogram and susceptibility of 20 clinical strains and a control strain (NCTC 6571) of *Staphylococcus aureus* to 10 different brands of ampiclox were determined by disk diffusion and tube broth dilution methods. The control strain was found sensitive to ampicillin, augmentin<sup>R</sup> and cloxacillin in the antibiogram, and to all the 10 brands of ampiclox, with the MIC's of either 0.125 or 0.25 µg/ml among the clinical strains. This result, compared with the MIC's obtained in the range of 0.125 µg/ml to > 60 µg/ml, varying among the brands of ampiclox against the 20 clinical strains, indicates contrasting inhibitory activity among the different brands but reflective of the worrisome level of resistance to antibiotics by *Staph. aureus*. However, this resistance to most of the brands of ampiclox could not be associated with brand variation having found the control strain sensitive to all the brands of ampiclox.

### INTRODUCTION

Ampiclox is a Beta-lactam combination antibiotic comprising ampicillin (an -aminobenzylpenicillin) and cloxacillin, both of which are still obtainable as individual penicillins. Ampicillin and cloxacillin represent an improvement over the parent penicillins (penicillin G, penicillin V and benzathine penicillin) in terms of stability in the presence of the gastric juice acid and (Beta-lactam drug inactivating enzymes - penicillinase and cephalosporinase and spectrum of

antimicrobial activity(1). Though ampicillin is susceptible to the action of (Beta-lactamase{2}), a deficiency that accounts for its combination with cloxacillin, thus producing a synergy in the antibacterial activity of ampiclox. However, there have been reports of multiple drug resistant staphylococci to antimicrobial agents(3,4,5). In this episode of multiple drug resistance (MDR) ampicillin, amoxicillin and cloxacillin (6) as well as other Beta-lactamase targeted antibiotics like ampiclox have

not been excluded: amoxicillin-clavulanate, methicillin, vancomycin, and cephalosporins (7,8,9,10,11,12, 13). The MDR in *Staphylococcus aureus* has been largely attributed to resistance genes borne on R-plasmid DNA(14). Notably, the hospital strains of this organism have been greatly implicated(15). *Staph aureus* is an important cause of community and hospital acquired infections (16,17). Besides plasmid, high-level resistance could also occur from a low level through a multistep process due to chromosomal gene alteration(18). Among the enormous number of antibiotics available, penicillin antibiotics are recognizably the most commonly used. This could explain the high level of production of these antibiotics in different forms-single, combination and different brands. Antibiotic prescribing has been observed to be in favour of some particular brands of antibiotics particularly, ampiclox(19). Hence, the need for this study to evaluate the antimicrobial potency of available different brands of ampiclox.

## **MATERIALS AND METHODS**

### **BACTERIOLOGY**

Strains of *Staphylococcus aureus* from different clinical sources, numbering 20, were collected from the Medical Microbiology Laboratory, University

College Hospital (U.C.H.), Ibadan and General Bacteriology Laboratory, Lagos University Teaching Hospital (LUTH), Lagos, Nigeria (Table 1). All the clinical strains were screened for purity by means of some coagulase test with reference to a control preserved on Nutrient agar (OXOID) slants at 4°C in a refrigerator until when they were needed.

### **ANTIBIOTICS**

Ten brands of ampiclox (powder for injection) were purchased from local pharmacy shops, and given codes as : EL, PH, JA, CL, ML, RS, SK, RH, TR.

**Antibiotic Disks** (Abtek Biological Ltd.): Augmentin<sup>R</sup> {30µg), Amoxicillin (25µg), Erythromycin (5µg), Tetracycline (10µg), Cotrimoxazole (25µg), Chloramphenicol (30µg), Cloxacillin (5µg), Gentamicin (10µg).

### **ANTIBIOGRAM**

The antibiotic susceptibility pattern of each strain of *Staph. aureus* was determined by the Kirby-Bauer's Disk-Diffusion method as previously described(21). The inoculum size of each strain was 10<sup>7</sup> cells/ml in 0.1ml taken from 10<sup>-2</sup> dilution of overnight broth culture, as determined in a pour-plate culture. All the culture and sensitivity plates were incubated at 37°C for 24hrs. Zones of growth inhibition were then observed and measured in millimeters (mm) to

determine sensitivity or resistance to a particular antibiotic disc (Table 2).

#### MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE BRANDS OF AMPICLOX

The tube-broth dilution method was used to determine MIC. Suitable high aq. stock concentrations of each brand of ampiclox were prepared to provide 60, 50, 20, and 4 $\mu$ g/ml, in four different tubes of 10ml nutrient broth (OXOD) each. Serial dilutions were made also in nutrient broth to give the following antibiotic concentrations: 30, 25, 10, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 and 0.031 $\mu$ g/ml. Each dilution was then inoculated with 0.1ml from 10<sup>-2</sup> aq. dilution (equivalent to 10<sup>7</sup> cells/ml) of an overnight broth culture. The mixtures were then incubated at 37°C for 24hrs, followed by observation for sensitivity or resistance revealed by the visibility of turbidity in each tube to determine the MIC.

#### RESULTS

In the antibiogram for the clinical strains of *Staph aureus*, amoxicillin, augmentin<sup>R</sup> (amoxicillin-clavulanate) and cloxacillin showed no zone of growth inhibition, but did so for the control strain, NCfC 6571. Gentamicin and chloraphenicol were relatively outstanding in their anti-staphylococcal activity (Table 2). Among the 10 brands of ampiclox, 9 brands exhibited reasonable activity of MIC 0.125 - 0.5 $\mu$ g/ml (Table 3) against 9 of the clinical strains (i.e. 45%) of *Staph. aureus* at different levels: BH (5 strains), SK (2 strains), TR (1 strain) RH (1 strain) and CL (4 strains). Thus, one coded EL lacked activity against any one of the clinical strains. These variations are reflected as percentage resistant pattern of the clinical strains (Table 4). However, everyone of the ampiclox brands produced MIC of either 0.125 $\mu$ g/ml or 0.25 $\mu$ g/ml against the control strain of *Staph. aureus*, NCfC 6571 (Table 3).

**TABLE 1: THE CLINICAL STRAINS OF STAPHYLOCOCCUS AUREUS**

Strain of	Clinical source/	Strain of	Clinical source/
<i>S. aureus</i>	Control	<i>S. aureus</i>	Control
0263	Endorcervical swab	086	HVS
0273	Wound swab	0342	Eye swab
0301	Ear Swab	0748	HVS
1122	Sputum	1937	Urethral swab
1503	Nasal swab	1950	Wound swab
1559	Eye swab	1970	Ear swab
2464	Sputum	1975	Sinus effluent
2994	Sputum	5626	Blood
3409	Wound swab	17322	Ear swab
3413	High Vaginal swab	NCTC 6571	Type culture
078	Wound swab		

a = National Collection of Type Cultures, London.

## DISCUSSION

Cloxacillin is used to treat B-lactamase (penicillinase) - producing staphylococci(6) due to the presence of isoxazolyl as an added group(1) in the antibiotic. The resistance to this antibiotic (cloxacillin) by all the 20 clinical strains in the antibiogram and by most of them in the ampiclox

brands indicates a strong possibility of the clinical strains as Blactamase bearers. The sensitivity noted for the control Oxford strain of *Staph. au reus* NCfC 6571 to cloxacillin both in the antibiogram and the ampiclox brands (at MIC of either 0.12511g/ml or 0.2511g/ml) supports this assertion.

**TABLE 2: ANTIBIOGRAM OF THE CONTROL NCTC 6571 AND CLINICAL STRAINS OF STAPHYLOCOCCUS AUREUS**

Strain of <i>S.aureus</i>	Antibiotic disk( $\mu$ .g)							
	Aug	Amx	Ery	Tet	Cxc	Gen	Cot	ChI
NCTC 6571	20.0*	18.0	22.2	25.5	21.5	23.1	6.4	14.0
078	**_	-	-	-	-	-	-	-
086	-	-	-	-	-	17	-	-
0342	-	-	-	-	-	-	-	-
0748	-	-	-	-	-	-	-	-
1937	-	-	-	-	-	16	-	-
1950	-	-	-	-	-	17	-	15
1970	-	-	-	-	-	15	-	-
1975	-	-	-	-	-	23	22	25
5626	-	-	-	-	-	-	-	-
17322	-	-	-	-	-	-	-	10
0263	-	-	-	-	-	16	-	-
0273	-	-	-	-	-	15	-	-
0301	-	-	-	-	-	13	-	-
1122	-	-	-	-	-	-	-	-
1503	-	-	-	-	-	12	10	10
1559	-	-	-	-	-	17	12	11
2464	-	-	-	-	-	24	22	18
2994	-	-	-	-	-	12	10	9.0
3409	-	-	-	-	-	13	11	10
3413	-	-	-	-	-	21	15	13

KEY: Aug: augmentin (30 $\mu$ g)

Ery: Erythromycin (5 $\mu$ g)

Cxc: Cloxacillin (10 $\mu$ g)

Cot: = Cotrimoxazole (10 $\mu$ g)

Amx = amoxicillin (25 $\mu$ g) .

Tet = Tetracycline (10 $\mu$ g)

Gen = Gentamicin (10 $\mu$ g)

ChI = Chloramphenicol (20 $\mu$ g)

\* = Zone of growth inhibition (Sensitivity) \*\* = No zone of growth inhibition (resistance).

**TABLE 3: MIC's OF BRANDS OF AMPICLOX AGAINST CLINICAL AND CONTROL STRAINS OF STAPHYLOCOCCUS AUREUS**

*Sa Strain	Brand of Ampiclox									
	BH	SK	ML	RH	RS	EL	PH	JA	TR	CL
0263	> 60	> 60	>60	> 60	1.0	> 60	1.0	30	50	20
5626	> 60	> 60	>60	> 60	1.0	> 60	1.0	30	50	30
0273	0.25	> 60	>60	> 60	2.0	2.0	2.0	> 60	> 60	. > 60
078	0.25	20	>60	> 60	2.0	50	0.25	0.5	5.0	0.25
0301	> 60	> 60	> 60	> 60	5.0	> 60	> 60	> 60	> 60	> 60
086	> 60	> 60	> 60	> 60	1.0	> 60	> 60	> 60	> 60	> 60
1122	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
0748	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
1503	2.0	> 60	> 60	> 60	2.0	> 60	> 60	1.0	2.0	10
1559	0.25	> 60	> 60	> 60	0.5	> 60	0.125	0.5	2.0	0.25
0342	0.125	0.5	20	5.0	0.25	20	0.125	1.0	5.0	0.5
2464	0.0	5.0	1.0	2.0	0.125	30	10	5.0	0.25	0.5
1937	10	0.125	0.25	1.0	0.5	20	0.25	0.125	1.0	0.125
1950	20	10.0	0.125	0.5	0.5	> 60	0.125	0.25	5.0	5.0
2994	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
1970	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
3409	10	> 60	> 60	> 60	4.0	> 60	> 60	10	> 60	10
1975	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
3413	2.0	> 60	> 60	> 60	0.5	> 60	> 60	10	> 60	5.0
3413	2.0	> 60	> 60	> 60	0.5	> 60	> 60	10	> 60	5.0
17322	025	10	> 60	> 60	> 60	> 60	0.5	> 60	> 60	> 60
NCTC 6571	0.125	0.125	0.125	0.125	0.25	0.5	0.25	0.25	0.5	0.125

\*Staph-aureus

**TABLE 4: RESISTANCE PATTERN OF THE CLINICAL STRAINS OF *STAPH. AURUES* AGAINST 10 BRANDS OF AMPICLOX .**

Brands of Ampiclox	No of resistant isolates	percentage (%)
BH	15	75
SK	18	90
ML	18	90
RH	19	95
RS	14	70
EL	20	100
PH	14	70
JA	16	80
TR	19	95
CL	15	75

The variation noted in the activity of 9 out of 10 brands of ampiclox against 9 out of 20 clinical strains of *Staph aureus* and the inactivity of one brand of ampiclox (EL) against none of the 20 clinical strains, corroborates the report of higher demand of one particular brand of ampiclox than the demand for other (19). There is need therefore, for continuous monitoring of the brands of antibiotics with respect to their activity on relevant bacteria from clinical sources. Having found the control strain, *Staph.aureus* NCfC 6571, sensitive to the 10 brands of ampiclox tested, the resistance noted in this study among the 20 clinical strains to most of the brands of ampiclox could not be associated with variation in the

brands.

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