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Effect of Cloxacillin Concentration in Ampiclox on the Suspectibility of some Clinical Bacteria

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ABSTRACT

Synergistic antimicrobial effect of various ratios of ampicillin/cloxacillin combinations were investigated on susceptible clinical bacterial isolates of *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Proteus* spp. Agar diffusion method was employed in the study to determine the potency of the varying ratios of ampicillin/cloxacillin combination. Variation in the zone diameters of inhibition were observed among the different varied ratios. In these case of ratio with higher concentration of cloxacillin, the zone diameter of inhibition is higher than those ratio with higher concentration of ampicillin. When cloxacillin and ampicillin were tested separately, cloxacillin was found to be very much effective than ampicillin especially against *S. aureus ithat is why they are used synergistically*. The study showed increase in the zone diameter of inhibition in all ratio with high concentration of cloxacillin, more than that of the present ratio of 250mg ampicillin and 250mg cloxacillin, but at statistical level, there was no significant different. Therefore, as such the present ratio 250mg ampicllin and 250mg cloxacillin is still considered as appropriate.

Keywords: *synergism, ampicillin, cloxacillin, antimicrobial.*

I. INTRODUCTION

Antibiotic synergism occurs when the effect of the combination of antibiotics is greater than the sum of the effect of the individual antibiotics. It is common for patients with medical problem to be taking many drugs combination which work synergically. The combination of ampicillin and cloxacillin in the ratio of 250mg each, to form ampiclox is very active against some bacterial strain which are known to be resistance to single antibiotic such as penicillin, and also the penecillinase producing strain, e.g. *Staphylococcus aureus.*

Synergetic effect of ampicillin and cloxacillin (β-lactam antibiotics) significantly reduce the less effectiveness of single antibiotic to some bacterial strain and also the penecillinase producing strain (Chinedum *et al.*, 2003). The combination of ampicillin and cloxacillin (ampiclox) produce a broad spectrum antibiotic activity against both gram positive and gram negative bacteria. Barreled, *et al.* (1977) demonstrated that in-vitro combination at which neither were previously effective will kill certain resistant gram negative bacteria, that has important clinical potential. Mackie and McCartney, (1989) also reported that two antibiotics acting together in vitro may have synergy when their antibacterial activity, whether bacteriostatic or bacteriocidal, is greatly exceeds that of the either drug alone.

II. MATERIALS AND METHODS

Drugs Sample: The antibiotics used (Ampiclox, Cloxacillin and Ampicillin) were purchased from registered drug dealer and pharmacist within Kano metropolis and carried to the laboratory for the investigation.

Test Organisms: The microorganisms used in the study were clinical isolates collected from pathology microbiology department of Mohd Abdullahi Wase Specialist Hospital Kano. They are: *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Proteus* spp.

Culture Media: Nutrient agar and Mueller Hinton agar were used in the study.

Preparation of Sensitivity Discs: Impregnated sterile paper discs 6mm in diameter of No. 1 Whatman filter paper containing the solution of the antibiotics of various combination of the combination were prepared as described by Kirby – Bauer (1966) and demonstrated by Arzai (2002).

Inoculum of Preparation: Inoculum preparation was done, as described by Monica (2002) and demonstrated by

Mukhtar and Okafor (2002), where 3 – 5 well isolated colonies of similar appearance to the test organisms were touched using sterile wire loop, and emulsified in 3-4ml of sterile physiological slime.

Bioassay Procedure: Agar diffusion method as described by Kirby – Bauer (1966), Monica (2002), and demonstrated by Mukhtar and Okafor (2002) and Scott (1989) was employed. Using sterile swab, the plate of Muller Hinton agar was inoculated in the test organisms and strike evenly over the medium in three direction. Impregnated, sterile Whatman filters paper discs containing the various concentration of the antibiotics were allanged and pressed firmly to the inoculated agar surface to ensure even contact. The plates were incubated aerobically at 37^0C for 24 hours. Diameters of zone of inhibition were measured and recorded in millimeter.

Statistical Analysis: Student's t-test was used to compare the result.

III. RESULT AND DISCUSSION

Table 1 shows the antimicrobial sensitivity pattern of ampcilox (500mg). it was already known that, the test organism were sensitive to ampiclox, however, it is now tested so as to see the effect of varying concentration of ampicillin – cloxacillin combination.

Table 1: Antibiotic sensitivity pattern of ampiclox 500mg (ampicillin 250mg and cloxacillin 250mg).

	Zone diameter of inhibition (mm)	Negative Control
E. coli	14.0	0.0
S. typhi	15.0	0.0
Proteus sp.	17.0	0.0
Staph. aureus	21.0	0.0

Table 2, shows the antibiotics sensitivity pattern of ampicillin – cloxacillin combination with high concentration of cloxacillin. The result of this study demonstrated that, the sensitivity pattern of ampiclox (500mg) and the various concentration of ampicillin – cloxacillin combination have shown an occational variation in the zone diameter of inhibition. The study have shown that, there is differences in the zone diameter of inhibition of *Staph. aureus* between combination with high concentration of cloxacillin and those with higher concentration of ampicillin. The zone diameter of inhibition is much higher in combination with higher concentration of Cloxacillin than those with higher concentration of ampicillin.

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Table 2: Antibiotics sensitivity pattern of ampicillin – cloxacillin combination with higher concentration of cloxacillin

	Zone diameter of inhibition (mm)						
Test Organism	**125/375**	**200/300**	**150/350**	**100/400**	**50/450**	**0/500**	**Negative control**
E. coli	16.0	16.0	16.0	15.0	14.0	13.0	0.0
S. typhi	18.0	16.0	16.0	15.0	13.0	17.0	0.0
Proteus sp.	18.0	15.0	17.0	15.0	19.0	18.0	0.0
Staph. aureus	19.0	14.0	15.0	35.0	35.0	40.0	0.0

This because, *Staph. aureus* is a penecillinase producing microorganisms, that has the ability to inactivate some penecillins and their derivatives including ampicillin, or make them less active. Therefore, the increase in zone diameter of inhibition in combination with higher concentration of cloxacillin is because cloxacillin is a semi – synthetics penicillin that has greater activity against *Staph. aureus*, which is due to the presence of β – lactaming which can in activate the β – lactamase enzyme produced by the bacteria.

The result also demonstrated that, the sensitivity pattern of various concentrations of ampicillin – cloxacillin combination to other test organisms (*E. coli* and *S. typhi*) have shown variation in zone diameter of inhibition in combination with higher concentration of cloxacillin, with exception of *Proteus* spp. which show higher zone diameter of inhibition in combination with higher concentration of ampicillin (Table 3).

Table 3: Antibiotics sensitivity pattern of ampicillin – cloxacillin combination with higher concentration of ampicillin.

	Zone diameter of inhibition (mm)						
Test Organism	**375/125**	**300/200**	**350/150**	**400/100**	**450/50**	**500/0**	**Negative control**
E. coli	17.0	19.0	17.0	15.0	17.0	14.0	0.0
S. typhi	20.0	19.0	17.0	19.0	20.0	22.0	0.0
Proteus sp.	36.0	35.0	38.0	36.0	37.0	31.0	0.0
Staph. aureus	20.0	20.0	19.0	20.0	19.0	13.0	0.0

The sensitivity pattern of the test organism to various concentrations of ampicillin – cloxacillin combination, agrees with similar report by Barrelet *et al* (1977) and Bonnside (1968), Von Os *et al* (1976) also demonstrated in – vitro activities of ampicillin, cloxacillin and their commercially available combination against a variety of gram – positive microorganism. Their study shows that, the activity of the combination against penicillin resistant staphylococcus is mainly due to the activity of cloxacillin.

The result indicated that, synergertic effect of ampicillin – cloxacillin combination is more pronounce in ampicillin resistance strain of *Staph. aureus.*

Despite the higher zone diameter of inhibition in combination with higher concentration of cloxacillin, when the results are compared statistically, using t-test, it revealed there was no significant difference between the higher or lower concentration of cloxacillin in Ampiclox (at 5% level of probability and 10df (i.e. 2.228) which is greater than the calculated value (0.019).

IV. CONCLUSION

Conclusively, the work has justified that, the present concentration of ampicillin – cloxacillin combination (250mg ampicillin and 250mg cloxacillin) acceptable by British Pharmacopoeia (BP) and United State Pharmacopiea (USP) and the various concentration of ampicillin – cloxacillin combinations has no significant difference. Eventhough increase in the concentration of cloxacillin in ampiclox lead to the increase in the zone diameter of inhibition, but still there is no significant difference since the inhibition zone is within the range.

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