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COMPARATIVE ANTIBACTERIAL STRENGTH ANALYSIS OF THREE COMMONLY USED ANTIBIOTICS IN AUCHI

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ABSTRACT

The sustainability and effectiveness of antibiotics commonly used for medication is endangered by the development of resistant pathogens. The main goal of this present study was to compare the antimicrobial strength of three commonly administered antibiotics in Auchi (singly and combined). The antibiotics tested for antibacterial activites were Ciprofloxacin, Amoxicillin and Ampiclox against four (4) clinical isolates: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Campylobacter jejuni*. Agar well diffusion method was used for the susceptibility testing as described by the Clinical and Laboratory Standards Institute. The Results obtained revealed very significant zones of inhibition for all the tested antibiotics against the bacterial isolates. The combination of ciprofloxacin-amoxicillin gave the highest inhibition zone diameter (IZD) against *Klebsiella pneumoniae and Campylobacter jejuni*, while Ciprofloxacin-ampiclox combination had the highest antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Amoxicillin-ampiclox combination had the least activity against all the test isolates used in this study. The antibiotic combinations of ciprofloxacin-amoxicillin showed synergy, ciprofloxacin-ampiclox was antagonistic, while Amoxicillin-ampiclox combination was indifferent against the test organisms. Although the antibiotics tested alone in this study were found to be effective, the use of combined therapy in the treatment of confirmed or suspected mixed infections should only be used when

necessary. Therefore, before the prescription of any antibiotic, a culture and sensitivity test must be conducted to determine the right antibiotics required for the treatment.

KEYWORDS: Antibacterial, antibiotics, comparative, resistance, inhibition zone diameter.

1.0 INTRODUCTION

Antibiotics are substances used for the control of infections and diseases caused by pathogens (Igbeneghu, 2013). They are categorized as narrow, broad, or extended-spectrum agents. Narrow spectrum antibiotics (e.g. penicillin G) are effective against Gram-positive bacteria, Broad-spectrum antibiotics, (such as tetracyclines and chloramphenicol) affect both Gram-positive and some Gram-negative bacteria while extended-spectrum antibiotics are chemically modified, to affects additional types of bacteria, usually Gram-negative bacteria (Kshetry *et al.*, 2016).

Different antibiotics have different mechanism of action; antibiotics affect microorganisms in several ways with variation from one antibiotic to the other (Dubey and Maheshwari, 2005). The different mechanisms includes; Inhibition of cell wall synthesis (ampicillin, cephalosporin, β-lactam, Vancomycin, Bacitracin), Inhibition of nucleic acid function (Nitroimidazole, Nitrofurans, Quinolones, Rifampicin) or intermediate metabolism (Sulphonamides, trimethoprin), Damage of cell membrane hence interfere with its function (Polymyxin, Polyene), Inhibition of protein synthesis (Aminoglycosides, Fenicols, Lincosamides, Macrolides, Streptogramins, Pleuromutilins, Tetracyclines) and Inhibition of respiration, that is, antagonism of metabolic pathways (Mah and Memish, 2000; Lambert, 2011).

Antibiotics are among the most commonly used medications globally and its discovery has helped in the control of pathogenic bacteria which are of enormous importance to global health until the development of antibiotic resistant pathogens which has endangered its sustainability and effectiveness (Wise *et al.*, 1998). Antibiotic resistance is becoming a worldwide problem posing a grave danger to humanity as various diseases and infections that were formerly treated with this substance are now difficult to control (Suree and Pana, 2015).The misuse of antibiotics has been reported as the main cause of antibiotic resistance (Väänänen *et al.*, 2006). These include: inappropriate prescription, abuse of antibiotics by patients, use of antibiotics in animals for growth promotion, and use of antibiotics for long-term care in facilities (Mah and Memish, 2000). However, the abuse of antibiotics by the public is an important risk factor for antibiotic resistance and for the treatment and management of infections and diseases (Melander *et al.*, 2000). Therefore, since antibiotics have remained the main-stay of clinical therapy worldwide (due to their role in management of common infectious diseases), it is crucial to gain an understanding of the susceptibility pattern of the commonly available antibiotics used by public in order to improve on their sustainability and effectiveness.

Auchi, the administrative headquarters of Etsako West Local Government Area, is located in the northern part of Edo State, South-South of Nigeria. It is the second-largest city in Edo State after Benin City, the State capital (fig 1.0).

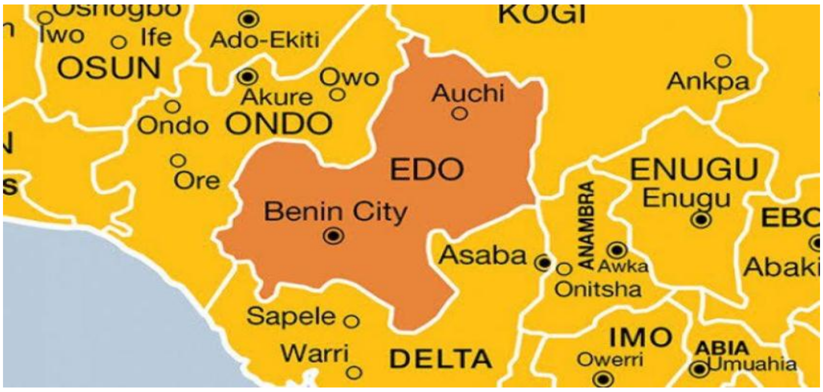

Fig. 1.0: The map of Edo state (highlighted) showing the location of Auchi.

This research is aimed at comparing the antimicrobial strength of three commonly used antibiotics in Auchi, in order to improve public knowledge and change the attitudes towards antibiotic use, as a strategy to maintain its effectiveness**.**

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Three different most commonly used antibiotics (Ciprofloxain, ampicillin and ampiclox) were purchased from pharmacies within Auchi and taken to the laboratory for analysis.

2.2 Source and Confirmation of Test Microorganism

Four pure clinical isolates which include *Escherichia coli*, *Staphylococcus aureus Klebsiella pneumoniae*, and *Campylobacter jejuni* were obtained from Microbiology Laboratory of Auchi Polytechnic Cottage hospital, Auchi, Edo State, Nigeria. All the isolates were authenticated and checked for purity, confirmed by Gram staining and biochemical test using routine laboratory techniques and maintained in agar slants at 37^{o}C.

2.3 Sterilization and disinfection

All glass wares were properly washed with detergent and rinsed with clean tap water. They were sterilized in hot air oven at 160^{o}C for 1hour. The inoculating loop was sterilized using the flame from a Bunsen burner. The flaming of the wire loop was repeatedly done at the end of every inoculation and the working bench was disinfected with swabs soaked in 70% ethanol.

2.4 Culture Media

The culture media used in this study were Mueller-Hinton agar and Nutrient Broth (Lab M Limited 1 Quest Park, Moss Hall Road, Heywood, and Lancashire BL9 7JJ, United Kingdom).They were prepared and sterilized by autoclaving at 121^{o}C for 15minutes according to manufacturer's specifications.

2.5 Standardization of test organisms

Broth cultures of the four (4) test organisms were prepared by plating the organisms in separate test tube containing 10 ml of nutrient broth and were incubated at 37^{o}C for 3 hours and standardized to Macfarland standard according to the method described by Suree and Pana, (2015).

2.6 Dilution of the antibiotics

Each of the antibiotics was dissolved in 10ml of sterile distilled water and diluted using two-fold serial dilution and then combined at 1:1 ratio, to determine their synergetic action against the test organisms.

2.7 Antimicrobial Screening

Agar well diffusion method was used to determine the antibacterial activity of the selected antibiotics. The 3-hour old standardized broth culture of each isolate was swabbed on Mueller-Hinton agar plate. The surface of the agar was completely swabbed with even coverage of the culture over the entire agar and the surface was allowed to dry for five minutes. Using a sterile cork borer, wells were created unto each appropriately labeled petri-dish. 0.1ml of the antibiotic solution was introduced into the wells on the inoculated agar surface in duplicates and incubated at 37^{o}C for 24 hours. After which, the average zone of inhibition in diameter was observed and measured using a metric ruler and reported in millimeters (mm) according to the method described by Clinical and Laboratory Standards Institute (2006).

3.0 RESULT

Table 3.1: ConfirmatoryBiochemical Characterization of the Test Clinical Isolates.

	Biochemical characteristic								Clinical isolates
Gram rxn	**Motility**	**Cat.**	**Coag.**	**Oxidase**	**Indole**	**Methylred**	**VP**	**Citrate**	
+	-	+	+	-	-	-	-	ND	*Staphylococcus aureus*
-	+	+	-	-	+	+	-	-	*Escherichia coli*
-	-	-	-	-	-	-	-	+	*Klebsiella pneumoniae*
-	+	+	ND	+	ND	ND	ND	ND	*Campylobacter jejuni*

Keys: + = present, - = Absent, Cat.=Catalase, Coag.=Coagulase ND = not determined

Table 3.2: Average inhibition zone diameter (mm) of the antibiotics against the selected clinical isolates.

Antibiotics	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Campylobacter jejuni*	*Escherichia coli*
Ciprofloxacin	44	27	40	34
Amoxillin	41	28	25	30
Ampiclox	36	36	27	33
Ciprofloxacin+Amoxicillin	51	37	44	37
Ciprofloxacin+Ampiclox	40	39	28	38
Amoxicillin+Ampiclox	33	25	23	25

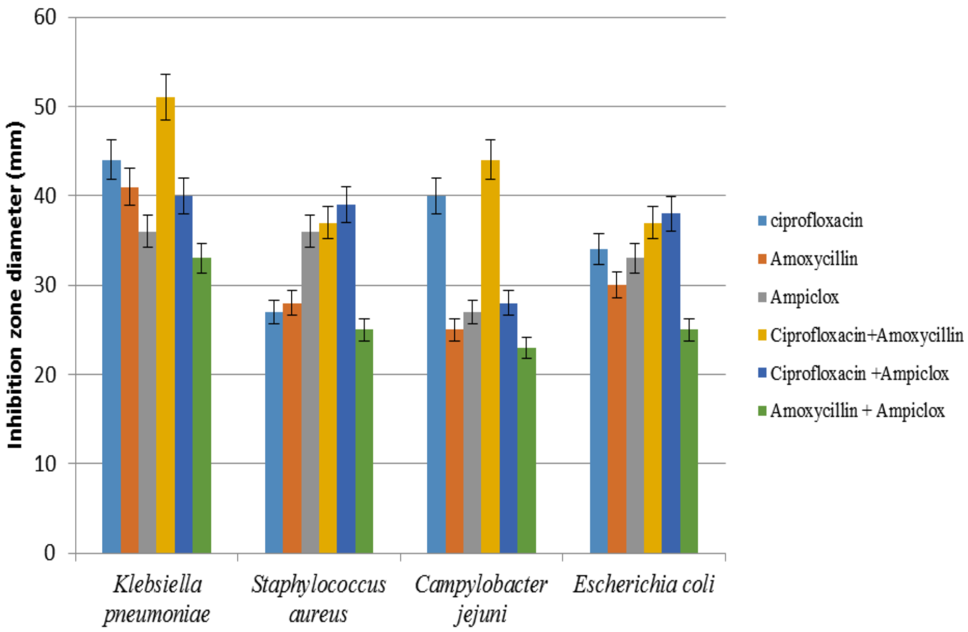

Fig 3.1: Chart showing the Inhibition zone diameter of the antibiotics against the selected isolates.

4.0 DISCUSSION

The result of the confirmatory Biochemical Characterization of the Test Clinical Isolates were shown in Table 3.1 and the results obtained for the antibacterial activities of the various commonly used antibiotics against the pure clinical isolates obtained for this study revealed several zones of inhibition as shown in Table 3.2. All the test organisms were susceptible to the selected antibiotics. The results of this study were similar to those reported by Kshetry *et al.*, (2016) that some bacteria isolated from different clinical samples at a tertiary care hospital in Nepal, were highly sensitive to vancomycin, Erythromycin, ciprofloxacin and streptomycin (Kshetry *et al.*, 2016).

Ciprofloxacin was actively effective against the test isolates with Inhibition zone diameter ranging from 27mm *(Staphylococcus aureus*) to 44mm (*Klebsiella pneumoniae*) as shown in figure 3.1. *Klebsiella pneumoniae* were highly sensitive to all the selected antibiotics tested both singly and combined which includes Ciprofloxacin, Amoxillin, Ampiclox, Ciprofloxacin+Amoxicillin, Ciprofloxacin+Ampiclox and Amoxicillin+Ampiclox. This was similar to those reported by Rao *et al*., (2014) that showed *Klebsiella* sp. to be sensitive to most of the antibiotic used in their study; tarivid (100%), peflaxin (70%), ciprofloxacin (80%), streptomycin (75%), imipenem (76.92%), levofloxacin (76.92%) and amikacin (76.92%).

The antibacterial activity of the combination of ciprofloxacin and amoxicillin have an inhibition zone of diameter ranging from 37mm (*Staphylococcus aureus* and *Escherichia coli*) to 51mm against *Klebsiella pneumoniae.* The combination of ciprofloxacin and ampiclox also had inhibition zone diameter ranging from 28mm (*Camphylobacter jejuni*) to 40mm *(Klebsiella pneumoniae*) as shown in figure 3.1. The antibacterial activity of the combination of amoxicillin and ampiclox with inhibition zone diameter ranging from 23mm (*Camphylobacter jejuni*) to 33mm (*Klebsiella pneumoniae*). All the tested antibiotics had high antibacterial activity against *Klebsiella pneumoniae* with the Ciprofloxacin-amoxicillin (51mm) combination being the highest followed by ciprofloxacin (44mm), amoxicillin (41mm), ciprofloxacin-ampiclox combination (40mm), ampiclox (36mm) and the least Amoxicillin-ampiclox combination (33mm). Ciprofloxacin-ampiclox combination had the highest antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Ciprofloxacin-amoxicillin combination had the highest antibacterial activity against *Camphylobacter jejuni.* This may be attributed to the mechanism of action of the quinolones (inhibition of DNA synthesis) and penicillins (inhibition of cell wall synthesis) which will reduce the transfer of resistance ((Suree and Pana, 2015). Amoxicillin-ampliclox combination had the least activity against all the test isolates used in this study this could be due to the abuse of antibiotics or the transfer of resistant gene because β-lactam (penicillin) drugs are most frequently prescribed antibiotics. The present study agrees with the findings of Angus, *et al.*, (2017), whose research revealed that the combination of Amoxicillion and Clavulanate or Ceftriaxone and sulbactam were recommended for empirical treatment of infections caused by extended-spectrum beta-lactamase producing bacteria in the hospital.

5.0 CONCLUSION

Antibiotics are important for bacterial infections control and they can be used singly or combined with one another for better result when prescribed by a physician. The ciprofloxacin-amoxycillin combination as tested in this research was found to be most effective against all the test organisms. Therefore for the treatment of any disease or infection, a culture and sensitivity test must be conducted to select the best antibiotics required for the treatment.

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