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Comparative Analysis of the Antimicrobial Strenght of Three Most Commonly Used Antibiotics in Awka Metropolis

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Abstract

Antibiotics are the drugs that either halt bacterial growth (bacteristatic) or kills the bacteria entirely. The comparative analyses of the three most common antibiotics used in Awka (Amoxycillin, Ciprofloxacin and Erythromycin) was carried out using *Staphylococcus aureus* and *Escherichia coli,* as the test organisms. Susceptibility/Sensitivity test is used to determine the antimicrobial activity of an antibacterial against an organism. Many methods can be used to carry out this test; broth dilution, antimicrobial gradient, disc diffusion and automated instrument method. In the course of this project work, Disc diffusion method was used to determine the antimicrobial strengths of the various antibiotics at different concentrations of the on the test organisms (*Staphylococcus aureus* and *Escherichia coli*). The antibacterial activity of the antibiotics procured from the pharmacy in Awka were tested in-vitro using the Antibiotics Disc Diffusion Method (Kirby-Bauer's method). The result showed that Ciprofloxacin had the highest antimicrobial effect on *Staphylococcus aureus* and *Escherichia coli,* with zones of inhibition, 32mm and 40mm respectively. Amoxycillin is also highly effective on *Staphyloccocus aureus* and a little bit effective on *Escherichia coli* with zones of inhibition 30mm and 29mm respectively. Erythromycin was effective on *Staphyloccocus aureus* with 22mm zone of inhibition and totally resistant to *Escherichia coli* with no zone of inhibition. Ciprofloxacin is the best antibiotic amongst the three analysed for the treatment of bacterial infections implicating *Staphylococcus aureus* and *Escherichia coli*. Amoxycillin is also effective on *Staphylococcus aureus* but not effective on *Escherichia coli* and finally Erythromycin has a little antimicrobial effect on *Staphylococcus aureus* but showed no effect on *Escherichia coli.* From the research, it can be recommended that whenever people are diagnosed of bacterial infections as a result of *Staphylococcus aureus* or *Escherichia coli*, the medical personnels are advised to prescribe Ciprofloxacin as it has the highest antimicrobial effect on the organisms than the other antibiotics it was compared with.

Keywords

Antibiotics, Antimicrobial Strength, Sensitivity, Resistance, Awka

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1. Introduction

An antibiotic also called an antibacterial is a type of antimicrobial drug [1], used in the treatment and prevention of bacterial infections [2]. They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity [3]. Antibiotics are not effective against viruses such as the common cold or influenza; drugs which inhibit viruses are termed antiviral drugs or antivirals rather than antibiotics.

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Sometimes the term antibiotic (which means "opposing life") is used to refer to any substance used against microbe [4]. However, the difference between antibiotics (ie, penicillin) and antimicrobials (ie, sulfonamide) is that the former is produced naturally, while the latter is synthetic (although both maintain the same goal of killing or preventing the growth of microorganisms). Some sources distinguish between antibacterial and antibiotic; antibacterials are used in soaps and disinfectants, while antibiotics are used as medicine [5].

Antibiotics can be broad Spectrum or Narrow spectrum; Broad spectrum antibiotics are those ones with activity against a wide range of gram positive and gram negative organisms while narrow spectrum antibiotics has activity against one or few type of bacteria [6].

Antibiotics effectiveness and easy access has led to their over-use, prompting bacteria to develop resistance [2]. This has led to widespread problems, so much as to prompt the World Health Organization to classify antimicrobial resistance as a "serious threat [that] is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country" [7].

The term 'broad spectrum' is applied to anti-bacterials, with activity against a wide range of Gram positive and Gram negative organisms. They include some β lactam antibiotics and the tetracyclines, aminoglycosides, sulphonamides, and chloramphenicol. Narrow spectrum antibiotics are those with activity against one or few types of bacteria, e.g. vancomycin against *staphylococci* and *enterococci*. In theory it is always better to use narrow spectrum antibiotics once the infective agent is known as this limits the detrimental effects on the normal bacterial flora [8].

Antibacterial resistance is the ability of bacteria to stop an Antibiotics from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others [9].

Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth [10].

For the comparison of the antimicrobial strength antibiotics on a test organism, sensitivity test which shows various zones of inhibition, is run and the diameter measured. The bigger the zone of inhibition, the higher the susceptibility [11].

Antimicrobial agents include naturally occurring antibiotics, synthetic derivatives of naturally occurring antibiotics (semi-synthetic antibiotics) and chemical antimicrobial compounds (chemotherapeutic agents), [16-17]. Generally, however, the term 'antibiotic' is used to describe antimicrobial agents (usually antibacterial) that can be used to treat infection. Compared with antibacterial agents, fewer antiviral and antifungal agents have been developed. Many antiviral agents have serious side-effects e.g. those used to treat HIV infection [11].

This work is therefore aimed at comparing the antimicrobial strenght of the three most common antibiotics used in Awka on some pathogenic microorganisms: *Staphylococcus aureus* and *Escherichia coli*.

2. Materials and Methods

2.1. Source of Test Organisms

The organisms used in this work: *Staphylococcus aureus* and *Escherichia coli*, were collected fromthe Federal Medical Centre, Asaba.

2.2. Source of Antibiotics

The antibiotics used in this analysis, Amoxycillin, Ciprofloxacin and Erythromicin were procured from a renowned pharmacy store in Awka.

2.3. Sample Area

Awka is a city in Anambra State, Nigeria. It is the capital of Anambra State and located 6.21 latitude and 7.07 longitude and it is situated at elevation 99 meters above sea level. Awka has a population of 167,738 (according to 2006 census) making it the 3rd biggest city in Anambra. It operates on the WAT time zone, which means that it follows the same time zone as Onitsha.

2.4. Identification of Test Organisms

The test organisms were sub-cultured onto Nutrient agar platesand biochemical tests were carried out on them. The biochemical tests carried out include: Gram staining, Indole Test, Catalase Test, Coagulase test.

2.5. Antibacterial Sensitivity/Susceptibility Test

The minimum inhibitory (MIC) concentration was taken as the lowest concentration of the different antibiotics on the disk that would inhibit the growth of bacteria in Nutrient agar.

The minimum inhibitory concentration of the various antibiotics used are shown in the table below.

Table 1. The minimum inhibitory concentrations of the various antibiotics used.

Antibiotics	Potency (MIC)
1. Amoxycillin (AM)	20µg
2. Ciprofloxacin (CPX)	5µg
3. Erythromycin (E)	15µg

The antibacterial activity of the antibiotics procured from the pharmacy in Awka were tested in-vitro using the Antibiotics Disc Diffusion Method (Kirby-Bauer'smethod). The Antibiotic discs already have the antibiotics incorporated in them at various potencies.

Nutrient agar plates were prepared and each plate was properly inoculated with the 24 hours culture of the test organism in nutrient broth using sterile swabsticks. With the petridish lids in place, they are kept for 3-5 minutes so that the surfaces of the agar dry.

Using a sterile forceps, the antibiotic discs were carefully placed on the inoculated plate ensuring the discs were kept 25mm away from each disc and at most 15mm away from the edges of the plate. The antibiotic discs were slightly pressed down to ensure adequate contact with the agar.

Within 15-30 minutes of the antibiotic discs application, the plates were inverted and incubated at 35°C for 16-18 hours.

After the incubation, the plates were carefully examined for confluent or near confluent growth and zones of inhibition. Using a ruler, the diameter of the zones of inhibition were measured in Millimeter (mm) from the underside of the plate.

For Confirmation, the Agar Well Diffusion Method was carried out, where a 2-fold serial dilution of the three antibiotics using sterile water as the diluent was done and it gave different concentrations viz: 80%, 60%, 40% and 20%.

Nutrient agar plates were prepared and each plate was properly inoculated with each test organism using sterile swabsticks.

Wells were madeusing a sterile cork borer and each well was carefully filled with different concentrations of the antibiotics. The plates were incubated at 37°C for 16-18 hours allowing the antibiotics to diffuse properly into the nutrient agar.

After the incubation, the plates were observed for zones of inhibition. The zones of inhibition were obtained by measuring the diameter using a ruler in Millimeter (mm).

The interpretation of the zones of inhibition that is whether an organism is susceptible, intermediate or resistant to an antibioticswas done based on the CLSI (Clinical Laboratory Standards Institute).

Table 2. National Committee for Clinical Laboratory Standards (NCCLS) standards for antimicrobial susceptibility testing.

Antimicrobial Agent	Resistant	Intermediate	Susceptible
Amoxicillin	≤19	-	≥20
Ciprofloxacin	≤15	16-20	≥21
Erythromycin	≤13	14-22	≥23

Figure 1. Antibiotics Diffusion Discs for gram positive organisms.

Figure 2. Antibiotics Diffusion Discs for gram negative organisms.

3. Results

The biochemical tests carried out on the test organisms show that *Staphyloccocus aureus* is coagulase positive, catalase positive and a gram positivecocci and *Escherichia coli* is coagulase negative, catalase negative, indole positive and a gram negative rod.

Table 3. The result of the biochemical test carried out in order to identify the organisms.

	Test Organisms	Gram Stain	Indole Test	Catalase Test	Coagulase Test
1.	Staphylococcus aureus+	NA	+	+	-
2.	Escherichia coli	-	+	-	-

Key:
NA=Not applicable
+=Positive
-=Negative

The sensitivity/ susceptibility test showed that *S. aureus* is susceptible to Amoxycillin, Ciprofloxacin and intermediate to Erythromycin with the diameter of zones of inhibition: 30mm, 32mm, 22mm respectively while *E. coli* is susceptible to Ciprofloxacin with zone of inhibition, 40mm, resistant to Amoxycillin, with zone of inhibition, 19mm and also resistant Erythromycin with no zone of inhibition.

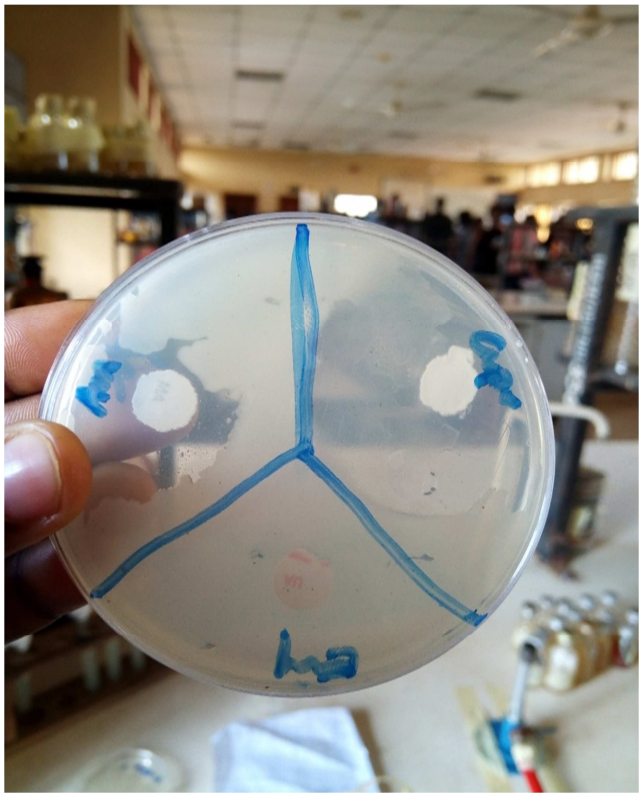

Figure 3. Culture plate showing the zones of inhibition (Paper disc method).

Table 4. The sensitivity of *Staphylococcus aureus* to Amoxycillin, Ciprofloxacin and Erythromycin.

	Antibiotics	Potency (µg)	Zone of Inhibition (mm)
1.	Amoxycillin	20	30
2.	Ciprofloxacin	5	32
3.	Erythromycin	15	22

Table 5. The sensitivity of *Escherichia coli* to Amoxycillin, Ciprofloxacin and Erythromycin.

	Antibiotics	Potency (µg)	Zone of Inhibition
1.	Amoxycillin	20	19
2.	Ciprofloxacin	5	40
3.	Erythromycin	15	No zone of inhibition

Interpretation of Zones of Inhibition

Using the table above, the organisms are reported as 'Resistant', 'Intermediate/Moderately susceptible', 'Susceptible'.

Resistant: A pathogen reported as 'resistant' implies that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose or site of infection.

Intermediate: A pathogen reported as intermediately susceptible suggests that the infection it has caused is likely to respond to treatment when the drug is used in larger doses than normal or when the drug is concentrated at the site of infection, e.g. in the urinary tract.

Susceptible: A pathogen reported as susceptible suggests that the infection it has caused is likely to respond to treatment when the drug to which it is susceptible is used in normal recommended doses and administered by an appropriate route [12].

4. Discussion

This analysis was carried out to compare the antimicrobial strenght of the three most common antibiotics used in Awka on some pathogenic microorganisms, *staphylococcus aureus* and *Escherichia coli*. The antibacterial spectrum and efficiency against the test organisms were demonstrated.

From the sensitivity test with *Staphylococcus aureus*, Ciprofloxacin has the highest zone of inhibition followed by Amoxycillin and Erythromycin with diameters, 32mm, 30mm and 22mm respectively. According to Table 5 above, the test organism is susceptible to Ciprofloxacin and Amoxycillin and intermediate to Erythromycin. *Staphylococcus aureus* is most sensitive to Ciprofloxacin. This was similar to the findings of [13].

With Escherichia coli, Ciprofloxacin has the highest zone of inhibition followed by Amoxycillin with diameters, 40mm and 22mm while Erythromycin showed no zone of inhibition. According to Table 5, the test organism is susceptible to Ciprofloxacin and resistant to Amoxycillin and Erythromycin. *Escherichia coli*is most sensitive to Ciprofloxacin. This was similar to the findings of [14-15].

5. Conclusion / Recommendation

It has been shown that the potency of antibiotics vary depending on the test organism. From analysis carried out, it can be concluded that Ciprofloxacin has the highest antimicrobial strength against *Staphylococcus aureus* and *Escherichia coli.* Amoxycillin is also effective on *Staphylococcus aureus* but not effective on *Escherichia coli* and finally Erythromycin has a little antimicrobial effect on *Staphylococcus aureus* but showed no effect on *Escherichia coli.*

From the analysis above, it can be recommended that whenever people are diagnosed of bacterial infection as a result of *Staphylococcus aureus* or *Escherichia coli*, the medical personnels are advised to prescribe Ciprofloxacin as it has the highest antimicrobial effect on the organisms than the other antibiotics it was compared with.

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