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STUDIES ON COMPARATIVE ANALYSIS OF ANTIMICROBIAL STRENGTH OF THREE OVER THE COUNTER COMMONLY USED ANTIBIOTICS: A CASE STUDY IN OWERRI MUNICIPAL.

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ABSTRACT: *Antibiotics are drugs that either halt bacterial growth or kills the bacteria entirely. The comparative analysis of these three most common antibiotics used in Owerri (Ceftriaxone, Erythromycin and Amoxicillin) was carried out using Staphylococcus aureus and Escherichia Coli as the test organisms. Susceptibility or 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 diffusion, disc diffusion and automated instrument method. In the course of this work, Disc diffusion method was used to determine the antimicrobial strengths of the various antibiotics. Antibiotics are not effective against viruses such as common cold or influenza, drugs which inhibits viruses at termed anti-viral drugs. Antibiotics can be broad specimen or narrow specimen. Broad specimen antibiotics are those one with activity against a wide range of gram positive and gram-negative organisms. While narrow specimen antibiotics has activity against one or few types of bacteria. Antibiotics effectiveness and easy access has led to wide spread problems, so much as to prompt the World Health Organization to classify antimicrobial resistance as a serious threat, that is no longer a prediction but already in occurrence in every region of the world, and has the potential to affect anyone of any age in any country.*

KEYWORDS: Antimicrobial strength antibiotics**,** Ceftriaxone, Erythromycin and Amoxicillin.

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INTRODUCTION

Antibiotics are chemicals, when the chemicals are put into the body, they stop the growth of all kinds of germs. They help the body to fight diseases. More than 3000 years ago, ancient people stumbled over the discovery that some molds could be used as a cure. The Egyptians, the Chinese and the Indians of central American would use molds to treat rashes and infected wound. At that time, they didn't understand either diseases or treatment. As time went on, people began to gain some insight of diseases. In the 1860, Louis Pasteur saw that many diseases were caused by bacteria. Later he discovered that we may be able to fight germs and other microbes. It was two German doctors, who were first to make an effective medication from microbes. Rudolf and Emmerich and Oscar has conducted their experiment in the 1890. Allmen did was to take the germ from infected bandages and grow them in a test tube. The would then isolate a particular gain that caused green infections in open wound. This germ was a bacteria called *Bacillus Pyoicyaneus.*

They put them into another test tube containing other types of bacteria, it was then it happened that the *bacillus* Pyocyneus wiped out the other disease germ. The germs that was killed were those that caused cholera, typhoid, diphtheria and anthna. From this, the two men created a medication that they called pyocinase, it was the first antibiotic used in hospitals. In 1928, Alexander Fleming, a Scottish scientist discovered penicillin, the first antibiotics. He was keeping in a peti disc when a speek of mold fell in, it caused the mold to grow on the nutrient Agar used to feed the bacteria. Fleming through the mold called penicillin notatum produced a substance that killed the bacteria and called it penicillin. However, he was not able to extract it from the broth in which he grew the mold. In 1945, Waksman used the word antibiotics for the first time and proposed that it can be defined as a chemical substance of microbial origin that possess antibiotic powers.He discovered a drug called Streptomycin. It originated from microbes found in soil and was a cure for many intestinal diseases. Now antibiotics like penicillin and Streptomycin was discovered. Each was effective against certain disease,but scientists wanted more. Doctors however, anted broad spectrum drug- that is, a single antibiotics that could cure many diseases, the search proved successful when laboratory discovered Aureomycin, which is a drug that does the job of penicillin and Streptomycin. In 1945, yet another laboratory came with one of the effective antibiotics ever found, Terramycin. This drug could be used against many bacteria diseases (Katzung, 1994).

LITERATURE REVIEW

ANTIBIOTIC

An antibiotic also called an antibacterial is a type of antimicrobial drug, used in the treatment, and prevention of bacterial infections. They may either kill or inhibit the growth. Health Organization to classify antimicrobial resistance as a "serious threat" that is no longer a prediction for the future.

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

Testing for antibiotic sensitivity is often done by Kirby Bauer method. Small wafers containing antibiotics are placed into a plate upon which bacteria are growing. If the bacteria are sensitive

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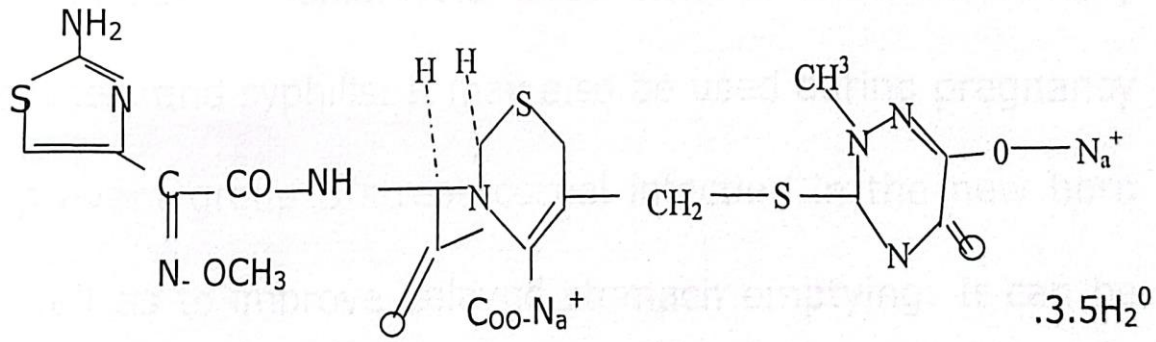
to the antibiotics, a clear ring or zone of inhibition is seen round the wafer indicating poor growth. From the comparison of the microbial strength of antibiotics on a test organism, sensitivity test which shows various zones of inhibition is run and the diameter measured. The bigger the zone of inhibition, the higher the susceptibility.

Ceftriaxone: Ceftriaxone is a broad spectrum antibiotic used to treat many kinds of bacterial infections including severe or life threatening forms such as meningitis. It is a sterile semi synthetic broad- spectrum Cephalopoda antibiotic for intravenous or intramuscular administration. The chemical formular of Ceftriaxone is C18H16NgN92O7S3, 3.5H2O.it has a calculated molecular weight of 661459 and the following structural formulae:

ANTIBIOTICS DESCRIPTION.



Ceftriaxone is a white to yellow crystallic powder which is readily soluble in water, sparingly soluble in methanol and very slightly soluble in ethanol. The PH of a 1% aqueous solution is approximately/6.7. Before instituting treatment with ceftriaxone, appropriate specimens should be obtained for isolation of the causative organism and for determination of its susceptibility to the drug.

Erythromycin: Erythromycin is also a board spectrum antibiotic, used to treat a wide variety of bacterial infections. It is known as a macrolide antibiotic. It works by stopping the growth of bacteria. Infections that can be treated with the use of erythromycin include: respiratory tract infections, skin infections, Chlamydia infections, pelvic inflammatory diseases, and syphilis. It may also be used during pregnancy to prevent group B streptococeal infection in the new born as well as to improve delayed stomach emptying. It can be given intravenously and by mouth. It is active against most gram positive bacteria.

Chemical formula C37 H67 NO13

Molar Mass 733.937g.mol-1

Amoxycilin: Amoxicillin is used to treat a wide variety of bacterial infections. This medication is a penicillin type antibiotic. It works by stopping the growth of bacteria such as pneumonia, bronchitis (infection of the air way tubes leading to the lungs) and infections of the ears, nose, throat,urinary tract and skin. It is a beta-Lactam antibiotic and can be administered orally or intravenously. Amoxicillin was a discovered in 1958 and came into medical use in 1972. It is on the World Health Organization's list of Essential Medications. It is one of the most commonly prescribed antibiotics in children. It is used in the treatment of a number of

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infections including acute Otitis media, Streptococcal Pharyngitis, Pneumonia, skin infections, urinary tract infections, salmonella infections, lyme disease and Chlamydia infections. Amoxicillin is a moderate spectrum bacteriolytic P-Lactam antibiotic in the amino-penicillin family used to treat susceptible gram positive and gram negative bacteria. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other B-Lactam antibiotics. Aureus can cause range of illness, from minor skin infections such as opimples, impecetige, boils, cellilits, follicuslitis,carbuncles, scalded skin syndrome, and abscess, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocerditod, toxic shock syndrome, bacteremia, and sepsis. It is also one of the five most common causes of hospital acquired infections and if often the cause of wound infections. S.aureus produces asexually by binary fission complete separation of the daughter cells is mediated by S. aureus autolysin, and in its absence or targeted inhibition, the daughter cells remain attached to one another and appear as clusters, and is catalase positive.

Escherichia Coli: E-Coli is a gram negative facultative anaerobic, rod- shaped coliform, bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms).

Most E.Coli strains are harmless, but some serotype (EPEC, ETEC, etc) can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents. The harmless strain are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K2 (which help in blood clot) and preventing colonisation of the intestine with pathogenic bacteria, having a symbiotic relationship. E-Coli is expelled into the environment within fecal matter. The bacteria grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.

Mode of **Transmission:** E-coli and other facultative anaerobes constitutes about 0.1% of the gut microbiota, and feca-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for faecal contamination.

The bacterium can be grown and cultured easily. E-Coli is a chemoheterotrophic whose chemically defined medium must include a source of carbon and energy.

MATERIALS AND METHODS

Sample Size/Sample Collection

In this study, clinical samples are collected and characterized by culture techniques, antibiotic resistance patterns, and susceptibility. A total of 5 specimen of urine and 5 specimen of throat swab and sputum, one collected to study *Staphylococcus aureus and e. coli* for characteristics results to culture media and biochemical tests.

Isolation and Identification of Test Organisms

Specimens were inoculated on to Mannitol Salt Agar and blood Agar, following overnight incubation, colonies are observed followed by gram staining, catalase test and all the primary screened strains will be subjected to various morphological and biochemical tests to ensure

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their identity. Using a wire loop, the samples are streaked for isolation on a plate of blood agar, it is then incubated upside down and steaked in the petri plate holder on the shelf for 37° for 18-24 hours. Also, for mannital salt agar, [MSA], same process is applied. After which the various strains are subjected to various morphological and biochemical tests to ensure that e-coli and s. aureus were isolated.

Biochemical test to identify e.coli

a. Indole Test: Colony was inoculated into peptone water broth and innocubated at 37°C of 24 hrs after incubation few drops of kovacs reagent were added the presence of punk colored rings indicates a positive results.

b. Oxidase Test: This test is done using filter paper moistened with few drops of a freshly prepared solution of tetramethl-phenylene-di-aminedihydrochloride. Especially, clump of cells are picked up from the slant growth with a sterile wooden stick and smeared on the moistened paper. Development of a violent or purple colour within 10 seconds.

RESEARCH RESULTS AND ANALYSIS

INTRODUCTION

This chapter reviews the results and analysis of the qualitative data and finding of the study. The findings are also discussed in the light of previous research findings and available literature, where applicable, in order to identify similarities and differences between this study and previous studies and literature.

Isolation of Test Organisms from Samples

Since several different type of normal flora may be present in a sample in addition to a possible pathogen, all microbes in the sample were isolated and identified, using a pure culture technique. Using a streak plating procedure in which the clinical sample was smeared onto a section of the agar in a petridish and then spread out in progressive steps.

Procedure with Sample a (Urine)

1. A 10 fold serial dilution of samples was made, following Agar preparation (Blood Agar).
2. A wire loop was sterilized on a Bunsen burner and let to cool.
3. The serially diluted sample labeled 10-2 was used and with the wire loop the samples are streak (spread) for isolation on the plate of Blood Agar.
4. It is then incubated upside down and stacked in a petri-plate holder on the shelf for 37°for 18-24 hours.

Same procedures were applied for sample (B (Sputum and Sample C (Throat Swab).

Following 24 hours incubation, it was observed that there was growth on all the Agar plates. Biochemical test was carried out using isolates to study staphylococcus aureus and E-coli for characteristic results.

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Identification

Bacterial isolates were identified using conventional methods based on their morphological, biochemical and physiological characteristics. The following biochemical and test were carried out to identify test organisms from sample.

Antibiotic susceptibility testing

The antibacterial activity of antibiotics procured from pharmacy in Owerri were tested using Disc Diffusion method (Kirby -Bauer's method).

Disc Preparation Procedures

1. Whatman filter papers were punched to a disc size of 0.5cm diameter, using a standard office paper perforating machine.
2. 0.5cm diameter disc were separated into three universal bottles of 4-disc each. They were all sterilized by autoclaving before impregnating the antibiotics.
3. The antibiotics used were each dissolved in appropriate volumes of sterilized distilled water.
4. Impregnation was achieved by putting 1ml of stock solution in a sterile plastic petri dish. The sterilized filter paper discs were then added to the solutions (4disk for each antibiotic stock solution) and allowed to absorb fully for 10 minutes and then dried in the oven at 40℃ for 15 minutes.

Sensitivity Testing

Three nutrient Agar plates were prepared and each plate was properly inoculated with the already prepared 24 hours pure culture of the test organisms, using sterile swab sticks. They are kept for 3-5 minutes (after covering the lids of the petro dish) so that the surface of the agar dry. Each plate inoculated was divided into 4 quadrant was placed an antibiotic disc. The three Nutrient Aga plates were inoculated each with each test organism. All plates were inoculated aerobically as 370c for 24hours. The zones of inhibition were measured for each disc and the average for each antibiotic was recorded.

Table 1: shows the biochemical characteristics of isolates

Samples	Sample A	Sample B	Sample C
Morphology	Round	Rod	Rod
Biochemical Tests			
Grams staining	+	-	-
Indole test	-	+	+
Catalase test	+	+	+
Citrate test	+	-	-
MR test	+	+	+
Oxidase test	-	-	-
Bacteria species identified	*Staphylococcus aureus*	*E. coli*	*E. coli*

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Table 2: Preparation of stock antibiotic solutions for filter paper disc impregnation

Antibiotic	Tablet Conc	Vol. of distilled water to dissolve tablet	Conc. Of Solution	Dilution Needed	Disc Conc
Erythromycin	250mg	25ml	10,000ug/ml	10-1	10ug
Amoxicillin	250mg	10ml	25,000u/ml	10-1	25ug
Ceftriaxone	250mg	75ml	300u/ml	10-2	30ug

Table 3: shows the zones of inhibition of each antibiotic on the test organism

Antibiotic Test organisms	Erythromycin	Ceftriaxone	Amoxycillin
Staphyloccocus aureus	22mm	39mm	30mm
Escherichia Coli	-	40mm	20mm

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Average zones of inhibition diameter on E. coli and staphylococcus aureus in nearest whole mm

RESULT INTERPRETATION

The antibacterial activity of the antibiotics procured from a pharmacy in Owerri were tested in-vitro using the antibiotic disc diffusion method. The result showed that amoxicillin is highly effective on Staphylococcus aureus and a little bit effective on Escherichia coli with zones of inhibition of 30mm and 29mm respectively. Erythromycin was effective on Staphylococcus aureaus with 22mm zone of inhibition and totally resistant to E.coli, with no zone of inhibition.Ceftriazone had the highest effect on Staphylococcus and E.coli with zones of inhibition measuring 39mm and 40mm respectively. Ceftriaxone is therefore the best antibiotic for treatment of bacterial infection implicating Staphylococcus and E.coli. Using the table above, the organisms are therefore reported as Resistant, intermediate and susceptible.

*E. coli was resistant to Erythromycin, with no zone of inhibition and intermediate to Amoxycilin but highly sensitive to ceftriaxone. This implies that the infection it causes will respond effectively to treatment with Cefmaxone and likely to respond to treatment with amoxicillin when the drug is used in large dose, or concentrated at the site of infection, but erythromycin will net respond to infections caused by E.coli.

Staphylococcus Aureus was highly sensitive to Ceftrixone and Amoxyilin and intermediate or moderately sensitive 2 Erythromycin. This implies that infections caused by S. Yureus can be treated with Ceftriaxone and will respond effectively, and likely to respond to treatment with Erthromycin, if drug is used in larger dose or concentrated at the site of infection.

DISCUSSION AND CONCLUSION

Sensitivity testing still remains a cornerstone in clinical bacteriology diagnosis. Isolation of a pathogen without knowledge of its antibiogram in face of wide spread resistance to commonly used drugs is valueless. The commercially available sensitivity disc are produced on the antibiotics manufactured by the drug company that distributes them. Some locally available, useful and sometimes cheaper antibiotics may not have commercial disc for them, making their sensitivity testing difficult. Local preparation of antibiotic sensitivity disc will introduce greater flexibility with minimum wastage.

It has been shown also that the potency of antibiotics vary, depending on the test organism. From the analysis carried out, it can be concluded that Ceftriaxone has the highest antimicrobial strength against *Staphylococcusaureus* and E. coli. Amoxicillin is also effective on Staphylococcus aureus but not effective on E.coli and finally, Erythromycin has a little antimicrobial effect on Staphylococcus aureus but showed on effect on E.coli.

From the analysis above, it can be recommended that whenever people are diagnosed of bacterial infection as a result of Staphylococcus aureus or E.coli, the medical personnel are advised to prescribe Ceftriaxone as it has the highest antimicrobial effect on the organism than other antibiotics it is compared with.

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