Antibacterial Activity of three Broad Spectrum Antibiotics against some Clinical Bacterial Isolates

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Abstract: Antibiotics, either produced by microorganisms or formulated synthetically have a dynamic attribute of inhibiting Growth or completely suppressing the toxic effect of microorganisms. This study aimed to compare the antibacterial strength of three common antibiotics: Ciprofloxacin, Gentamycin and Erythromycin against *Escherichia coli, Pseudomonas aeruginosa*and *Staphylococcus aureus*isolated from clinical samples such as Nasal swab, wound swab, urine and high vaginal swab.The sensitivity test was carried out using the Disc diffusion method. A total of 25 isolates were obtained from these samples after culturing. Of the 25 isolates, 5(20%) yielded *pseudomonas aeruginosa,* 10(40%) yielded *Staphylococcus aureus*and *Escherichia coli* respectively. Gentamycin demonstrated the highest antibacterial activity against the test organisms with 100% activity against *Pseudomonas aeruginosa* and 90 and 70% activity against *E. coli* and *S. aureus*. It was followed by ciprofloxacin which had 60 and 70% activity against *E. coli* and *S. aureus*respectively. Erythromycin was the antibiotic with the least activity, but it showed higher activity (80%) than ciprofloxacin (40%) against *Pseudomonas aeruginosa.* Therefore gentamycin is a better choice of antibacterial therapy against infections causedby*E. coli, P. aeruginosa*and *S. aureus.*It exhibited the highest antibacterial effect on these organisms than the other antibiotics tested.
Keywords: Analysis, Antibacterial strength, Broad spectrum, antibiotics, clinical isolates.

INTRODUCTION

Antibiotics are the drugs that inhibit bacterial Growth or kill the bacteria entirely (Xinhua, 2015). They are produced naturally by microorganisms or formulated synthetically and are used to treat and prevent bacterial infections (NHS, 2015). Availability and obtainability of commercially available broad-spectrum antibiotics causing multi-ding resistance remains a key global health issue (Khan *et al.,* 2011).

Antibiotics have been used for decades but due to their frequent administration in humans, common environmentally existing bacteria are quickly becoming resistant to treatment with these drugs (Hawser *et al.,* 2007).

Broad-spectrum antibiotics have an exhaustive range of coverage that arrive at the effectiveness of these medicines against both Gram-negative and Gram-positive bacteria (Ibeawuchi and Mbata, 2002; Chopra *et al.,* 2002).

Escherichia coli (gram-negative bacteria) is one of the most frequent causes of many common bacterial infections, including cholecystitis, Bacteremia, cholangitis, urinary tract infections (UTI) and traveller's diarrhoea and other clinical infections such as neonatal meningitis and pneumonia (Bhavsar and Krilov, 2015). *P. aeruginosa* also a gram-negative bacteria has become an important cause of Gram-positive infection. The most serious infections caused by *P. aeruginosa* include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia and septicemia. It mostly causes infections in hospitalized patients (Gellatty and Hancock, 2013). *S.aureus* is a gram-positive bacterium and causative agent of much infectious diseases such as skin infections, bacteremia, endocarditis, pneumonia and food poisoning. It was originally a leading nosocomial pathogen (Arumugan *et al.,* 2017).

The increasing prevalence of inappropriate antibiotic prescription and rising resistance is one of most pressing problems healthcare services face today (Costelloe *et al.,* 2010). Available antibiotics seem to be a limited resource. There sistance seems to be emerging faster than the availability of new antibiotics, a phenomenon now recognized as a major threat to public health (Morris, 2007; Vincent, 2011).

In general practice, there are worries about some common infections which are becoming increasingly hard to treat. That illness due to resistant bacteria may take longer to resolve (Butler *et al.,* 2006). This indicates that the inappropriate prescription by physicians for their patients may effect the effectiveness of antibiotics given to all patients. The goal is to use antibiotics in a careful way and avoid risk, reserving antibiotics that are not intended for first-line use until the older and more commonly used medications fail (Hay *et al.,* 2005). In addition, the adverse effect profiles of various antibiotics differ, so the administration of these drugs to individual patients must be based on the risk-benefit estimation for each patient (Walsh, 2003).

Hence, in the present study, broad-spectrum antibiotics, namely; Ciprofloxacin, Erythromycin and Gentamycin are used for investigation. Erythromycin: Belongs to the Macrolide class of antibiotics. It is used to treat a number of bacterial infections such as respiratory tract infections, skin infections, Chlamydia infections, pelvic inflammatory disease, and syphilis (Stephen *et al.,* 2011). Gentamycin: They are an aminoglycoside class of antibiotics used to treat several kinds of bacterial infections; this may include bone infections, endocarditis, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections and sepsis among others (Stephen *et al.,* 2011).Ciprofloxacin belongs to a class of drugs called quinolone antibiotics. It is used to treat a number of bacterial infections. This includes bone and joints infections, Intra-abdominal infections, certain types of infections diarrhoea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections. The dosage and the length of treatment are based on the medical condition of the patients and their response to treatment(Stephen *et al.,* 2011).

Reduced susceptibility to broad-spectrum drugs has become a major problem mostly in Nigeria. A country-wide analysis of different clinical isolates of bacteria with different brands of various broad-spectrum antibiotics will help the future. A country-wide analysis of different clinical isolates of bacteria with different brands of various broad-spectrum antibiotics will help assess the performance and therapeutic standards. This study was undertaken to determine the antibacterial strength of three commonly used broad spectrum antibiotics against some clinical isolates: *E. coli, Pseudomonas aeruginosa* and *S. aureus.*

MATERIALS AND METHODS

Source of test organisms: Clinical samples ofthenasal swab (10), wound swab (20), urine (20) and high vaginal swab (HVS) (20were collected from Madonna Catholic Hospital, Umuahia, Abia State to isolate *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa* which were the organisms chosen for the study. Nasal swabs, wound stabs and HVS swabs were collected using a sterile moisten swab sticks. In addition, early morning urine samples were collected with sterile urine containers.

Isolation and identification of test isolates: The specimens were cultured on Mac Conkey agar, nutrient agar (both Oxoid, UK) and blood agar by streaking on the culture plates. The plates were incubated for growth 37^0C for 24hrs.After incubation, the plates were observed for growth and morphological and biochemical tests were carried out to identify and authenticate the isolated organisms. The morphological and biochemical screening carried out includes Gram staining, Catalase test, Coagulase test, Oxidase test, TSI test, Citrate test, Urease test and Motility,Indole, Urease (MIU) test.

Gram staining: This differentiates bacterial species into two groups: Gram-positive and Gram-negative based on the cell walls, chemical and physical properties. An dried smear on a grease-free slide is heat-fixed, allowed to dry, and then flooded with crystal violet for 1 min. It was washed with water and then flooded with iodine for 1 min, wash off with water and water decolourized with acetone for few seconds and wash off immediately with water. Safranin (a counter stain) was applied for 1 min and wash off

with water. The prepared stain film was dry and viewed under the x100 objective lens with oil immersion dropped in the slide. Purple violet colour - Gram-positive, while pink colour is Gram-negative. (Cheesbrough, 2006).

Catalase test: A drop of water was placed on a glass slide using a Pasteur pipette with a wire loop. A colony from the culture was transferred onto the water to make a thick smear. 2 drops of hydrogen peroxide were transferred and on the smear with a Pasteur pipette. The smear was observed for the presence of bubbles. The presence of gas bubblesindicates a positive result, while no bubbles indicate a negative result. (Owuama, 2015).

Coagulase test: A drop of distilled water was placed at each end of a glass slide. A colony from the culture was transferred onto the water and emulsified. A loopful of EDTA-treated plasma was added to the suspension and mixed gently. Within 10 seconds it was examined for the presence of agglutination (clumping). Presence of clot = positive result, No clot = Negative result. (Owuama, 2015)

Oxidase Test: A few drops of fresh oxidase reagent was applied to a piece of filter paper. A colony from the culture was smeared on the fitter paper. The fitter paper was observed for colour change. Within 10-15 seconds, purple colour = positive, No colour change = negative (Owuama, 2015)

Citrate text: Pure bacterial colonies were picked up from a straight wine inoculated into a slope of Simmons citrate agar and incubated overnight at 37^0c**.** The growth of the organism on the slant and a change in colour of the medium from green to blue indicate a positive reaction.In contrast a negative reaction is indicated by the lack of Growth of organisms on the slant and no obvious colour change (Amadi *et al,* 2018).

Motility, Indole, Urease (MIU) test: This was carried out with the motility, indole, urease (MIU) media base dispensed into tubes in slant form. The test organism was inoculated by streaking the slant surface and stabbing the butt with a sterile wire loop and straight wire respectively. After incubation for 24 hours, a positive result for motility was noted by the organism's growth deviation from its inoculation line (haziness in the tube). Non motile organisms generally grow within the stab line (line of inoculation) leaving the surrounding medium clear, urease positive organisms turned the media bright red-blue due to the hydrolysis of urea in the presence of indicator phenol red. The organism's ability to utilize indole was tested by inserting a drop of indole reagent into the tube housing the menu media base and the inoculated organisms. A positive cherry red line on the surface of the slant a few minutes after making the drop incubated over night at 37^0c (Cheesbrough, 2006).

TSI (Triple Sugar Iron) Test: TSI agar contains three sugars (1.0% each of sucrose and lactose, 0.1% glucose) Phenol red detecting carbohydrate fermentation and ferrous ammonium sulfate for detection of hydrogen sulfide production. The TSI tube is inoculated by streaking the organisms on a slant and stabbing the butt with the inoculum. Fermentation is detected by gas and a visible colour change (from red to yellow) of the phenol red. The production of hydrogen sulfide is indicated by the presence of a residue that blackens the medium in the butt of the tube. Thus, a bacterium that can ferment glucose but not lactose or sucrose will produce a red slant and a yellow butt in a TSI tube, whereas, lactose or sucrose fermentation will produce a yellow slant and butt at the end of incubation. However, if an organism cannot utilize any of the three sugars, it will use only the amino acids/proteins which will cause the slant of the tube to become red as the colour of the butt remains unchanged(Amadi *et al,* 2018; Cheesbrough, 2006).

Antibiotic sensitivity testing: Three (3) broad-spectrum antibiotics (Ciprofloxacin (5µg), Gentamycin (10µg) and Erythromycin (15µg)manufactured by Antibiotic Ltd, UK) were used for the study. These antibiotics were procured from a renowned pharmacy in Umuahia.

Mueller-Hinton agar (Oxoid, UK) was used for sensitivity testing. The Kirby-Bauer disc diffusion technique was used. The colonies were compared with 0.5 Mcfarland standard in saline. Swabs were streaked uniformly on Muller-Hinton plates to obtain uniform growth. Antibiotic discs were placed on the surface of the media using sterile forceps. A little pressure was used to ensure firm contact with the agar plate. Plates were examined for a zone of inhibition after incubation at 35^0C for 24hrs by measuring the diameter using a ruler from the back of the plate. The zone sizes of each plate were interpreted using the guidelines by clinical and laboratory standards institute (CLSI. 2016).

Table1: Clinical andLaboratory Standards Institute (CLSI) breakpoint for antimicrobial susceptibility testing.

Antimicrobial agent	Resistant	Intermediate	Sensitive
Ciprofloxacin	≤15	16 – 20	≥21
Erythromycin	≤ 13	14 – 22	≥ 23
Gentamycin	≤ 12	13 – 14	≥ 15

RESULTS

The study compared the antibacterial strength of three broad spectrum antibiotics commonly used in treatment against some clinical isolates of *E. coli, P. aeruginosa* and *S. aureus* obtained from various clinical samples. A total of 25 isolates were obtained from various sources, including wound swabs, urine cultures, High vaginal swabs, and Nasal swabs.

Table 1 shows the frequency of occurrence of the test organisms from the different clinical samples. *E. coli* was isolated from all the specimens except from the Nasal swab (40%). *P. aeruginosa* was isolated from only the wound specimen (20%). *Staphylococcus aureus* was isolated from the wound, Nasal swab and urine (40%). Thus, a total of 25 isolates were isolated from the clinical samples.

The susceptibility pattern of *E. coli* to the antibiotics tested is shown in Table 2. All the isolates of *E. coli* from the HVS showed 100% susceptibility to the three drugs tested.In addition, 50% of the isolates from the wound were sensitive to Gentamycin and Erythromycin. All isolates from urine samples were sensitive to Gentamycin. Gentamycin exhibited the highest activity (90%) followed by Ciprofloxacin (60%) while Erythromycin showed the least activity (50%).

Table 3 depicts thesusceptibilitypattern of *S. aureus* to the antibiotics. 75% of the isolates from the wound were sensitive to Gentamycin, 50% from the same source (wound) were susceptible to Ciprofloxacin and Erythromycin, 80%of the isolates from Nasal Swab were susceptible to ciprofloxacin and Gentamycin. The isolate fromtheurine sample was sensitive to Ciprofloxacin and Erythromycin but was resistant to Gentamycin. Ciprofloxacin and Gentamycin exhibited the highest activity (70%) respectively and minimal activity (60%) recorded against Erythromycin.

Table 4 shows the antibiotic susceptibility pattern of *Pseudomonas aeruginosa.* Gentamycin exhibited the highest activity (100%) followed by Erythromycin (80%) with the least activity being recorded against Ciprofloxacin (40%). Amongst the three antibiotics, Gentamycin exhibited the highest inhibitory effect against the test isolates. Ciprofloxacin followed it although Erythromycin showed higher activity than Ciprofloxacin against *P. aeruginosa.*

Table 1: Frequency of occurrence of the test isolates from the different clinical samples

	Isolates (%)			
Source	*E. coli*	*P. aeruginosa*	*S. aureus*	Total
Wound	2(20)	5(100)	4(40)	11(44)
HVS	2(20)	0(0)	0(0)	2(8)
Nasal swab	0(0)	0(0)	5(50)	5(20)
Urine	6(60)	0(0)	1(10)	7(28)
Total	**10(40)**	**5(20)**	**10(40)**	**25(100)**

Table 2: Antibiotic Sensitivity Pattern of *E. coli*

		Isolates (%)		
Source	*No of Positive Growth*	*Ciprofloxacin*	*Gentamycin*	Erythromycin
Wound	2	0(0)	1(50)	1(50)
HVS	2	2(100)	2(100)	2(100)
Nasal swab	0(0)	0(0)	0(0)	0(0)
Urine	6	4(66.7)	6(100)	2(33.3)
Total	**10**	**6(60)**	**9(90)**	**5(50)**

Table 3: Antibiotic Sensitivity pattern of *S. aureus*

		Isolates (%)		
Source	*No of Positive Growth*	*Ciprofloxacin*	*Gentamycin*	Erythromycin
Wound	4	2(50)	3(75)	2(50)
HVS	0(0)	0(0)	0(0)	0(0)
Nasal swab	5	4(80)	4(80)	3(60)
Urine	1	1(100)	0	1(100)
Total	**10**	**7(70)**	**7(70)**	**6(60)**

Table 4: Antibiotic sensitivity pattern of *P. aeruginosa*

		Isolates (%)		
Source	*No of Positive Growth*	*Ciprofloxacin*	*Gentamycin*	Erythromycin
Wound	5	2(40)	5(100)	4(80)
HVS	0(0)	0(0)	0(0)	0(0)
Nasal swab	0(0)	0(0)	0(0)	0(0)
Urine	0(0)	0(0)	0(0)	0(0)
Total	**5**	**2(40)**	**5(100)**	**4(80)**

DISCUSSION

This study showed the highest sensitivity rate of *E. coli* to Gentamycin (90%) and the least sensitivity to Erythromycin (50%). It was recorded a study conducted at Riyadh hospital that *E. coli* resistance was increased from 10% in 2001 to 22% in 2005 (Babay, 2007). The resistance reported against ciprofloxacin by *E. coli* ranged between 20 – 30% in different countries (Glupezynski *et al.,* 2001).It is similar to what was obtained in this study where *E. coli* recorded 40% resistance to Ciprofloxacin.

S. aureus isolates were more susceptible to Gentamycin and Ciprofloxacin. The isolates of *S. aureus*in the current study showed less sensitivity to Erythromycin. This extent of susceptibility is similar to different studies conducted in different parts of the world (Wiley, 2012; Okafor *et al.,* 2018).

Gentamycin resistance among *S. aureus* isolates is comparable to that reported in 2000 – 2002 from the United States 51%, Canada 24.1%, Italy 58.6%, Germany 26.1% and France 40.5% (Jones *et al.,* 2004). Thus, there been an increase in resistance to fluoroquinolones among isolates of *S. aureus* in recent years. However, the fourth generation fluoroquinolones may reduce this growing and dangerous process of increasing resistance (Goldstein *et al.,* 1999).

The minimal level of susceptibility of the test organisms to Ciprofloxacin and Erythromycin may be because these antibiotics have been in use for because these antibiotics have been in use for longer. Also their oral route of administration is known to affect their rate of absorption into the blood stream (Stephen *et al.,* 2011). It could also be attributed to the low cost and irrational use of antibiotics for conditions that may not clinically indicate their use and over-the-counter sale of antibiotics in pharmacies without prescription by authorized practitioners (WHO, 2014; Jones, 2014).

Generally, the variation found in the susceptibility patterns of these commonly used antibiotics in this study could be attributed to the prevailing usage and abuse of the drugs in the area under study. The lower sensitivity to the commonly used drugs might indicate that the prescribers' dependence indicates the prescribers' dependence on these drugs in contrast to gentamycin, which is less commonly used. This further suggests the relation between antibiotic usage and the level of drug resistance encountered (Xinhua, 2015). The use of antibiotics must be restricted and monitored to reduce or completely decline the resistance. For proper patient care and prevention of treatment failures, constant surveillance and antibiotic sensitivity testing need to be implemented.

CONCLUSION

This study showed that the efficacy of antibiotics vary depending on the organisms been tested Gentamicin has the highest potency against *E. coli, S. aureus* and *P. aeruginosa* (90%, 70% and 100% respectively). Ciprofloxacin is also effective on *S. aureus* but less effective on *P. aeruginosa*, finally Erythromycin has a good antimicrobial effect on *P. aeruginosa* with little effect on *E. coli.*

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