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Evaluation of Antimicrobial Activity of Some Commonly Used Antibiotics on *E. coli* and *Klebsiella* Species

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ABSTRACT

Many microorganisms have developed resistance to commonly used antibiotics, constituting a major challenge in management of microbial infections. This study investigates the efficacy of selected antibiotics against two significant bacterial pathogens. Clinical isolates of *E. coli* and *Klebsiella* species were obtained from Microbiology department of specialist hospital Gombe and subjected to antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method. The antibiotics tested included Gentamicin, Ofloxacin, Augmentin, Ceftriaxone, Erythromycin, Cloxacillin, Cefuroxime, and Ceftazidime. The results revealed that *E. coli* exhibited sensitivity to Gentamicin, Ofloxacin, Augmentin, and Ceftriaxone, but resistance to Erythromycin, Cloxacillin, and Ceftazidime. Conversely, *Klebsiella* species were sensitive to Erythromycin, Cefuroxime, and Ceftazidime, with resistance noted against Gentamicin, Augmentin, and Ceftriaxone. Cefuroxime emerged as the most effective antibiotic against both bacterial species, showing no resistance in either. These findings underscore the critical need for regular antibiotic susceptibility testing to ensure the effective treatment of infections caused by these pathogens, as resistance patterns can significantly impact clinical outcomes.

Keywords: *Escherichia coli*, *Klebsiella* species, antibiotic susceptibility, Kirby-Bauer Disc Diffusion, Antibiotic Resistance.

INTRODUCTION

The struggle of mankind against infectious diseases is well known. The discovery of antibiotics led to optimism that infections could be controlled and prevented. However, infectious diseases remain a leading cause of morbidity and mortality, particularly in developing countries. This ongoing challenge is largely due to the emergence and spread of antimicrobial resistance (AMR), which is recognized as a major obstacle in the treatment of microbial infections both in hospitals and communities (Akeem et al., 2014). The Centers for Disease Control and Prevention (CDC) has identified AMR as one of the world's most urgent public health problems, as resistant infections result in prolonged illness, increased healthcare costs, and higher mortality (CDC, 2022).

Among the wide range of bacteria implicated in AMR, *Escherichia coli* (*E. coli*) and *Klebsiella* species are of significant concern. These Gram-negative organisms are commonly associated with hospital- and community-acquired infections, including urinary tract infections, pneumonia, and gastrointestinal diseases. Both organisms have demonstrated a high ability to acquire resistance to various antibiotics, especially β-lactams and fluoroquinolones, making treatment increasingly difficult.

In Nigeria, the indiscriminate use of antibiotics, lack of effective diagnostic tools, and weak enforcement of antimicrobial policies contribute to the rising prevalence of resistant bacterial strains. Thus, continuous evaluation of the antimicrobial susceptibility patterns of common pathogens is essential to guide rational antibiotic use and reduce treatment failure. This study aims to evaluate the activity of selected commonly used antibiotics on *E. coli* and *Klebsiella* species isolated from clinical samples in Gombe State, Nigeria. Antibiotic susceptibility

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testing was conducted using the Kirby-Bauer disc diffusion method to determine the resistance patterns of the isolates. The findings are expected to provide relevant data to support and inform treatment choices and promote responsible antibiotic use in clinical settings.

MATERIALS AND METHODS

Study Area

The study was conducted at the Department of Biological Science Laboratory, Federal University Kashere, Akko LGA Gombe State.

Sample Collection

Collection of Antibiotics Sensitivity Discs

Different brands of commercially prepared antibiotics sensitivity disc were purchased from Pharmaceutical Store. Such antibiotics sensitivity disc includes: Ampicillin, Amoxicillin, Augumentin, Ciprofloxacin, Erythromycin, Gentamycin, Tetracycline, chloramphenicol, Cefixime and Streptomycin. They were transported to the laboratory for labeling and storage. The pharmaceutical detail of these sensitivity discs (brand name, production date, expiring data, NAFDAC Reg. no) was noted and recorded.

Collection of Test Organisms

Clinical bacterial isolates were collected from microbiology department of specialist hospital Gombe. These isolates include: *E. coli* and *Klebsiella* species isolated from various samples, include; urine, stool, urogenital etc.

Sample Size

Total of 20 clinical bacterial isolates (*E. coli* and *Klebsiella* Spp) were collected from Microbiology Department of Specialist Hospital Gombe.

Identification of the Test Organisms

Gram Staining

Using test organisms, smears were made on a clean glass slide. The smear was allowed to air dry and passed three times over Bunsen flame which fix the preparation to the slide. The smear was stained, using Gram staining technique and examined microscopically under ×100 objectives to identify gram positive and gram-negative isolates (Akeem *et al*., 2012).

Biochemical Identification

The bacterial isolates collected were confirmed using the following biochemical tests: coagulase, catalase, motility, urease, indole and triple sugar iron test according to the procedures described by (Chesbrough, 2004).

Catalase Test

Catalase productions by the test organism was detected by picking colony of the test organism and inoculate on drop of hydrogen peroxide contained on a center of a clean microscopic slide. Catalase positive species show active bubbling while non-catalase production specie shows no bubbling (Chambers *et al*., 2014).

Coagulase Test

A drop of distilled water was placed on glass slide; a colony of the test organism was emulsified on the drop to make a thick suspension. A loop full of plasma was added to the suspensions and mix gently. Coagulase positive organism showed clumping within seconds while negative was not clumped within seconds (Lakshmi *et al*., 2013).

Motility Test

The test organism was placed on a drop of normal saline contained in a center of clean microscopic slide. The preparation was covered with a cover slip and examined microscopically for motile organisms using X10 and X40 objectives (Shame *et al*., 2017).

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Urease Test

The test organism was grown on a medium containing urea and indicator phenol red for 24 hours, urease positive organism showed pink coloration (Lalit *et al*., 2014).

Citrate Utilization Test

A sterile wire loop was used to inoculate the citrate medium with a saline suspension of the test organism and then the butt was stab. This was incubated at 35^{o}C for 24 hrs. enteric bacteria showed blue coloration on the media after incubation (Mandell *et al*., 2018).

Indole Test

The organism was inoculated in a bijou bottle containing 3ml of sterile peptone water and incubate at 37 degree for 24 hours. 0.5ml of kovac's reagent was added and shake gently after incubation. A red surface layer was produced (Georgy *et al*., 2004).

Triple Sugar Iron Test

Triple sugar iron agar slants were inoculated with the test organisms and covered with cotton plugs to allow proper aeration. After incubation at 37°C for 24 hours, sugar fermentation patterns were observed and interpreted as follows: No sugar fermentation: Red slant (alkaline) and red butt (alkaline), typical of *Pseudomonas aeruginosa*., Glucose fermentation only: Red slant (alkaline) and yellow butt (acidic), indicating limited acid production; characteristic of *Salmonella spp.* and *Shigella spp.*, Lactose and/or sucrose fermentation: Yellow slant (acidic) and yellow butt (acidic), sometimes with gas production at the base; typical of *Escherichia coli* and *Klebsiella* species (Nusrin et al., 2012).

Oxidase Test

A clean glass rude was used to emulsify a colony of the test organism on a piece of filter paper in a clean Petri dish containing 3 drops of freshly prepared oxidase reagent. Oxidase positive organism showed blue or purple coloration (Suruchi *et al*., 2011).

Preservation of the Test Organisms

Nutrient agar slants were prepared as instructed by the manufacturer, autoclaved at 121 degree Celsius for 15 minutes. This was allowed to dry in slanted position and stored at 40 degrees Celsius (40^{o}C). The test organism was inoculated on the surface of the slant using a sterile wire loop. This was also stored at 40^{o}C (Okore *et al*., 2016).

Preparation of Inoculums

Subcultures

Subcultures were made by carefully picking one colony using sterilize inoculation loop, this was inoculated on the surface of nutrient agar, a confluent bacterial growth appears on the medium. Such pure cultures were used for identification and antibiotic sensitivity tests. 3 to 5 colonies of the organism tested were picked by using a sterile wire loop emulsify in 3ml of sterile normal saline in which density of the bacterial suspension was adjusted by matching it with turbidity standard of barium chloride (McFarland's) (Anthony *et al*., 2018).

Antibiotics Susceptibility Test

Antimicrobial sensitivity testing was performed for each bacterial isolate using Mueller Hinton Agar (MHA) (Oxford, England) by the Kirby Bauer disc diffusion method following standard procedure. Sensitivity agar plates was dried in the hot oven until no visible excess moisture was observed on the surface. A sterile swab stick was dipped in the suspension of the prepared inoculums and then excess fluid was removed by pressing and rotating it against the side of the tube above the level of the suspension. The swab was streak evenly over the surface of the medium in three directions, rotating the plate to ensure even distribution. With the Petri dish lid in place, the surface of the agar was allowed to dry for 3-5 minutes. Sterile forceps were used to place the

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appropriate antibiotics discs on the surface of inoculated plates. The plates were incubated at 37°C for 24 hours and then examined for the presence of zones of inhibition of bacterial growth around antibiotic disc. Zone of inhibitions were measured by a ruler on the underside of the plate and interpret according to the standards of the 2015 clinical and laboratory standards institute (Bennett *et al*., 2017).

Statistical Analysis

The data obtained from the antibiotic susceptibility tests were statistically analyzed using the independent sample t-test to compare the mean zones of inhibition between *Escherichia coli* and *Klebsiella* species for each antibiotic. This analysis was conducted to determine whether the observed differences in antibiotic sensitivity between the two bacterial species were statistically significant. Results were considered significant at $p < 0.05$

RESULTS AND DISCUSSION

This study evaluated the effectiveness of commonly used antibiotics against *Escherichia coli* and *Klebsiella* species isolated from clinical samples. The findings highlight varying susceptibility patterns between the two bacterial species, underscoring the growing concern over antimicrobial resistance. The results showed that *E. coli* was highly sensitive to Gentamicin, Ofloxacin, Augmentin, and Ceftriaxone . This is consistent with previous studies such as Akinola et al. (2022) and Kibret & Abera (2011), which also reported high sensitivity of *E. coli* to these antibiotics (Table 1). The effectiveness of these antibiotics may be attributed to their broad-spectrum activity and mechanisms of action, such as inhibition of protein synthesis and cell wall formation. However, resistance to Erythromycin, Cloxacillin, and Ceftazidime was observed, which reflects global reports of increasing resistance in Enterobacteriaceae to macrolides and beta-lactams.

Table 1: Shows Antibiotic Susceptibility Pattern for *E. coli* species of Bacterial Isolate

Antibiotics	Disk content (*μg*)	Interpretive categories and MIC Break point nearest (mm)			Test result (mm)	Remark
		S	I	R		
Erythromycin	15	≥21	16-20	≤20	18	Resistant
Gentamicin	10	≥15	13-14	≤12	17	Sensitive
Cloxacillin	15	≥19	17-18	≤16	12	Resistant
Ceptriaxone	30	≥23	20-22	≤19	24	Sensitive
Ofloxacin	5	≥16	13-15	≤12	18	Sensitive
Cefuroxime	30	≥18	15-17	≤14	16	Intermediate
Augmentin	30	≥18	14-17	≤13	20	Sensitive
Cefrazidine	30	≥21	18-20	≤17	22	Resistant

Key: MIC - Minimum inhibitory concentration; Mm - millimeter; *μg* - microgram

On the other hand, *Klebsiella* species showed sensitivity to Erythromycin, Cefuroxime, and Ceftazidime. Resistance to Gentamicin, Augmentin, and Ceftriaxone in *Klebsiella* isolates suggests the presence of extended-spectrum beta-lactamases (ESBLs), which are known to degrade cephalosporins and render them ineffective (Table 2). This aligns with studies by Gang et al. (2013) and Torimiro et al. (2018), who also reported resistance of *Klebsiella* to beta-lactam antibiotics due to ESBL production. Interestingly, Cefuroxime emerged as the most effective antibiotic against both bacterial species, with no resistance observed. This suggests that Cefuroxime may still be a viable treatment option in settings where resistance to other antibiotics is prevalent.

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However, intermediate sensitivity observed in some cases indicates a potential shift toward resistance if misuse continues.

Table 2: Shows Antibiotics susceptibility pattern for *Klebsiella* species of bacterial isolate

Antibiotics	Disk content (*μg*)	Interpretive categories and MIC Break point nearest (mm)			Test result (mm)	Remark
		S	I	R		
Erythromycin	15	≥21	16-20	≤15	22	Sensitive
Gentamicin	10	≥15	13-14	≤12	10	Resistant
Cloxacillin	15	≥19	17-18	≤20	21	Intermediate
Ceptriaxone	30	≥23	20-22	≤13	11	Resistant
Ofloxacin	5	≥16	13-15	≤12	14	Intermediate
Cefuroxime	30	≥18	15-17	≤14	24	Sensitive
Augmentin	30	≥18	14-17	≤13	12	Resistant
Cefrazidine	30	≥21	18-20	≤17	22	Sensitive

Key: MIC - Minimum inhibitory concentration; Mm - millimeter *μg* - microgram

The observed differences in antibiotic response between *E. coli* and *Klebsiella* may be due to inherent structural and genetic differences (Table 3). For example, *Klebsiella* possesses a thick polysaccharide capsule that can impede antibiotic penetration, while *E. coli* may rely more on efflux pumps and plasmid-mediated resistance genes. These findings emphasize the importance of routine antibiotic susceptibility testing before initiating treatment. Empirical therapy without laboratory guidance may contribute to treatment failure and the further emergence of resistant strains. Additionally, the data support the need for continuous surveillance of resistance patterns and rational use of antibiotics in both clinical and community settings.

Table 3: Shows comparison of antibiotic susceptibility pattern between *E. coli* and *Klebsiella* specie of bacterial isolate

Antibiotics	*E. coli*	*Klebsiella* species
Erythromycin	(R)	(S)
Gentamicin	(S)	(R)
Cloxacillin	(R)	(I)
Ceptriaxone	(S)	(R)
Ofloxacin	(S)	(I)
Cefuroxime	(S)	(S)
Augmentin	(S)	(R)
Cefrazidine	(R)	(S)

Key: MIC - Minimum inhibitory concentration; Mm - millimeter; *μg* - microgram

CONCLUSION

The outcomes of this study showed that the susceptibility of eight (8) commonly used antibiotics was evaluated against *Escherichia coli* and *Klebsiella* species. Cefuroxime remained fully active against both *E. coli* and *Klebsiella*, with no resistance recorded. Gentamicin, Ofloxacin, Augmentin, and Ceftriaxone demonstrated activity against *E. coli*, as evidenced by the measured zones of inhibition and based on Clinical and Laboratory Standards Institute (CLSI) breakpoints, indicating sensitivity. In contrast, little or no activity was observed for Erythromycin, Cloxacillin, and Ceftazidime against *E. coli*, suggesting resistance. Cefuroxime yielded an intermediate result, indicating moderate effectiveness. For *Klebsiella* species, Erythromycin, Ceftazidime, and Cefuroxime were effective, showing large zones of inhibition consistent with CLSI standards. These antibiotics can therefore be considered sensitive for *Klebsiella*. However, Gentamicin, Augmentin, and Ceftriaxone were found to be ineffective against *Klebsiella*, indicating resistance.

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