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PREVALENCE AND ANTIBACTERIAL RESISTANCE PROFILE OF *Escherichia coli* AND *Klebsiella* species ISOLATES OF URINARY TRACT INFECTION TO COMMON ANTIBIOTICS

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ABSTRACT
Urinary tract infection (UTI) is caused mainly by both Gram positive and negative bacteria including Escherichia coli and Klebsiella species. Urinary tract infection (UTI) caused by multi-drug resistant bacteria is increasing worldwide and has become a major public health concern that requires global attention. In order to promote better treatment outcome of UTI and increase awareness of antibiotic resistance, this study was conducted among UTI patients of Barau Dikko teaching hospital Kaduna to determine the prevalence and analyze the antimicrobial resistance of Escherichia coli and Klebsiella species to common antibiotics being used for their treatment. Result from this study shows the prevalence of Escherichia coli to be 75% while Klebsiella species was 59.85%. Escherichia coli was resistance to the common antimicrobial drugs like Chloramphenicol 30µg (100%), Septrin (30µg)(100%), Sparfloxacin (10µg)(43%) Gentamycin 30µg (60%), Ciprofloxacin 30µg (40%), Pefloxacin (30µg)(55%), Augumentin (10µg)(100%), Amoxacillin (30µg)(100%), Streptomycin (30µg)(10%) and Tarivid (10µg)(80%). Klebsiella species was resistant to Chloramphenicol 30µg (60%), Septri(30µg)(100%), Sparfloxacin (10µg) (25%), Ciprofloxacin 30µg (65%), Pefloxacin (30µg)(50%), Augumentin (10µg)(100%), Amoxacillin (30µg)(30%), Streptomycin (30µg)(30%) and Tarivid (10µg)(100%). Both uropathogens analysed had P-values less than 0.05 indicating that their resistance to antibiotics is statistically significant. The occurrence of bacterial Urinary tract infection with a higher resistance rate for commonly used antimicrobials leaves the clinicians with very few options to choose drugs used for empirical treatment of UTIs. Therefore, there is the need for scientists to put more efforts in exploring alternative drugs or herbs to tackle UTI infection effectively.
Keywords: antibiotic, Eschericia coli, Klebsiella species, Prevalaence, resistance, UTI

INTRODUCTION

The Urinary tract is the name given to the internal organs that collect, store and expel urine from the body, while the urinary tract infection (UTI) can be defined as condition in which bacteria are multiplying and attacking the urinary tract regardless of the position along the tract (Sule *et al*., 2016; Klein and Hultgren, 2020). Patients suffering from asymptomatic UTI are commonly treated with antibiotics; these treatments can result in long-term alteration of the normal microbiota of the vagina and gastrointestinal tract and in the development of multidrug-resistant microorganisms (Kostakioti *et al.,* 2012; Garoy *et al.,* 2019; Agbawo *et al*., 2020). The availability of niches that are no longer filled by the altered microbiota can increase the risk of colonization with multidrug-resistant uropathogens. Importantly, the 'golden era' of antibiotics is waning, and the need for rationally designed and alternative treatments is therefore increasing (Christaki *et al.,* 2020 & Mukubwa *et al.,* 2023). Recent studies have used RNA sequencing to directly analyze uropathogens from the urine of women experiencing symptomatic UTIs. These studies, together with basic science and improved animal models, have been crucial in enabling us to understand the molecular details of how uropathogens adhere, colonize and adapt to the nutritionally limited bladder environment; evade immune surveillance; and persist and disseminate in the urinary tract (Kostakioti *et al.,* 2012).

Although microorganisms use a diverse mechanism to get hold of drug resistance through horizontal gene transfer (plasmids, transposons and bacteriophages), recombination of foreign DNA in bacterial chromosome and mutations in diverse chromosomal locus (Bouamri *et* al., 2015;

Sule *et al.,* 2016). The empirical usage of ciprofloxacin and cephalosporins has been threatened by the emergence of broad-spectrum beta-lactamases (Rahman *et al.,* 2019). However, antibiotic resistances are diverse to respective communities (Sabir *et al.,* 2014). *Klebsiella* spp are often resistant to many antibiotics, including cephalosporins and aminoglycosides (Nas *et al.,* 2019). β-lactam antimicrobial agents are most common treatment option for such infections.

Importantly, the 'golden era' of antibiotics is waning, and the need for rationally designed and alternative treatments is therefore increasing. Recent studies have used RNA sequencing to directly analyze uropathogens from the urine of women experiencing symptomatic UTIs (Khelfaoui *et al.,* 2020; Odongo *et al.,* 2020). These studies, together with basic science and improved animal models, have been crucial in enabling us to understand the molecular details of how uropathogens adhere, colonize and adapt to the nutritionally limited bladder environment; evade immune surveillance; and persist and disseminate in the urinary tract (Kostakioti *et al.,* 2012 & Cunha *et al.,* 2016).

The aim of this study was to determine the antibacterial resistance profile of *Escherichia coli* and *klebsiella* species isolates of urinary tract infection to common antibiotics

MATERIALS AND METHODS

The sample size for the study was determined from a standard epidemiology formula for minimum sample size calculation by (Nas *et al.,* 2019).

The sample size was calculated using the formula below;

$$n = \frac{Ɛ^2 p(1-p)}{i^2}$$

Where Ɛ is the confidence interval, P is the prevalence rate, i is the level of precision and n is the sample size.

n=?

Ɛ = 1.96

p= 9.5%

i= 5%

$$n = \frac{(1.96)^2 \times 0.095\ (1-0.095)}{(5\%)}$$

$$n = \frac{3.8416 \times 0.085875}{0.0025}$$

n= 132.26

Therefore, number of samples collected was: 132.26 ≈ 132.

A total of 132 samples were collected from UTI patients from Barrau Dikko Teaching Hospital, Kaduna and was analysed for microbial susceptibility to various antibiotics.

Isolation and Identification of Bacteria

Samples of urine collected were tested within half an hour of collection. All samples were inoculated using a sterilized wire loop. Where 20µl of the Urine samples were cultured using streaking method on Cystine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar plates after which the plates were incubated at 37 °C for 24 hours in an inverted position and colony growth was observed. *E. coli* appeared yellowish on CLED agar while *Klebsiella* species appeared mucoid pink on MacConkey agar. Gram reactions of the isolates indicated that the isolates were both Gram negative. Various Biochemical tests like indole, methyl red, Vogues Proskauer, citrate, oxidase and urease test were also done on the isolates to confirm their identity (Cheesebrough, 2006).

Standardization of Inoculum

The method of Oyeleke and Manga (2008) was used to standardize the organisms. The isolates being tested were sub-cultured on Mueller Hilton Agar (MHA) that was prepared according to the manufacturer's instruction at 37°C overnight. Colonies from the overnight growth on the MHA were inoculated into a tube containing Mueller Hinton broth until the turbidity is equivalent to the density of 0.5 McFarland standards (1.5 x10 CFU/ml).

Antibiotic susceptibility test

Antibiotic susceptibility test was performed using Kirby Bauer disk diffusion method in respect to European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014). Bio-Rad antibiotic disk that were used are; amoxicillin / clavulanic acid (AMC) (20/10 µg), cefoxitine (FOX) (30 µg), cefotaxime (CTX) (30 µg), ceftazidime (CAZ) (30 µg) , imipeneme (IMP) (10 µg), aztreoname (TMJ) (30 µg), cefepime (FEP) (30 µg) fosfomycine (FOS) (10 µg), gentamicine (CN) (10 µg)), amikacine (AK) (30 µg), tobramycine (TOB) (10 µg), nalidixic acid (NA) (30 µg), ciprofloxacin (CIP) (5 µg), ofloxacin (OFX) (5 µg) and trimethoprimsulfamethoxazole (SXT) (1.25 / 23.75 µg).

Standardized cultures of *E. coli* and *K. pneumoniae* were spread on prepared MHA using sterile cotton swab three to four times over the entire surface of the agar, performing the streaking each time and rotating the plate about

60° to ensure even distribution of the inoculums. The plates were allowed to dry for 3-5 minutes before applying the antibiotics disks. Moreover, Forceps was used to place the proper antimicrobial-impregnated disks on the surface of the MHA agar culture plate and inverted for overnight at 37℃. The plates were then observed for susceptibility to the test antibiotics. Zones of inhibition of antibiotics against the test isolates were recorded as susceptible or resistant based on CLSI standards (Mukubwa *et al.,* 2023).

Statistical analysis

All outcome data were analysed using Statistical Package for Social Sciences (SPSS) version 18.0software and Microsoft Excel 2010. The difference between the resistance patterns of pathogens was determined using Chi-square test of Pearson. All differences in which the probability of the null hypothesis was $p < 0.05$ were considered significant.

RESULTS

Biochemical Characteristics and Colonial Morphology of the Bacterial Isolates

The table below shows the biochemical characteristics and colonial morphology of the bacterial isolates. The isolates were confirmed to be *Escheric|hia coli* and *Klebsiella pneumoniae*.

Table 1: Cultural and Biochemical Characteristics of the Bacterial Isolates

Isolate	Colony morphology	Gram Reaction	CI	NI	Mot	MR	VP	IN	Lac	Probable Organism
A	Yellowish colonies on CLED	-	-	+	+	+	-	+	+	*Escherichia coli*
B	Pinkish colonies on MCA	-	+	+	-	-	+	-	+	*Klebsiella pneumoniae*

Keys:
A= *Escherichia coli*
B= *Klebsiella pneumoniae*
+: positive; **-**: Negative; Citr: Citrate; MRi: Methyl Red; VPi: Vogas Proskauer; Lac: Lactose; Mot.: Motility; EMB: Eusine methylene blue; MCA: MacConkey Agar.

Out of 132 urine samples examined 99(75%) were positive for *Escherichia coli* while 79(59.85%) were positive for *Klebsiella pneumoniae* as confirmed using Gram staining and biochemical tests. Results on Table 3 presents the susceptibility pattern of *E. coli* and *Klebsiella pneumoniae* isolates to antibiotics and CLSI breakpoint for each organism to the antibiotic agents were used to determine their sensitivity. Both isolates showed resistance to all the antibiotics tested against them except Gentamycin which only *Klebsiella pneumoniae* was sensitive to. Result on antibiotic resistance percentage of *Escherichia coli* and *Klebsiella pneumoniae* shows 100% and 60% resistance to Chloramphenicol, 43% and 25% was for Sparfloxacin while 60% and 0% was for Gentamycin respectively. Also, Ciprofloxacin had 40% and 65% resistances, pefloxacin had 55% and 50% resistances, Amoxicillin had 100% and 30% resistances, Streptomycin had 10% and 30% resistances and also, Tarivid had 80% and 100% resistances by *E. coli* and *Klebsiella pneumonia* respectively. However, both organisms were 100% resistant to Septrin and Augmentin.

Table 2: Percentage occurrence of *Escherichia coli* and *Klebsiella* species isolates from urine of UTI patients (n=132)

Isolates	**Number positive**	**Percentage positive (%)**	**P value**
E. coli	99	75.00	0.0165
K. pneumonia	79	59.85	

Table 3: Antibacterial sensitivity of *Escherichia coli* and *Klebsiella* species

Antibiotics	***CLSI**	**Mean Zone of inhibition (mm)**			
		Klebsiella pneumonia	**Interpretation**	***Escherichia coli***	**Interpretation**
Chloramphenicol (30µg)	≥18	11.24±0.35^{ab}	Resistant	14.5±0.71^{abcd}	Resistant
Ciprofloxacin (30µg)	≥21	13.25±0.35^{c}	Resistant	15.0±0.00^{aefg}	Resistant
Septrin (30µg)	≥15	0.00±0.00^{d}	Resistant	0.0±0.00^{hi}	Resistant
Sparfloxacin (10µg)	≥30	10.75±0.35^{a}	Resistant	12.75±1.06^{bj}	Resistant
Amoxacillin (30µg)	≥18	8.5±0.71	Resistant	0.00±0.00^{hj}	Resistant
Augmentin (10µg)	≥17	0.0±0.00^{d}	Resistant	0.00±0.00^{ik}	Resistant
Gentamycin (30µg)	≥15	15.5±0.00^{ef}	Sensitive	14.75±0.35^{ce}	Resistant
Pefloxacin (30µg)	≥23	12.5±0.71^{bc}	Resistant	15.0±0.00^{dfkl}	Resistant
Tarivid (10µg)	≥17	15.0±0.00^{eg}	Resistant	9.0±0.71	Resistant
Streptomycin (30µg)	≥23	15.0±0.71^{fg}	Resistant	16.75±0.35^{gl}	Resistant
	P value	0.0283		0.0267	

Key: *CLSI = CLSI breakpoint for Sensitivity

Table 4: Antibiotic Resistance percentage

Organism	Chloramphenicol (30µg)	Septrin (30 µg)	Sparfloxacin (10µg)	Gentamycin (30µg)	Ciprofloxacin (30µg)	Pefloxacin (30µg)	Augumentin (10µg)	Amoxacillin (30µg)	Streptomycin (30µg)	Tarivid (10 µg)
E. coli	100	100	43	60	40	55	100	100	10	80
Klebsiella pneumoniae	60	100	25	0	65	50	100	30	30	100

Key: Chlo **=**

DISCUSSION

The most common causative agent of Urinary tract infections in the current study is *Escherichia coli* 99 (75%), followed by *Klebsiella pneumoniae* 79 (59.85%) isolates. *Escherichia coli* had the highest percentage occurrence because it has the ability to adhere, grow and resist against host defense that finally result in colonization and infection of urinary tract (Rahman *et al*., 2019) and this conform favorably with similar studies conducted by Nordamann *et al*., (2019) and Mukubwa *et al.,* (2023). Mukubwa *et al.,* (2023) in his study reported prevalence of 57% and 14.8% for *E.coli* and *Klebsiella species* respectively. However, in contrast to the findings in this study, *E. coli* accounted for less than 20% of UTI cases as reported by Odongo *et al.,* (2020). In this study, *E. coli* and *Klebsiella pneumonia* isolated from UTI showed resistance to all the common antibiotics tested against ranging from Chloramphenicol, Sparfloxacin, Ciprofloxacin, Pefloxin, Amoxicillin, Streptomycin, Tarivid, Septrin and Augmentin while only *Klebsiella pneumonia* was sensitive to Gentamycin. This report is in correspondence with earlier studies conducted by Mukubwa *et al.,* (2023) who reported that the urinary *E. coli* and *K. pneumoniae* isolates in their study showed high resistance to most of the first and second line oral and parenteral antibiotics such as cotrimoxazole, ofloxacin, ciprofloxacin, levofloxacin, amoxicillin-

clavulanate and cefuroxime commonly used in Kinshasa, confirming the existence and challenges of antimicrobial resistance of bacterial uropathogens in Democratic Republic of Congo. The urinary *E. coli* and *K. pneumoniae* isolates in this study showed highest resistance of 100% to Septrin and Augmentin while showing remarkable resistance to all other antibiotics tested against, confirming the existence and challenges of antimicrobial resistance in bacterial uropathogens in Kaduna, Nigeria. Since *Klebsiella pneumonia* was sensitive to Gentamycin, it indicates that *Klebsiella pneumonia* can treated using Gentamycin. This antibiotic resistance is a warning sign of emerging resistance by *E. coli* and *Klebsiella pneumaniae* infections (Gupta *et al.,* 2011 & Amawi *et al.,* 2021).

CONCLUSION

The prevalence of *Escherichia coli* in the urinary tract infection (UTI) samples tested in this study was 99 (75%) while that of *Klebsiella pneumoniae* was 79 (59.85%). Both isolates in study showed 100% resistance to Septrin and Augmentin while *Klebsiella Pnuemoniae* was sensitive to Gentamicine having zone of inhibition of 15.5 ± 0.00^{ef}. This study indicates an alarming rate of resistance of *E.coli* and *Klebsiella species* associated with urinary tract infections to common antibiotics. Therefore, immediate action is needed to prevent these resistant bacteria from spreading in both healthcare and community settings through the development of new antibiotics and vaccines. High antibiotic usage should also be reduced and proper sensitivity tests should be administered to patients before prescription of appropriate antibiotics.

Conflict of interest

There was no conflict of interest among the authors

Author's contributions

This research was a collaborative work where all the authors were involved in the sample collection process, culturing and reviewing and processing of the result.

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