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Detection of Antibiotic Resistance in Gram-Negative Uropathogens Isolated from Suspected Urinary Tract Infection Patients in Kano State, Nigeria.

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Abstract

Urinary tract infection (UTI) is caused mostly by different bacteria of family Enterobacteriaceae in hospital and communities acquired infection. Emerging increasing resistance has been observed among uropathogens. The aim of the research was to evaluate current antibiotic resistance pattern of gram-negative bacteria causing UTI among patients attending Aminu Kano Teaching Hospitals in Kano, Nigeria. This research was conducted at Aminu Kano teaching hospital, Kano State, Nigeria. Two hundred and four urine samples (204) of patients who were suspected of UTI were included as the study population. The urine sample was inoculated on cysteine lactose electrolyte deficient (CLED) agar plates and incubated at 37^{0}C for 24 hours. Identification was based on standard microbiological methods. The susceptibility testing was done by modified Kirby Bauer technique. Clinical laboratory standard institute (CLSI) 2024 guidelines were used for screening and confirmation of extended spectrum beta lactamase (ESBL) production. Double disk synergy method was used for confirmation of ESB. Out of 204 urine processed, 43 (65.2%) were gram negative and 41 were Enterobacteriaceae, isolation rates were Escherichia coli 17 (39.5%), Citrobacter spp 8(18.6%), Klebsiella pneumonia 7(16.3%), K. oxytoca 4 (9.3%), Enterobacter 3 (7.0%), Pseudomonas aeruginosa 1 (2.3%), Acinetobacter baumanni 1 (2.3%), Proteus vulgaris 1 (2.3%), Yersinia spp 1 (2.3%). Susceptibility showed that the isolates were highly susceptible to gentamycin (86.04%) and nitrofuratoin (67.5%). Resistance to third generation cephalosporins varies from 48.84% - 75.2%, while amoxicillin has 72.1% resistance. Among the isolates 9 were ESBL producers. Prevalence of ESBL was 66.7% for E. coli, 22.2 *% for K. pneumonia,* 11.1% *for citrobacter spp. It is recommended for continuous surveillance of resistant rate among bacterial isolates causing UTI to ensure proper recommendation for the treatment of the disease.*

Keywords: ESBL, UTI, CLED, Uropathogens.

INTRODUCTION

A bacterial infection of any part of the urinary system is referred to as a urinary tract infection (UTI), and is one of the most common bacterial illness seen by clinicians worldwide (Abalkhail *et al.,* 2022).

One of the leading antibacterial resistance mechanisms for many UTI causing gram-negative bacteria is extended-spectrum β- lactamase enzyme possession that hydrolyses the β-lactam

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ring of antibacterial (Belete, 2020). ESBL are plasmid mediated enzymes that confer resistance to penicillin, third generation cephalosporins and aztreonam, but they are reportedly to be inhibited by clavulanic acid (Chinyere *et al.,* 2020).
In this 21st century, antibiotics, particularly those from the β-lactam class, are crucial to the practice of medicine; nearly two thirds of recent hospital prescriptions include these drugs (Bush & Bradford, 2020).

Increased antibiotic use in human and use in animal production are the two major causes of emergence of resistant bacteria in hospitals, human communities, and also animal farms (WHO, 2019).

Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines, making infections harder to treat and increasing the risk of disease spread, severe illness and death (WHO, 2024).

MATERIALS AND METHODS

Study area
The study was conducted at Aminu Kano Teaching Hospital, Kano Nigeria. Aminu Kano Teaching Hospital is a 500-bed hospital, located along Zaria road Kano. It has latitude: 11, 9664322 and longitude: 85487286, it belongs to the government of the federal republic of Nigeria, and run under the supervision of the federal ministry of health.

Study population
The study population were patients attending Aminu Kano teaching hospital, Kano, Nigeria

Inclusion criteria
The study included patients with UTI of all ages attending Aminu Kano teaching hospital.

Exclusion criteria
Non-UTI patients under antibiotic treatment

Sample Size Determination
The sample size was calculated and determined using the formula stated for the calculation of minimum sample size use for human subjects (Cochran, 1977; Naing *et al.,* 2006)

$$N = \frac{Z^2pq}{d^2}$$

Where, N= Sample size, Z= Statistics for a level of 95% confidence interval= 1.96, P= prevalence rate of 15.8%, d = level of significance (allowable error) = 5% or 0.05
q= 1-p, Total sample size n =204

Ethical Approval
Ethical approval to conduct the research was obtained from ethical committee of AKTH (AKTH/MAC/SUB/12A/P-3/VI/1974)

Data Collection
Demographic data was collected using a designed questionnaire, before collecting patients 'samples, patients were given a thorough explanation of the study and their agreement to take part was requested.

Sample Collection
The container was labelled with patient ID and was given to the patient; the patient was directed to wash their hands before the sampling. The patient was instructed on how to collect the urine sample (midstream urine) and how to handle the container.

Sample Processing
Laboratory analysis of the samples including macroscopic examination was conducted, to check out the colour, turbidity and presence of blood (Chinyere *et al.,* 2020). Uncentrfuged urine samples were mixed by rotating the plastic container before inoculating the midstream urine samples using calibrated wire loop (0.001mL) into CLED. After cultures were incubated overnight under aerobic condition at 37^0C for 24hrs; colonies were counted to check the presence of significant growth. Colony counts yielding bacterial growth of $\geq 10^5$ CFU/mL of urine was regarded as significant bacteriuria, but specimens that produced $< 10^5$ CFU/mL was considered insignificant (Belete, 2020).

Identification of bacterial species was done using colony characteristics, gram staining reaction, and biochemical tests following standard procedures. The gram-negative bacteria were identified by indole production, H_2S production in triple sugar iron (TSI), citrate utilization, urease test, motility and carbohydrate utilization tests (Belete, 2020).

Susceptibility Test
The disk diffusion method introduced by Kirby and Bauer (1956), was adopted .
The isolated bacterial colonies were selected, and suspended into the growth media, and standardized through turbidity test. The standardized suspension was then inoculated onto the solidified agar plate, and the antibiotic disk was placed on the inoculated plate. The disc containing the antibiotic was allowed to diffuse through the solidified agar, resulting in the formation of an inhibition zones after the overnight incubation at 37 ^{o}C (Khan, 2019).

Screening for an ESBL Production
Initial screening for ESBL was done by the diameters of zones of inhibition produced by ceftazidime (30µg) and cefotaxime (30µg) found to be within the CLSI screening guidelines. These breakpoints indicative of thought for ESBL production are: for CAZ <22mm, for CTX <21mm. Phenotypic detection of ESBL production was confirmed by double disk synergy test according to CLSI guideline (2024).

Detection of ESBL production by Double –Disk synergy (DDS) Test
Suspected ESBL producers were further confirmed by double disk synergy (DDS) test. Amoxiclav disk was placed in between third generation of cephalosporins (ceftriazone and ceftazidime or cefotaxime) in Muller-Hinton agar plate with a distance of 20 mm apart from centre of the amoxiclav disk. The inoculated plate was incubated at 37^0C for 24hrs. A clear extension of the edge of the inhibition zone of cephalosporin disks towards amoxiclav disk was interpreted as ESBL production of that isolated bacteria (Mitiku *et al.,* 2019).

RESULTS
Out of 204 suspected UTI samples collected from AKTH Kano, 66 (32.35%) growth of the isolates were obtained after considering the macroscopy and the number of colonies on CLED plate. The 66 (32.35%) isolates were identified using Gram's staining technique and Biochemical characterization test 43 (65.2%) isolates were gram- negative bacilli while 23(34.3%) isolates were gram- positive cocci (**table 1**).

Table 1: Gram Staining of Bacterial Isolates

Gram's Reaction	No. of Observed	Percentage of Occurrence
Gram positive cocci	23	34.8
Gram negative bacilli	43	65.2
Total	66	100

Out of 43 gram-negative bacteria isolates. *Escherichia coli* has the highest occurrence 17(39.5%) **(Table 2).** *Citrobacter* was the second most occurrences with value of 8(18.6%) followed by *Klebsiella pneumonia* with value of (16.3%), followed by *Klebsiella oxytoca* with value of 4 (9.3%) and *Enterobacter spp* , Proteus vulgaris, Yersinia all have value of 1 (2.3) (**Table 2**). *Pseudomonas aeruginosa, Acinotobacter baumanni, Proteus vulgaris, Yersinia* all have value of 1 (2.3%) **(Table 2).**

Table 2: Occurrence of Gram-negative Bacteria Based on Biochemical Reaction

Isolates	Frequency	Percentage of occurrence (%)
Escherichia coli	17	39.5
Citrobacter species	8	18.6
Klebsiella pneumoniae	7	16.3
Klebsiella oxytoca	4	9.3
Enterobacter	3	7.0
Pseudomonas aeruginosa	1	2.3
Acinetobacter baumanni	1	2.3
Proteus vulgaris	1	2.3
Yersinia species	1	2.3
Total	**43**	**100**

In this study, both *Escherichia coli and Klebsiella pneumonia* shared several similarities in their antibiotic sensitivity test profile, in the sense that most of the isolate were multidrug and extended drug-resistant bacteria (**Table 3).**

Table 3: Zones of inhibition of antibiotic susceptibility test measured in mm

Isolates	antibacterial agents					
	F(300µg)	CAZ(30µg)	AMC(30µg)	CRO(30µg)	CTX (30µg)	CN(30µg)
E.coli	22	0	0	0	0	20
K. neumoniae	0	8	7	8	0	0
K. oxytoca	8	9	7	9	8	0
Citrobacter	28	10	0	8	9	18
Enterobacter	17	0	0	0	0	16
P. aeruginosa	0	0	0	0	0	71
A. baumanni	19	7	8`	10	12	0
p. vulgaris	7	9	11	8	12	21
Yersinia spp	22	10	0	17	13	25

Key: AMC=Amoxicillin-clavulanate, CAZ=Ceftazidime,CRO=Ceftriazone,CTX=Cefotaxime, CN=Gentamycin, F=Nitrofuratoin

In the screening for ESBL using CLSI breakpoint, nine (9) species of *Escherichia coli* were screened, only 6 (35.3%) were found to be resistant to third generation of cephalosporins (Ceftriaxone, Cefotaxime, Ceftazidime), six (6) species of *Klebsiella pneumonia* were screened and 2 (28.6%) were found to be resistant to third generation of cephalosporins, five (5) species of *Citrobacter* were screened and only one 1(12.5%) were found to be resistant to third generation of cephalosporins and three (3) species of *Enterobacter* was screened and all were susceptible to third generation of cephalosporins (Table 4).

Table 4: Screening for ESBLs production among isolates based CLSI Breakpoint

Isolates	Multi-Drug Resistant Isolates	Number of Suspected ESBL isolates	Percentage (%) of Suspected ESBL isolates
Escherichia coli	9	6	66.7
K lebsiella pneumonia	6	2	22.2
Citrobacter	5	1	11.1
Enterobacter spp	3	0	0
Total	**23**	**9**	**100**

In the confirmatory test done using double disk synergy test, six (6) *Escherichia coli,* two (2) *Klebsiella pneumonia* and one (1) *Citrobacter* spp, that were initially screened to be resistant to third generation of cephalosporins were subjected to confirmatory test using double disk synergy test and they were all positive for ESBL. Their percentage among Enterobacteriaceae are 14.63%, 4.88% and 2.44% respectively **(Table 5).**

Table 5: Confirmation of ESBLs production among Enterobacteriaceae isolates based on DDST Method

Enterobacteriaceae Isolates (n=41)	Number of ESBL Screened Isolates using DDST	Number of positive ESBL Isolates	% of positive ESBL Among Enterobacteriaceae
Escherichia coli	6	6	66.7
K. pneumonia	2	2	22.2
Citrobacter	1	1	11.1
Total	**9**	**9**	**100**

The age group 30-40 has the highest occurrence of gram-negative bacteria causing UTI 14(32.6%) followed by 20-30 ages, 11(25.6%), followed by 10-20 age group, 5(11.6%) and

40-50 age group, 3 (7.0%) **(Table 6).** In the distribution of isolate among sex, females have the highest occurrence of 26 (60.5%) and males have 17 (39.5%). Female also has the highest prevalence of *E. coli* 11(64.7%) while the male has 6 (35.3%). In the distribution of isolates among inpatient and out patients, out patients had the highest occurrence with percentage of 41 (95.35%) while the inpatients had 2 (4.65%) **(Table 6).**

Table 6: Distribution of UTI Infection among Demographic Characteristics

Demographic Data	Frequency	Percentage
Age Group		
0-10	4	9.3
10-20	5	11.6
20-30	11	25.6
30-40	14	32.6
40-50	3	7.0
50-60	3	7.0
70-80	1	2.3
80-90	1	2.3
90-100	1	2.3
Total	43	100
Sex		
Male	17	39.5
Female	26	60.5
Total	43	100
***Escherichia coli* among sexes**		
Male	6	35.3
Female	11	64.7
Total	17	100
Inpatients/outpatients		
Inpatients	2	4.65
Outpatients	41	95.35
Total	43	100

DISCUSSION

Recent evidence indicates a rising trend of antibiotic resistance among common bacterial pathogens in Nigeria (Abubakar and Sulaiman, 2018). Only few hospitals in Nigeria have sustainable antibiotic resistance and infection control units and only few data are available in accessible forms regarding antibiotic resistance in Nigerian hospitals (Babatola *et al.,* 2021). One of the growing antibacterial resistance mechanisms for many UTI causing Gram-negative bacteria is extended-spectrum β-lactamase enzyme possession that hydrolyses the β-lactam ring of antibacterial agent (Belete, 2020).

This study was carried out to study prevalence, antibiotic resistance, the phenotypic detection of resistance enzymes ESBL possessing bacteria and antibiotic susceptibility patter n of the gram-negative isolates at Aminu Kano teaching Hospital Kano. In this study, the predominant bacteria among the bacterial isolates was *Escherichia coli* which account for 17(39.5%) and the occurrence was observed more with female sex, this is because the female urethra is shorter than the male urethra, and it is closer to the anus. This shorter distance allows bacteria, particularly *Escherichia coli* from the gastrointestinal tract (normal floral), to enter the urinary tract more easily in females and cause auto infection. In males, the longer urethra provides a greater distance for bacteria to travel, reducing the risk of infection (Paul, 2024). Pregnant women are more prone to UTIs due to changes in the urinary tract and hormonal fluctuations during pregnancy, which can make them more susceptible to *Escherichia coli* colonization (El-Kashif, 2019). This finding is similar to the study conducted by Ezugwu *et al. (*2021) at Enugu State Nigeria for the Prevalence of urinary tract. In another

research by Faloye *et al.,* (2025) in Akure, Ondo State, Nigeria the UTI prevalence of 31.97% was somewhat similar to this study. UTI prevalence study by Egbebi *et al.* (2025) in Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria revealed prevalence of 22.2% which differ from that of this study.

Infections and associated risk factors among pregnant women in Enugu Metropolis, which showed that prevalence of *Escherichite cali* among women was 40.6%. *Klebsiella* species have 11(25.6%) occurrence and *Citrobacter* species have 8(18,6%) occurrence and they were the second and third most occurrence isolates in this study which accounted for and respectively, This study is in agreement with Apms00B 01"Antimicrobial Susceptibility Pattern of EESBL Producing Uropathogens" conducted at AKTH, North western Nigeria by Usman *et al.* (2023), and Extended Spectrum Beta Lactamase Production and Multi-Drug Resistance among Enterobacteriaceae" conducted at Addis Ababa Ethiopia by Teklu *et al.* (2019), the two referenced study revealed that *Klebsiella* species and *Citrobacter* species were second and third predominant bacteria causing UTI. This study revealed high occurrence of UTI among age group 31-40 followed by 20-30 age group and the most populated sex within that age range were the females, this is because; females within the child bearing age are more prone to UTI infection due to; pregnancy, hormonal changes. Frequent sexual intercourse and use of spermatocyte (Zanaj and Vogli, 2022). This finding is in agreement with a study conducted by Laari, *et al.* (2022) on maternal age and stage of pregnancy as determinants of UTI in pregnancy at Ghana, the study concluded that women at child bearing age was at high risk of urinary tract infection. Most of the gram-negatives (95.335%) were from outpatient, this indicating the community transmission of the bacteria, and this call for the need for proper personal hygiene to control the transmission. This finding was contrary to a research done by Odoki *et al.,* (2019) at Busheny District Uganda on prevalence of bacterial UTI and associated factors among patients attending hospitals in Bushenyi District, Uganda, found the prevalence of inpatient (57%) to be higher than outpatient (43%) respectively.

The prevalence of Extended Spectrum beta-lactamase among Enterobacteriaceae from this study was found to be 21.95% among Enterobacteriaceae; this isa bit higher than some previous researches. ESBL prevalence in a study by Morufat *et al*., (2025) in sub- Saharan africa was found to be 20.76% which was in agreement with this research. The prevalence of ESBL in another research conducted in Abakaliki Ebonyi State, Nigeria by Thaddaeus *et al.,* (2025) was found to be 17.7% which was lower compared to that of this study. On antimicrobial susceptibility pattern of ESBL producing uropathogens conducted at AKTH, North western Nigeria, by Usman *et al.* 2023 who found the prevalence to be 39.5. ESBL-producing gram-negative isolates from urine and wound specimens in a tertiary health facility conducted by Ogefere *et al.,* (2019) at Southern 'Nigeria who found the prevalence in urine to be 41.6%, prevalence Of ESBLs Producing bacteria isolated from UTI Patients attending some selected hospitals in Minna, Nigeria, conducted by Iseghohi *et al.* (2020) who found the prevalence to be 25%.

CONCLUSION

This study showed that the gram-negative uropathogens isolated harboured ESBL enzyme. Based on this result obtained, Gentamicin and nitrofurantoin were considered as appropriate anti bacteria for empirical treatment of UTIs in the study area. Additionally, from the result; the gram-negative isolates showed high resistance to Amoxicillin and third generation of cephalosporins. The findings of this study also revealed that UTIs is predominantly caused by *Escherichia coli, Klebsiella* species and *Citrobacter* species.

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