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Antimicrobial Susceptibility and Determination of ESBL Producing Multidrug Resistance Uropathogenic *E. coli* and *Klebsiella* sp. Isolated in some Selected Hospitals in Kano State.

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Abstract

Background: *The persistent exposure of bacterial strains to a multitude of β-lactams has induced a dynamic and continuous production and mutation of β-lactamases in bacteria, expanding their activity even against the third and fourth generation cephalosporins such as ceftazidime, cefotaxime and cefepime and against aztreonam. The presence of ESBL has been associated with increased mortality, longer duration of hospitalization and increased hospital cost (Hang et al., 2019).*

Aim: *The study was aimed to determine ESBL producing isolates among multidrug resistance uropathogenic isolates of E. coli and Klebsiella sp isolated from the three major hospitals in Kano State, Nigeria. A total of one hundred and thirty-one (131) suspected Klebsiella sp. and E coli isolates were collected from the Microbiology laboratory of all the 3 study sites i.e., Murtala Muhammad Specialist Hospital (MMSH), Muhammad Abdullahi Wase Teaching Hospital (MAWTH) and Aminu Kano teaching hospital (AKTH) all in Kano State, North West Nigeria. Isolates were subjected to Gram staining, morphological and biochemical characterization as well as MicrogenTM Gram negative Identification A system. Antibiotic susceptibility testing was conducted using modified Kirby-Bauer disc- diffusion method.*

Results: *Result showed that all the isolates were susceptible to gentamicin (100%), 75% and 67% susceptible to ciprofloxacin and amoxicillin-clavulanic acid respectively. The resistance pattern of the isolates was observed to be cephalothin (77.5%), cefpodoxime (72.5%), ampicillin (57.5 %), tetracycline (52.5%), sulfamethoxazole-trimethoprim (50%), ceftriaxone (40%), amoxicillin-clavulanic acid (32.5%) and ciprofloxacin (25%). Klebsiella sp. showed higher resistance to cephalexin (90%), ampicillin (90%), cefpodoxime (80%), and then tetracycline (60%) while resistance of E. coli to cephalexin, cefpodoxime, and tetracycline were 73.3%, 70%, and 50% respectively. Out of the 22 MDR isolates, 14 (63.6%) were E. coli and 8 (36.4%) were Klebsiella sp. Eight (8) (20 %) were XDR with no PDR strain detected. Eight (8) of these XDR were E. coli while 6 were Klebsiella sp.*

Conclusion: *The outcome of this study has also demonstrated the palpable presence of ESBL producing bacteria strains from the major hospitals within Kano metropolis.*

Keywords*: Antibiotics, Escherichia coli, Klebsiella, Kano, susceptibility pattern*

Introduction

The emergence and spread of drug-resistant microbes is far more rapid than the introduction of new medicine into clinical practice (Ling, 2015). The introduction of antibiotics around World War II represented a revolution in therapeutic medicine and has saved millions of lives. When penicillin was introduced in the 1940s a "golden age" of antibiotic discovery began and during the next three decades several new classes of antimicrobial agents with different targets of action were introduced to the market (Peirano *et al*., 2012).

Antibiotic resistance is a worldwide problem that can cross international boundaries and spread between continents very easily and speedily. World heath readers have described antibiotics resistant organisms as "nightmare bacteria" that pose a "catastrophic threat" major threat to people across the world. The wrong use of is the single most important factor leading to antibiotic resistance around the world (CDC, 2013). The emergence of antibiotic resistance in the management of UTI is a serious public health issue, particularly in the developing countries where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation (Agbagwa *et al*., 2022). Besides, antibiotics are available over the counter in all over Nigeria which contributes to the misuse and overuse of antibiotics by the common people resulting treatment failure, increased length of hospital stay, increase in term and magnitude of mortality etc and above all the emergence of bacterial resistance knowledge about common uropathogens and their regional susceptibility pattern is crucial to optimize the therapeutic strategy (Mamun *et al*., 2016). The emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms, leading to antimicrobial resistance, continues to threaten our ability to treat common infections. Especially alarming is the rapid global spread of multi- and pan-resistant bacteria (also known as "superbugs") that cause infections that are not treatable with existing antimicrobial medicines such as antibiotics (Cheesbrough, 2012). There is an obvious increase in the prevalence of multi drug resistance (MDR) and ESBL producing *E. coli* isolates from human sources which has been observed throughout the globe (Nyirabahizi *et al*., 2020).

Materials and Methods

Ethical approval

Ethical clearance with the number AKTH/OFH/1232/25 and MOH/OFF/797/T.I/72 were obtained from the AKTH and Kano State Ministry of Health ethical committee for sample collection respectively.

Sampling site

The samples were collected from Murtala Muhammad Specialist Hospital (MMSH), Muhammad Abdullahi Wase Teaching Hospital (MAWTH), and Aminu Kano teaching Hospital (AKTH)

respectively, all in Kano metropolis. Kano State (city) is located northwest of the country between latitude $11^0$58' and $12^0$01' North and longitude $8^0$29' and $8^0$31' East.

Sample collection and sample size

The study was conducted on *Klebsiella* sp. and *E. coli* isolates obtained from uropathogenic patients with urinary tract infection, collected from the microbiology laboratory of each of the above-mentioned hospitals. A total of one hundred and thirty-one (131) suspected *Klebsiella* sp. and *E coli* isolates were collected from the Microbiology laboratory of all the 3 study sites i.e., Murtala Muhammad Specialist Hospital (MMSH), Muhammad Abdullahi Wase Teaching Hospital (MAWTH) and Aminu Kano teaching hospital (AKTH) all in Kano State, North West Nigeria. The sample size is based on the number of available isolates collected and purified within the period of sample collection as the research is not epidemiological research. The isolates were collected within the period of 4 months (May 2016 to November, 2016). The isolates were transported immediately to the laboratory for analysis after collection from the study site.

Characterization of the Isolates

Morphological characteristics such as shape, size, opacity, pigment production and turbidity of the 24 hours bacterial cultures (colony) were observed after purifying the isolates on nutrient agar. The identity of these bacterial isolates was confirmed through Gram staining reaction and conventional biochemical tests (as described by the manual for Microgen GN ID A panel kit, and the software as well), and also on differential media; (Eosin Methylene Blue agar and MacConkey agar).The series of biochemical tests include carbohydrate fermentation test, indole test, methyl red and Voges Proskauer tests, citrate utilization test, hydrogen sulphide production and motility tests (Farasat *et al*., 2012).

Identification of the isolates

Isolates giving atypical responses to any of the above-named tests were examined further using MicrogenTM Gram negative Identification A system. The data obtained by the Microgen GN-ID A micro well strip was designed to generate a 4-digit octal code which was used to interpret the result by the Microgen Identification System Software.

Antibiotic susceptibility testing

Susceptibility of the isolates to some commonly used antibiotics was determined using the modified Kirby-Bauer disc- diffusion method on Mueller Hinton agar as recommended by Clinical Laboratory Institute Standards (CLSI, 2016). The bacterial isolates were grown for 18 to 24 hours on nutrient agar. They were suspended in 2 ml sterile normal saline and turbidity adjusted to match McFarland Opacity Standard No 0.5 (equivalent to 1.5 x 10^8 bacterial density). Bacterial suspensions of 0.1 ml were dispensed on the surface of the Mueller-Hinton agar plate and spread evenly using a sterile spreader. The inoculum was allowed to dry for 5 min and antibiotic discs were dispensed on the surface of the media and incubated aerobically at 37^{o}C for 18 hrs. Results were interpreted as percent susceptible (%S) or resistant (% R), according to the approved clinical breakpoints (CLSI, 2016). Intermediate isolates were counted as susceptible to all the agents. The susceptibility tests were standardized using *E coli* ATCC 25922 obtained from Microbiology

Laboratory of Aminu Kano Teaching Hospital. The following antimicrobial agents (single discs, Oxoid Ltd., Basingstoke, Hamphire, England) were tested; Ampicillin (10µg), Cephalothin (30µg), Cefpodoxime (10µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Trimethoprim/Sulphamethoxazole (25µg), Tetracycline (30µg) Gentamicin (30µg) and amoxicillin-clavulanic acid (25µg) (CLSI, 2016).

Identification of multidrug resistance (MDR), extensively multidrug resistant and pan-drug resistant strains

The number of antibiotic each bacterium was resistant to in the disc diffusion test was noted for identification of multidrug resistant strains. Multidrug resistance (MDR), extensively multidrug resistance and pan-drug resistance isolates were identified according to the guidelines recommended by joint initiative of the European Centre for Disease Prevention and Control (ECDC) and the centers for Disease Control and Prevention (CDC) (Magiorakos *et al.*, 2012). According to the guidelines, MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively multidrug resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pandrug resistant (PDR) was defined as non-susceptibility to all agents in all categories of antimicrobial agents (Magiorakos *et al.*, 2012). MDR was also taken as resistant to four or more antibiotics tested (Ezekiel *et al*., 2011; Apum *et al*., 2018).

Detection of ESBLs Producing Bacteria

The double disc synergy test (DDST) was performed. Discs of cefpodoxime and ceftriaxone were placed at a distance of 20 mm to amoxicillin-clavulanic acid on a Mueller Hinton agar plate earlier inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37 ^{O}C for 18 hrs. Organisms were confirmed ESBLs producers if synergy between cefpodoxime and ceftriaxone and amoxicillin associated with clavulanic acid is detected i.e zones of inhibitions of 5 mm or greater obtained when compared with discs without clavulante (Tande *et al.*, 2009; Tawfik *et al.*, 2012).

Results

Sampling sites and population

Total number of isolates collected from each study site was shown in Table 1, where 43 (44.3%) isolates were collected from Murtala Muhammad Specialist Hospital, (MMSH), 30 (30.9%) isolates collected from Muhammad Abdullahi Wase Teaching hospital, (MAWTH), and 24 (24.7%) isolates were collected from Aminu Kano teaching Hospital (AKTH), Kano, respectively. After sub culturing, 18 (8.25%) of the isolates that yielded no growth on the media were not included in further analysis while 113 (91.75%) isolates yielded different cultural characteristics on the growth medium. The 113 suspected isolates that showed viable growth were subjected to Gram staining were 97 (85.8%) bacterial isolates were confirmed to be the Gram negative while 16 (14.2%) were Gram positive isolates.

Table 1: Total number of Isolates collected from each of the sampling site.

Sampling area	No. of isolates	Purified	Non purified	Gram -	Gram +
MMSH	56	50	08	41	09
AKTH	32	29	4	26	3
MAWSH	43	34	6	30	4
Total	**131**	**113**	**18**	**97**	**19**

Key: MMSH = Murtala Muhammad Specialist Hospital, MAWSH = Muhammad Abdullahi Wase Specialist Hospital.

Identification of the Gram-negative isolates

On subjecting all the 97 confirmed Gram-negative isolates (85.8%) to purification on differential culture media, 69 isolates were identified as members of the Enterobacteriaceae family while 28 were Pseudomonas species and other gram negative non-enterobacteria as shown in Table 2. The isolates were later subjected to biochemical tests for further identification and characterization. The results of the biochemical test confirmed the 30 (30.9%) *E. coli*, 10 (10.3%) *Klebsiella* sp., 29 (29.9%) other Enterobacteriaceae while 28 (28.9%) were non- Enterobacteriaceae. The *E. coli* and *Klebsiella* sp. were further characterized using microgen GNA ID kit. Of the 10 *Klebsiella* sp. obtained, 8 (80%) were *K. oxytoca* and 2 (20%) were confirmed to be *K. pneumoniae.*

Table 2: Distribution of organisms among presumptively identified *E. coli* and *Klebsiella* sp.

S/N	Organisms	Number	Percentage (%)
1	*E. coli*	30	30.9
2	*Klebsiella Species*	10	10.3
3	Other Enterobacteriaceae	29	29.9
4	Non-Enterobacteriaceae	28	28.9
	Total	**97**	**100**

Antibiotic susceptibility pattern of the isolates

The antibiotic susceptibility pattern of the isolates (*Klebsiella* sp. and *E coli*) is presented in Table 3. The result showed that all the isolates were susceptible to gentamicin (100%), 75% and 67% susceptible to ciprofloxacin and amoxicillin-clavulanic acid respectively. The resistance pattern of the isolates was observed to be cephalothin (77.5%), cefpodoxime (72.5%), ampicillin (57.5 %), tetracycline (52.5%), sulfamethoxazole-trimethoprim (50%), ceftriaxone (40%), amoxicillin-clavulanic acid (32.5%) and ciprofloxacin (25%). *Klebsiella* sp. showed higher resistance to cephalexin (90%), ampicillin (90%), cefpodoxime (80%), and then tetracycline (60%) while resistance of *E. coli* to cephalexin, cefpodoxime, and tetracycline were 73.3%, 70%, and 50% respectively. The activity of each of the tested antibiotic was compared between *E. coli* and *Klebsiella* sp in the Table below (Table 4).

Table 3: Antibiotic susceptibility of *E coli* and *Klebsiella* sp isolated from the three hospitals.

Antibiotic class/ Structural group	Abbreviation	Disc content (µg)	Number (%) resistant organisms	Number (%) susceptible organisms
Ampicillin (β-lactam –amino-penicillin)	AMP	10	23(57.5)	17 (42.5)
Cephalothin (β-lactam 1st generation cephalosporin)	KF	30	31(77.5)	9 (22.5)
Cefpodoxime (β-lactam 3rd generation cephalosporin)	CPD	10	29(72.5)	11 (27.5)
Ceftriaxone (β-lactam 3rd generation cephalosporin)	CRO	30	16(40)	24 (60)
Ciprofloxacin (fluroquinolone)	CIP	5	10(25)	30 (75)
Sulfamethoxazole trimethoprim (sulphonamide)	SXT	25	20(50)	20 (50)
Tetracycline (tetracycline)	TE	30	21(52.5)	19 (47.5)
Gentamycin (aminoglycoside)	CN	30	0(0)	40 (100)
Amoxicillin- clavulanic acid (β-lactam β-lactamase inhibitors)	AMC	25	13(32.5)	27 (67.5)

Key: AMP-Ampicillin; KF- Cephalothin; CPD- Cefpodoxime; CRO-Ceftriaxone; CIP-Ciprofloxacin; SXT- Sulfamethoxazole-trimethoprim; TET-Tetracycline; GN-Gentamicin; AMC-Amoxicillin-clavulanic acid.

Table 4: Susceptibility to antibiotics between *E. coli* and *Klebsiella* sp

Antibiotics	Isolate	Resistance	Sensitive
CRO	*E. coli*	12(12)	18(18)
	Klebsiella sp.	4(4)	6(6)
CPD	*E. coli*	21(21.8)	9(8.3)
	Klebsiella sp.	8(7.3)	2(2.8)
AMC	*E. coli*	11 (9.8)	19 (20.3)
	Klebsiella sp.	2 (3.3)	8 (6.8)
SXT	*E. coli*	14(15)	16(15)
	Klebsiella sp.	6(5)	4(5)
KF	*E. coli*	22(23.3)	8(6.8)
	Klebsiella sp.	9(7.8)	1(2.3)
AMP	*E. coli*	14(17.3)	16(12.8)
	Klebsiella sp.	9(5.8)	1(4.3)
CIP	*E. coli*	8(7.5)	22(22.5)

	Klebsiella sp.	2(2.5)	8(7.5)
TET	*E. coli*	15(15.8)	15(14.3)
	Klebsiella sp.	6(5.3)	4(4.8)

Key: AMP-Ampicillin; KF- Cephalothin; CPD- Cefpodoxime; CRO-Ceftriaxone; CIP-Ciprofloxacin; SXT- Sulfamethoxazole-trimethoprim; TE-Tetracycline; GN-Gentamicin; AMC-Amoxicillin-clavulanic acid.

Multidrug drug resistance pattern

The Multidrug drug Resistance Pattern of the isolates is presented in the Table 5. The result showed that 22 isolates out of 40 were multi-drug resistant (MDR) isolates while 18 were non-multidrug isolates (NMDR).

Table 5: Multidrug drug Resistance Pattern of pooled isolates from the three Hospitals in Kano, Nigeria**.**

S/N	Isolates	NART	GART	Resistance pattern	Inference
1	K4	4	4	CPD, KF, AMP, TET	MDR
2	2025E	7	6	CRO, CPD, SXT, KF, AMP, CIP, TET	MDR
3	2084E	6	6	CPD, SXT, KF, AMP, CIP, TET	MDR
4	2123E	8	7	CRO, CPD, AMC, SXT, KF, AMP, CIP, TET	MDR
5	11E	5	4	CRO, CPD, KF, AMP, TET	MDR
6	38E	5	4	SXT, KF, AMC, AMP, TET	MDR
7	3E	3	3	SXT, KF, TET	NMDR
8	2181E	7	6	CRO, CPD, SXT, KF, AMP, CIP, TET	MDR
9	2101E	6	6	CPD, AMC, SXT, KF, AMP, TET	MDR
10	28E	7	6	CRO, CPD, SXT, KF, AMP, CIP, TET	MDR
11	18E	7	6	CRO, CPD, SXT, KF, AMP, CIP, TET	MDR
12	2E	4	4	CPD, SXT, KF, AMP	MDR
13	3K	1	1	CPD	NMDR
14	1630K	5	5	CPD, SXT, KF, AMP, TET	MDR
15	212E	2	2	KF, AMP	NMDR
16	225E	5	5	CPD, AMC, SXT, KF, AMP	MDR
17	143E	2	2	KF, TET	NMDR
18	231E	0	0	-	NDMR
19	KI	2	2	KF, AMP	NMDR
20	K61	6	6	CRO, SXT, KF, AMP, CIP, TET,	MDR
21	K16	5	5	CPD, SXT, KF, AMP, TET	MDR
22	K18	5	4	CRO, CPD, SXT, KF, AMP	MDR
23	K2	4	4	CPD, AMC, KF, AMP	MDR
24	K26	7	6	CRO, CPD, SXT, KF, AMP CIP ,TET	MDR

25	K23	7	6	CRO, CPD, AMC, SXT, KF, AMP, TET	MDR
26	227	4	4	SXT, KF, AMP, TET	MDR
27	34	3	3	CPD, SXT, KF	NMDR
28	33	7	6	CRO, CPD, AMC, SXT, KF, AMP, TET	MDR
29	20	3	3	AMC, KF, TET	NMDR
30	10	3	3	CPD, KF, TET	NMDR
31	14	3	2	CRO, CPD	NMDR
32	13	2	1	CRO, CPD	NMDR
33	24	3	2	CRO, CPD, AMC	NMDR
34	30	4	4	CPD, AMC, KF, CIP	MDR
35	26	1	1	CRO	NMDR
36	2083	3	3	CPD, AMC, KF	NMDR
37	8	2	2	CPD, AMC	NMDR
38	1	2	1	CRO, CPD	NMDR
39	32	3	2	CPD, AMC, KF	NMDR
40	2106	1	1	AMC	NMDR

Key: AMP-Ampicillin; KF- Cephalothin; CPD- Cefpodoxime; CRO-Ceftriaxone; CIP-Ciprofloxacin; SXT- Sulfamethoxazole-trimethoprim; TE-Tetracycline; GN-Gentamicin; AMC-Amoxicillin-clavulanic acid. NART- Number of antibiotic resistant to; CART- Categories of antibiotic resistant to; MDR- Multidrug resistance; NMDR- Non-multidrug resistance.

Multidrug resistance, extended multidrug resistance and pandrug resistance

The distribution of multidrug resistance, extended multidrug resistance and pandrug resistance isolates is presented below in Table 6. Out of the 22 MDR isolates, 14 (63.6%) were *E. coli* and 8 (36.4%) were *Klebsiella* sp. Eight (8) (20 %) were XDR with no PDR strain detected. Eight (8) of these XDR were *E. coli* while 6 were *Klebsiella* sp.

Table 6: Multidrug resistance, extended multidrug resistance and pandrug resistance isolates

Isolate	No. NMDR	No. of MDR	No. of XDR
E. coli	16	8	6
Klebsiella sp.	2	6	2
Total	**18**	**14**	**8**

Key: MDR = Multidrug resistance, **NMDR =** Non-multidrug resistance, **XDR =** Extended drug resistance

Table 7: Distribution of MDR isolates across the three Hospital

S/N	Hospital	MDR isolates (%)
1	MMSH	12 (54.6)
2	AKTH	5 (22.7)
3	MAWSH	5 (22.7)
	TOTAL	**22 (100)**

ESBL Producing isolates Among the Multidrug resistant isolates

The multidrug resistant isolates (22) were further subjected to Extended Spectrum Beta Lactamase detection using Clinical Laboratory Standard Institute (CLSI) break point for screening test, where only 15 (68.2 %) were found to be ESBL positive and 7 (31.8 %) were non ESBL producing Gram negative enterobacteriaceae. After the confirmatory test for the ESBL detection, only 9 isolates (40.9%) out of the 22 isolates screened were positively confirmed to be ESBL producers based on double disc synergy test (DDST) (Table 8).

Table 8: Distribution of extended spectrum Beta lactamase producers among the MDR *Klebsiella* sp. and *E. coli* isolates

Isolates	No. of screened MDR	No. Positive	No of confirmed ESBL (%)
E. coli	14	9	5(55.6)
Klebsiella sp.	8	6	4(44.40)
Total	**22**	**15**	**9(100)**

Of the 9 positive ESBL producers, 4 (44.4%) were *Klebsiella* sp. while 5 (55.6%) were *E. coli* isolates (Table 4.16). Out of the 9 confirmed MDR ESBL producers, 4 (44.4%) were obtained from MMSH, 2 (22.2%) from AKTH and 3 (33.3%) were from MAWSH. (Fig 1)

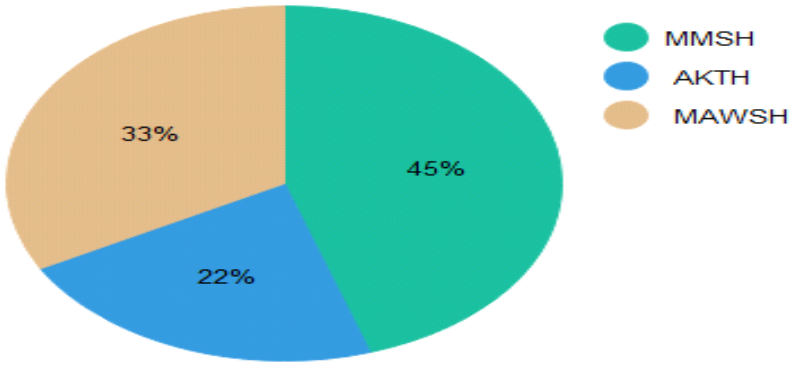

Figure 1: Distribution of multi drug MDR ESBL isolates across the three hospitals

Discussion

The emergence of antibiotic resistance in the management of UTI is a serious public health issue, particularly in the developing countries where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation (Agbagwa *et al*., 2022). The study was aimed to determine antibiotic susceptibility pattern and multidrug resistance uropathogenic isolates of *E. coli* and *Klebsiella* sp against the commonly prescribed antibiotics. From the study, out of the 131 isolates collected from all the three (3) study sites, Murtala Muhammad specialized hospital has the highest number of isolates collected with 56 (42.8%) followed by Muhammad Abdullahi Wase specialized hospital with 32 (24.4%). Aminu Kano teaching Hospital has the least number with 43 (32.8%). Out of the 131 total isolates collected from all the study sites, 18 (13.7%) isolates collected were either having no growth on subculture or shows growth on culture media with various cultural characteristics. Despite the fact that all the isolates are said to be Gram negative bacteria during collection, it was observed that only 97 (85.8%) bacterial isolates were confirmed to be Gram negative bacteria while 16 (14.2 %) were Gram positive isolates. This indeed is a problem of concern as erroneous laboratory result might let lead to false prescription, treatment failure, antibiotics resistance and subsequently high morbidity or even mortality.

The most common uropathogens in our study were *E. coli* (30.9 %) and *Klebsiella* sp. (10.3%). It supports the previous findings indicating that *E. coli* is the principal etiological agent of UTI, accounting for 60.02% of UTI. In another study, it was reported that predominant uropathogens are *E. coli* followed by *Klebsiella* sp. which also support our study (Shrestha *et al*., 2020). Our finding also correlates with the finding of Yusuf *et al* 2016 where most common Enterobacteriaceae isolated in AKTH Kano were *E. coli* followed by *Klebsiella* sp. It also correlates with other studies like Agyepong *et al.* (2018) (24.5%), Basak *et al.* (2016) (35%), Folgori *et al.* (2016) (67.6%), where most common gram-negative bacteria isolated were *E. coli.*

The biochemical identification of *E. coli* and *Klebsiella* sp. using Microgen Identification kit showed that 30.9 % and 10.3% of the isolates were *E. coli* and *Klebsiella* sp. respectively while 29.9 % where other Enterobacteriaceae, 28.9 % were non Enterobacteriaceae. The antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Agbagwa *et al*., 2022). According to this study, best activity was found in gentamicin (Aminoglycoside) and ciprofloxacin (Fluoroquinolone) with 100% and 75% susceptible to the pooled isolates respectively. The Aminoglycosides are bactericidal agents that inhibit bacterial protein synthesis and have been found to be particularly potent against the *Enterobacteriaceae* among other aerobic Gram-negative rods (Ramadan *et al*., 2020). Amador *et al.* (2018) obtained 93.5% susceptibility of *E. coli* isolates to aminoglycoside. In this study, none of the *Klebsiella* sp. and *E. coli* isolates had resistance to gentamicin. Gentamicin was found to be reasonably high efficacious agent among all antimicrobials used to almost all the *Klebsiella* sp. and *E. coli* in the current setting, similar results were also reported from other studies (Kothari and Sagar, 2008; Soholy *et al.*, 2009).

Multi drug resistant (MDR) Gram negative bacilli induced infections have been reported with an increasing frequency in tertiary health care providers in Nigeria and they have been found to be

associated with a significant morbidity and mortality (Yusuf *et al*., 2012). Multidrug resistance (MDR) was taken as resistance to four or more antibiotics tested (Ezekiel *et al*., 2011). ESBLs production is increasingly an important cause of transferable multidrug resistance in Gram-negative bacteria throughout the World (Ramadan *et al*., 2020). In this study, the prevalence of MDR, XDR and PDR strains in our study are 55 %, 20 % and 0 % respectively. Study by Basak *et al*. (2016) showed 33.5% MDR strains, 12.1% XDR and no PDR strains which is lower than our findings. Olivera *et al.* (2019) observed MDR prevalence to be 36%. Bhatt *et al*. (2013) in their study found out prevalence of XDR and PDR strains as 8.1 % and 0.9 % respectively. Adrizain *et al.* (2018) showed MDR and XDR prevalence as 28.7% and 4.7% among pediatrics patients from blood culture pathogens. In the study done by Agyepong *et al.* (2018) in Ghana, prevalence of MDR strains was 89.5% which is higher than the value obtained in our study. In the study of Bajpai *et al.* (2014), percentage of MDR strains (75.8%) among uropathogens are higher than our observations for MDR and PDR strains whereas percentage of XDR strains (12%) is lower than our findings. The percentage distribution of MDR isolates in MMSH, AKTH and MAWSH in Kano were 54%, 23% and 23% respectively. The distribution is higher in MMSH than AKTH and MAWSH. This could be due to the fact that MMSH is the largest hospital in term of patient's population in Kano and majority of the patients are poor and illiterate as such they are more likely involve in self-medication and hardly visit hospital at the early unset of infections which could lead to transmission of resistant isolates between people. The association of MDR and non-MDR strains in the three hospitals was found to be not statistically significant ($P<0.05$). The higher prevalence in our study could be due to the fact that the three hospitals are operating as teaching Hospitals where most of the patients are referred ones having prior exposure of antibiotics which could be a predictor factor for development of multi drug resistance.

The results of ESBL production suggest the presence of ESBL in 40.9 % of the 22 multidrug resistance *E. coli* and *Klebsiella* sp. that were resistant to at least four (4) or more groups of antibiotics. The high prevalence of ESBL among the MDR isolates in the three major hospitals in Kano has negative health implication. The prevalence of ESBL is high when compared with the findings of Garba *et al*. (2012) who first reported 9.3%. From then, higher reports of these enzymes among clinical isolates in Kano have been made even among the immune- compromised patients such as tuberculosis and cancer patients (Eyitayo *et al.,* 2009). In 2016, prevalence of ESBL in Kano was reported to be 14.4% according to Yusuf *et al.*, (2016). The high prevalence of ESBL among *E coli* and *Klebsiella* sp. in this study is of clinical importance as these species are among the most frequent Gram-negative bacteria involved in hospital acquired infections and other nosocomial outbreaks in Kano, Nigeria.

Conclusion

Our study had given overview of the common uropathogens in Hospital settings in Kano State, North west Nigeria. *E. coli* and *Klebsiella* sp. which were the major Pathogens in the study hospitals are largely resistant to cephalothin, cefpodoxime, ampicillin and tetracycline but highly susceptible to gentamycin, ciprofloxacin, amoxicillin clavulanic acid. Most useful antibiotic was gentamicin effective against *Klebsiella* sp. and *E. coli* can be considered as the alternative option in the empirical treatment of UTI. Moreover, high level of MDR and emergence of XDR strain were observed with no pandrug resistance isolates. Hence it is our responsibility to prevent the

development of MDR, XDR and potential PDR organisms, which will be impossible to handle with the low availability of new and broader spectrum antibiotics in the development pipeline. Phenotypic evaluation for the presence of ESBL using double disc diffusion method of the isolates showed 40.9% of the MDR isolates expressed ESBL phenotypically

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Conflict of Interest
The authors declare no conflict of interest exist

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