

Antibiotic resistant Uropathogenic *Klebsiella* spp. and *E coli* isolates and their Multidrug resistance pattern in three major Hospitals in Kano State

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Abstract

Many bacterial species have been reported to develop resistance to antibiotics commonly prescribed for urinary tract infections. Therefore, the need to search for natural alternative for remedy of this problem cannot be overemphasized. One hundred and thirty-one (131) suspected *E. coli* and *Klebsiella* sp. were collected from patients with suspected urinary tract infection from the three major hospitals in Kano State. Purification and identification of the bacterial isolates were carried out using standard microbiological methods. Basic biochemical tests and MicroGen identification kit were used for identification of the isolates. The antibiotic susceptibilities of the *E. coli* and *Klebsiella* sp. were determined using the modified Kirby-Bauer diffusion method and the multidrug resistant isolates, extended drug resistance as well as pandrug resistance were determined using standard microbiological methods. Multiple antibiotic resistance (MAR) index is a measure of the extent of antimicrobial agent resistance for the isolates in the group studied. It was calculated as a/b , where a represents the number of antibiotics to which the isolates were resistant and b represents the total number of antibiotics to which the isolate was exposed. Out of the 131 isolates collected from the three major hospitals, 42.8%, 24.4% and 32.8% were obtained from Murtala Muhammad specialist hospital, Aminu teaching hospital and Muhammad Abdullahi Wase teaching hospital respectively. The biochemical tests showed that 30 (30.9 %) *E. coli*, 10 (10.3) % *Klebsiella* sp, 29 (29 %), other Enterobacteriaceae, 28 (28.9) % non-Enterobacteriaceae. Of the 10 *Klebsiella* sp, 8 (80 %) were *K. oxytoca* while 2 (20 %) were *K. pneumoniae*. All the *Klebsiella* sp. and *E. coli* isolates ($n=40$) were susceptible to gentamicin (100%), 75 % susceptible to ciprofloxacin and 67% to amoxicillin-clavulanic acid. The proportion of the isolates resistant to cephalothin was 77.5%, 72.5% to ceftiofloxime, 57.5% to ampicillin, and 52.5 % to tetracycline. A total of 23 (57.5 %) of the isolates were multidrug resistant while 8 (20 %) of the multidrug isolates were extended drug resistant (XDR) with none being PDR. The isolates showed a high (77.5%) pre-exposure to antibiotics tested in this study at $MARI \geq 0.3$. The *Klebsiella* spp. showed high (80%) pre-exposure to the antibiotics tested in this study than *E. coli* (76.7%) at $MARI \geq 0.3$. MAR index varied considerably, the lowest index was 0.1 and the highest MAR index was 1.0. Which suggests that the isolates originated from environment where antibiotics are often used.

Keywords: Multiple antibiotic index, *E. coli*, *Klebsiella* sp, UTI.

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections in humans (Bischoff *et al.*, 2018). It is estimated that 40 % of women and 12 % of men experience a minimum one symptomatic UTI episode during their lifetimes, and 27 to 48 % of the affected women suffer from recurrent UTIs (Micali *et al.*, 2014). UTIs comprise about 40 % of all hospital-acquired infections and 50% of bacterial infections that contribute to increased morbidity causing prolonged hospitalization (Asadi Karam *et al.*, 2019). Complicated UTIs are also associated with immunosuppression and previous antibiotic exposure. This category of UTIs increases the risk of chronic and/or recurrent infections. Uncomplicated UTIs are found in patients who have no anatomical urinary tract abnormalities and do not use the urinary tract instrumentation. The increasing number of MDR isolates from UTIs of outpatients makes treatment more difficult. Risk factors of MDR isolated from UTIs include prior use of anti-microbials, hospitalization, genitourinary disturbances, age, and recurrent UTIs (Walker *et al.*, 2016).

Although *Escherichia coli* normally inhabits the intestines, certain strains of *E coli* can cause intestinal infections that produce bloody, watery, or inflammatory diarrhea (traveler's diarrhea). In children, diarrhea caused by certain strains of *E coli* O157:H7 may lead to destruction of red blood cells and kidney failure (hemolytic-uremic syndrome). *E coli* can also cause urinary tract infections (particularly in women) and bacteremia and meningitis in newborns (Flament-Simon *et al.*, 2020). Members of genera belonging to the *Enterobacteriaceae* family have earned a reputation placing them among the most pathogenic and most often encountered organisms in clinical microbiology. The incidence of community-acquired pneumonia attributed to *K. pneumonia* has decreased over the years, however its role as a common healthcare-associated pathogen causing infections of the urinary tract, bloodstream, pneumonia and intra-abdominal infections has become exceedingly common (Marr and Russo, 2019). *Klebsiella pneumoniae* also causes bacteremia, and a substantial percentage of nosocomial infections, pneumonia, urinary tract infections, septicemia and soft tissue infections (Rodrigues *et al.*, 2018).

Antimicrobial resistance (AMR) is a global public health concern, with the World Health Organization designating it a leading health threat (WHO, 2021). The 2022 GRAM Global Burden Report (2022), calculated bacterial AMR to be associated with 4.95 million deaths in 2019, including 1.27 million attributable deaths, placing AMR as a leading cause of mortality globally. Antibiotic resistance (AR) continues to pose a great threat to public health in both developed and developing countries (WHO, 2021). Infections caused by AR bacteria, especially multi-drug resistant organisms, can lead to serious health problems such as prolonged hospitalization, treatment failure and deaths (WHO, 2021).

Methodology

Collection of bacterial isolates

The *Klebsiella* sp. and *E coli* isolates from patients with Urinary tract infections were obtained from the Microbiology Laboratories of each of the three major hospital in Kano metropolis which

are Murtala Muhammad Specialist Hospital (MMSH), Aminu Kano Teaching Hospital (AKTH) and Muhammad Abdullahi Wase Specialists Hospital (MAWSH).

Sample size

The samples were selected using convenience sampling method and only samples which were said to be confirmed *Klebsiella* sp. and *E. coli* isolates were collected within time frame of isolates collection. The isolates were collected within the period of 4 months.

Purification of the isolates

The *Klebsiella* spp. and *E. coli* isolates were purified using standard microbiological method as described by Cheesbrough (2012). Colonies which appear pinkish-red on MacConkey agar plates were considered lactose fermenters (Chapin and Lauderable, 2007). The colonies were further streaked on sterile EMB plate and incubated at 37 °C for 24 hours. Colonies with greenish-black appearance with metallic sheen on EMB Agar and those with dark purple appearance with no sheen were considered as presumptive *E. coli* and *Klebsiella* sp. respectively. The isolates were purified by repeated streaking on plates until pure culture is obtained before storing on nutrient agar slants at 4 °C as working and stock cultures (Ogunshe *et al.*, 2006).

Characterization of the Isolates

The presumptive positive isolates collected from the laboratory of the hospitals that showed positive results to *E. coli* and *Klebsiella* sp. on media were further subjected to a series of biochemical tests, which include carbohydrate fermentation test, indole test, methyl red and Voges Proskaur 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 Microgen™ 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.

Inoculum Preparation

The preserved isolates were sub – cultured on prepared nutrient agar (biotech, England) plates and incubated for 24 hours at 37 °C so as to obtain confluent growth for sensitivity test. A loopful of the isolates from Nutrient agar was dispensed in a sterile normal saline to match the 0.5 McFarland standards for susceptibility tests as described by CLSI (2022).

Standardization of the Inoculum.

Using inoculum loop, enough material from an over-night culture of the test organism was transferred in to a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described in Clinical Laboratory Standard Institute (CLSI, 2022).

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, 2022). The bacterial isolates were grown for 18 to 24 hrs 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 1.5×10^8 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 °C for 18 hrs. The diameter of zone of inhibition was measured using metre rule in mm and the zones were compared with Standard antibiotics interpretative chart according to the approved clinical breakpoints (CLSI, 2022).

Calculation of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR) index is a measure of the extent of antimicrobial agent resistance for the isolates in the group studied.

It was calculated as a/b , where a represents the number of antibiotics to which the isolates were resistant and b represents the total number of antibiotics to which the isolate was exposed (Apun *et al.*, 2018).

RESULT

After sub culturing on nutrient agar, 18 (13.7%) of the isolates that yielded no growth on the media were not included in further analysis while 113 (86.3%) isolates that yielded different cultural characteristics on the growth medium were used for further analysis (Table 1).

Table 1: Frequency of Purified isolates collected from the hospitals

Isolate	Frequency	Percentage (%)
NG	18	13.7
Valid Growth	113	86.3
Total	131	100

KEY: NG- No Growth

One hundred and thirteen (113) (86.3 %) of the 131 isolates were able to grow on sub culturing on the media.

Despite the fact that all the isolates are said to be Gram negative bacteria during collection, it was observed that 97 (85.8%) bacterial isolates were confirmed to be the Gram negative while 16 (14.2%) were Gram positive isolates (Table 2).

Table 2: Frequency distribution of the Gram-negative bacteria from the study sites

Bacteria	MMSH	AKTH	MAWSH	Frequency n (%)
Gram negative	41	26	30	97 (85.8)
Gram positive	9	3	4	16 (14.2)
Total	50	29	34	113 (100)

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 3.

Table 3: Distribution of organisms among presumptively identified *E. coli* and *Klebsiella* sp.

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

The Antimicrobial Susceptibility Patterns of the study isolates

The antibiotic susceptibility pattern of the isolates (*Klebsiella* spp. and *E coli*) was determined using commonly prescribed antibiotics. All the isolates were 100%, 75% and 67% susceptible to gentamicin, 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%), sulphamethoxazole-trimetoprim (50%), ceftriaxone (40%), amoxicillin-clavulanic acid (32.5%) and ciprofloxacin (25%) as shown in Table 4.

Table 4: Antibiotic Susceptibility of the *Klebsiella* spp. And *E coli* isolates from the Three Hospitals (n= 40)

Antibiotic class	Antibiotic class	Disc content (µg)	Number (%) resistant organisms
Ampicillin	Penicillin	10	23(57.5)
Cephalothin	Cephalosporin	30	31(77.5)
Cefpodoxime	Cephalosporin	10	29(72.5)
Ceftriaxone	Cephalosporin	30	16(40)
Ciprofloxacin	Flouoroquinone	5	10(25)
Sulphamethoxazole trimetoprim	Sulphonamide	25	20(50)
Tetracycline	Tetracyclines	30	21(52.5)
Gentamicin	Aminoglycoside	30	0(0)
Amoxicillin- clavulanic acid (β-lactam β-lactamase inhibitors)	Penicillin	25	13(32.5)

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

The isolates showed a high (77.5%) pre-exposure to antibiotics tested in this study at MARI \geq 0.3. The *Klebsiella* sp. showed high (80%) pre-exposure to the antibiotics tested in this study than *E. coli* (76.7%) at MARI \geq 0.3 (Table 5). MAR index varied considerably, the lowest index was 0.1 and the highest MAR index was 1.0.

Table 5: Multiple Antibiotic Resistance Index (MARI) of *E. coli* and *Klebsiella* sp. from the three Hospitals in Kano, Nigeria

MARI	NUMBER OF ISOLATES (%)		
	ALL ISOLATES (n=40)	<i>E. coli</i> (n=30)	<i>Klebsiella</i> sp. (n=10)
0.1	3(7.5)	2(6.7)	1(10)
0.2	6(15)	5(16.7)	1(10)
0.3	8(20)	8(26.7)	0(0)
0.4	6(15)	4(13.3)	2(20)
0.5	0(0)	0(0)	0(0)
0.6	5(12.5)	2(6.7)	3(30)
0.7	3(7.5)	2(6.7)	1(10)
0.8	7(17.5)	5(16.7)	2(20)
0.9	1(2.5)	1(3.3)	0(0)
1.0	1(2.5)	1(3.3)	0(0)

The majority of the isolates belongs to a MAR index of 0.3 followed by 0.8. Similar result was found in *E. coli* but in *Klebsiella* sp, majority belong to MAR index of 0.6 (Fig 1)

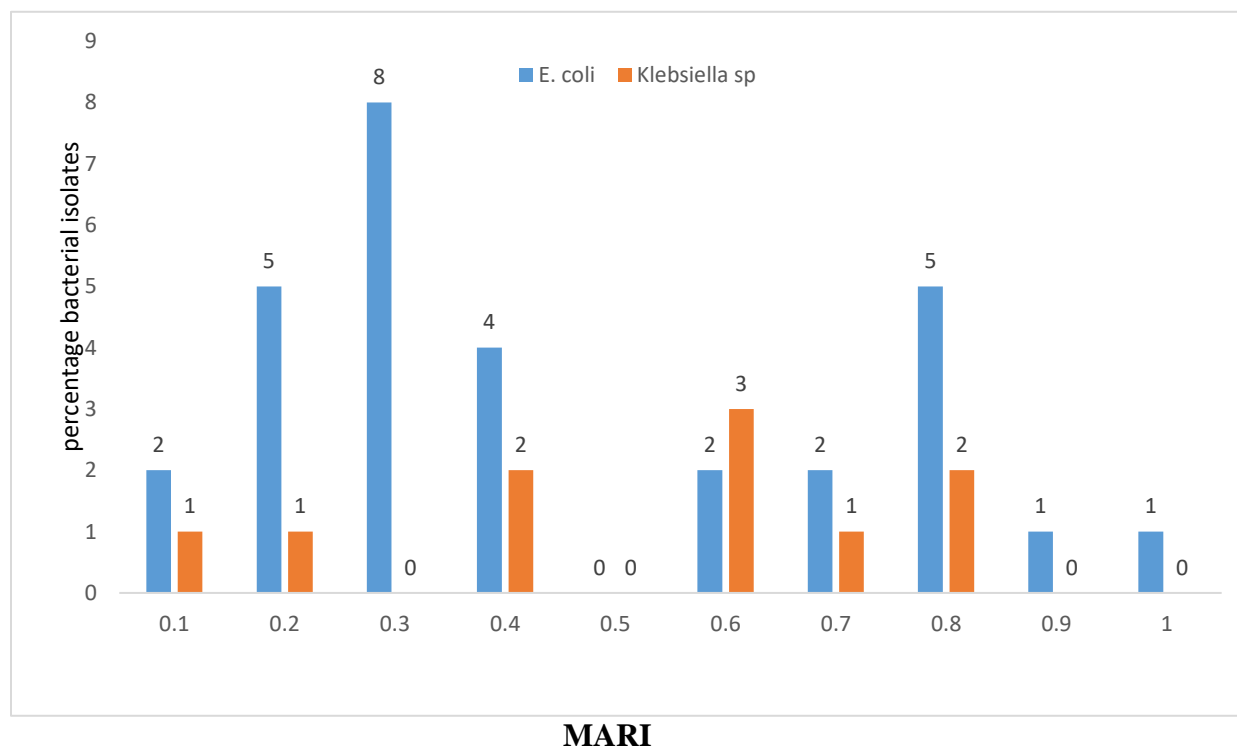


Figure 1: Multiple antibiotic resistance (MAR) index of *E. coli* and *Klebsiella* sp.

Discussion

Out of the 131 total isolates collected from all the study sites, 113 (86.3 %) isolates collected showed growth on sub cultured 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 lead to false prescription, treatment failure, antibiotics resistance and subsequently high morbidity or even mortality. This is because large number of patients with several infections mostly visits the hospital as its services are not expensive and also many referrals from other hospitals within and outside Kano are being made there. Even though, all the hospitals are owned by government, only MMSH rendered some services free or at cheaper rate than the others and is also easily accessible because of its location. The high percentage of false presumptive bacterial isolates observed in this study might be associated to lack of laboratory facilities which makes the laboratory diagnosis of infectious diseases difficult in many parts of the African continent and hence influencing the development of clinical algorithms that has induced a collapse management in clinical settings (Bagheri *et al.*, 2011). Hence the need for capacity building, enhancing universal access to rapid and accurate laboratory diagnostic kits is necessary to control false clinical report. The percentage of ciprofloxacin resistance observed in this study was 25%, the same range of resistance of *E. coli* and *Klebsiella* sp. to ciprofloxacin in the study was also observed to be 26.7% and 20% respectively. High prevalence of ciprofloxacin resistance (84%) has been reported in *E. coli* in Chinese (Yang *et al.*, 2018). Increase in an irrational consumption rate, transmission of resistant isolates between people, self-medication and noncompliance with medication and sales of substandard drug have been reported of ciprofloxacin. Twenty-one (52.5%) of the isolates were resistant to tetracycline while 20 (50 %) were resistant to the action of co-trimoxazole. Tetracycline resistance is already emerging in clinical isolates in many communities (Agbagwa *et al.*, 2022). Tetracycline belong to a family of broad-spectrum antibiotics that include tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline, and a number of other semisynthetic derivatives. These antibiotics inhibit protein synthesis in Gram-positive and Gram-negative bacteria. Since their introduction in late 1950s, they have been widely used in clinical and veterinary medicine, as well as for prophylaxis and growth promotion in food animals. In our study, 50 % *E. coli* and 60% *Klebsiella* sp. isolates, were resistant to tetracycline, resistance of this class of antibiotics is widespread because of the possible misuse and overuse of these drugs, limiting their utility in treating infections (Agbagwa *et al.*, 2022). Ampicillin resistance was 57.5% with 23 resistant organisms as shown in this study. This shows that almost all the organisms that are resistant to ampicillin are equally resistant to cephalothin while the organisms that were resistant to just one antibiotic were resistant to either ampicillin or cephalothin. Ampicillin resistance is increasingly common and at an alarming rate. Ampicillin is a commonly used broad-spectrum aminopenicillin which inhibits the final stage of bacterial cell wall synthesis and ultimately leads to cell lysis. Its usefulness is however, limited by its susceptibility to β -lactamase hydrolysis produced by the organism (Jain *et al.*, 2008).

Multiple antibiotic resistance (MAR) index is a measure of the extent of antimicrobial agent resistance for the isolates in the group studied (Apun *et al.*, 2018). It gives an indirect suggestion of the probable source of the organism (Olayinka *et al.*, 2004). MAR index values greater than 0.2 indicate that the isolates were recovered from samples originating from high-risk sources (Effah *et al.*, 2020). Most probably, there are no strict rules concerning antibiotic prescriptions and usage in such areas. In this study, high percentage (77.5%) of MARI at ≥ 0.3 was observed which generally suggests that the isolates originated from environment where antibiotics are often used (Christopher *et al.*, 2013), though the *Klebsiella* sp. showed a high (80%) pre-exposure to the antibiotics tested in this study than those *E. coli* isolates (76.7%) at MARI ≥ 0.3 . This was also expressed in the high percentage of MDR (77.5%) ([*Klebsiella* sp. (80%) and *E. coli* (76.7%)] expressed by the isolates in this study. The MAR index also revealed that majority of *E. coli* isolates belong to the group with MAR index above 0.5. This might be an indication that large proportion of the bacterial isolates have been pre-exposed to several antibiotics. A combination of microbial characteristics such as selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug resistant organisms might be the cause of this high resistance (Bryce *et al.*, 2016). Increase in irrational consumption of antibiotics and transmission of resistant isolates between people could also lead to this factor (Obaidat *et al.*, 2018).

Conclusion

Our study had given overview of the common uropathogens in Hospital settings in Kano State, North west Nigeria. The *E. coli* and *Klebsiella* sp. isolates which were the major Pathogens in the studied hospitals were largely resistant to cephalothin, cefpodoxime, ampicillin and tetracycline but highly susceptible to gentamycin, ciprofloxacin, amoxicillin clavulanic acid. A high proportion of *Klebsiella* sp. and *E. coli* isolates which were MDR were ESBL producers. The MARI data revealed that isolates with lowest and highest MAR index are present in our environment which is a major health risk. The high MARI indicate that the isolates originate from area where antibiotic is abused.

Recommendation

Periodic reassessment of *in vitro* susceptibility pattern of urinary pathogens to serve as a guide for antibiotic therapy since these organisms exhibit resistance to first-line drugs used for UTI infection

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CONFLICT OF INTEREST

The authors declare no conflict of interest exist

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