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ORIGINAL ARTICLE

Detection of blaNDM-1 gene in *Escherichia coli* and *Klebsiella pneumoniae* Producing Extended Spectrum β-lactamase (ESBL) Isolated from Urine Samples

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ABSTRACT

Background: *The most bacterial species showing resistance to almost all commonly used antibiotics are members of the Enterobacteriaceae family, including Klebsiella pneumoniae and Escherichia coli. One of the few treatment of emerging MDR bacteria is carbapenem.* ***Objective:*** *We aimed to find out the occurrence the carbapenem-resistant blaNDM-1 gene in ESBL-producing E. coli and K. pneumoniae isolated from urinary tract infections.* ***Methodology:*** *We tested four hundreds and sixty patients with UTIs at the Urology department of Beni-Suef university Hospital. The urine samples are subjected for bacteriological culture and identification of isolated oganisms. Isolates were tested for ESBL and MBL (Metallo-β lactamase) producing Escherichia coli and Klebsiella pneumoniae. We used polymerase chain reaction (PCR) to check whether the bla-NDM-1 gene was present in the MBL generating colonies.* ***Results:*** *E. coli was detected in 155 (33.7%) urine samples and K. pneumoniae in 92 (20%). E. coli had a prevalence rate of 38.1% for ESBL and K. pneumoniae had a rate of 39.1%. Additionally, 53.33 percent of the E. coli and 76.7 percent of the K. pneumoniae showed phenotypic positivity for MBL production. The percentage of E. coli and K. pneumoniae that tested positive for BlaNDM-1 was 71.8 and 75.0 percent, respectively, according to PCR results.* ***Conclusion:*** *Findings indicate that the blaNDM-1 gene is more common than previously thought. Hospitals and communities must immediately implement screening tests to determine the prevalence of blaNDM-1 dissemination.*

INTRODUCTION

In most cases, bacteria are to blame when it comes to UTIs. In cases of UTIs, gram-positive bacteria make up around 15-20%, whereas gram-negative bacteria account for 80-85% [1]. The most common kind of bacteria that may cause UTIs in the population is uropathogenic *E. coli*. *Staphylococcus, Klebsiella, Enterobacter, Pseudomonas,* and *Enterococcus* are also frequently isolated [2].

The majority of doctors prescribe beta-lactam medications to patients who have had an illness due to *E. coli* or *Klebsiella pneumonia* [3]. However, ESBL-producing bacteria have recently emerged as a major public health issue [4].

Infections caused by Gram-negative bacteria that have developed resistance to many drugs have left carbapenems as a last resort[5]. Nevertheless, carbapenem-resistant bacteria have been on the increase globally, thanks to individuals self-medicating or overusing without a proper diagnosis [6].

Among the most potent and widely distributed metallo-beta lactamase (MBL) enzymes *in E. coli, K. pneumoniae*, and other Gram-negative bacilli is the New Delhi Metallo-β-lactamase (NDM-1) [7].

In 2009, the blaNDM-1 gene was first detected in a Swedish patient who had contracted a UTI in India from carbapenem-resistant *Klebsiella pneumoniae* [8]. After first locating the gene in a 180 kb plasmid [9], researchers discovered that it was present in plasmids ranging from 50 to 500 kb in a number of Gram-negative bacteria [10].

METHODOLOGY

Study design:

The present study is a cross-sectional study that was carried out at Beni-Suef University Hospital between August 2022 and April 2023 and included a total of 460 patients suffering from urinary tract infections attending the Outpatient and Inpatient of Urology Departments.

Demographic characteristics and clinical features of the studied cases:

Thorough history taking regarding socio-demographic characteristics, history of risk factors associated with UTI, and clinical features related to UTI.

Collection of urine samples:

Mid-stream urine specimens were collected aseptically into a sterile container. Urine samples were centrifuged, and the sediment was examined microscopically as a wet preparation to detect significant pyuria (WBC count exceeds 10/HPF).

Urine Culture:

Urine samples that showed significant pyuria were cultured on CLED agar. If a culture did not show any growth after another 24 hours, it was considered negative.

Identification of bacterial isolates:

E. coli and *K. pneumoniae* were identified by their colonial morphology on the utilized medium, Gram staining, and biochemical profile (Oxoid, UK).

Antibiotic susceptibility testing:

We used the traditional Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) to determine the target isolates' antibiograms, and we interpreted the results according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [11].

The antibiotics used were: ampicillin (AMP = 30 µg), cefixime (CFM = 5 µg), imipenem (IPM = 10 µg), ciprofloxacin (CIP = 5 µg), amikacin (AMK = 30 µg), nalidixic acid (NAL = 30 µg), amoxicillin (AMX = 10 µg), gentamicin (GN = 30 µg), doripenem (DOR = 30 µg), levofloxacin (LEV = 5 µg), nitrofurantoin (NIT = 10 µg), ceftazidime (CAZ = 30 µg) and cefotaxime (CTX = 30 µg).

The isolates were classified, according to their resistance pattern, into multidrug-resistant (MDR), extreme-drug resistant (XDR), and pan-drug resistant (PDR). MDR bacteria are resistant to three or more classes of antibiotics, while XDR bacteria are resistant to multiple classes but still susceptible to agents in one or two classes, while PDR bacteria are non-susceptible to all agents in all antimicrobial classes [12].

ESBL screening test:

The ESBL screening test followed the guidelines set by the CLSI, 2023[11] by using the standard disc diffusion method using ceftazidime (30 µg) and cefotaxime (30 µg) discs from Oxoid, UK. Two antibiotic discs were placed 20 mm apart, and then the mixture was incubated at 35±2°C for 16-18 hours. Isolates were considered likely to develop ESBL if their inhibition zone widths were less than 22 mm for ceftazidime (30 µg) and less than or equal to 27 mm for cefotaxime (30 µg). Combination disk test (CDT) was used to further confirm the presence of ESBLs, following the guidelines set by CLSI[11].

Phenotypic Verification of ESBL Producers:

ESBL detection was performed using the combination disc technique on MHA medium, in accordance with CLSI recommendations[11]. Each isolate was cultured, with two antibiotics (cefotaxime 30 µg and ceftazidime 30 µg) administered individually on one side of the plate and in conjunction with clavulanic acid 10 µg on the other side. An increase of > 5 mm in the diameter of the zone of inhibition on the side of mixed discs, compared to findings without clavulanic acid, was deemed indicative of ESBL positivity.

Detection of metallo-β-Lactamases:

A mixed disk diffusion technique was done on MHA medium to identify MBLs. The indicator was 0.5 µg EDTA and the disk was 10 µg meropenem. In order to detect MBL-positive isolates, the plates were examined after an incubation time of 18-24 hours for a difference of at least 7 mm in the zone of inhibition between the combination disks (meropenem + EDTA) and the meropenem disk alone. The phenotypically verified MBL isolates were stored in glycerol broth at a temperature of -80ºC.

Plasmid extraction:

Stored isolates on glycerol broth were thawed on the bench at room temperature and plated on MacConkey agar. After overnight incubation, 3-5 colonies were inoculated in 5 ml nutrient broth and incubated for 16 hours for plasmid extraction using a plasmid extraction kit (Geneaid Biotech Ltd, Taiwan), cat. No. PDH100.

BlaNDM-1 gene detection by PCR:

Amplification of the DNA was done using One µl of plasmid DNA was added to 10.0 µl of a master mix PCR (applied biotechnology, Catalogue number: ABT003), and 1 µl of each primer, as shown in table (1), with 7 µl deionized water were subsequently added to make a final volume of 20 µl. Using a programmable thermal controller PCR machine (Biometra, Germany), reactions underwent an initial denaturation at 94°C for 10 minutes, followed by 35 cycles of amplification, consisting of denaturation for 30 seconds at 94°C; annealing for 40 seconds at 56°C, extension for 50 seconds at 72°C; and a final 10 minutes extension at 72°C.

Amplicons were visualized under UV light transilluminator (Whatman, Biometra, Germany) after electrophoresis through 1.5% agarose gel (Invitrogen, USA) containing 0.05 mg/l ethidium bromide (Fluska, USA) at 125 volts for 45 minutes in 1X tbe (Tris/Borate/EDTA) The samples were run alongside a 100-1500 bp ladder (Fischer Scientific, USA) that served as a molecular weight marker and an amplified product corresponding to 475 bp was considered positive.

Table 1: Primers for amplification of blaNDM-1 gene [13]

Primers were supplied by (williwfort, UK)

NDM F	GGGCAGTCGCTTCCAACGGT
NDM R	GTAGTGCTCAGTGTCGGCAT

Statistical analysis:

Data were then collected, inputted, and encoded into SPSS version 27 for Windows. Numerical data were expressed as mean and standard deviation, whilst

categorical variables were conveyed as count and percentage. Comparisons between two subgroups were performed using chi-squared or Fisher's exact test, contingent on the expected values in the cells, whereas comparisons involving more than two subgroups used chi-squared or exact tests dependent on the anticipated values in the cells.

* A P value of 0.05 or less was considered significant.

RESULTS

The mean age of the studied patients was 36.9±18 years, ranging from 6 to 80 years. More than half of the participants were females (62%) **(Figure 1).** 66.7% had a history of receiving medications for UTI previously, 62% were catheterized, 53.3% were diabetics, and 43.9% had a history of urinary tract infection. The most common clinical complaints were frequency of micturition, dysuria, and abdominal pain **(Table 2).**

Table 2: Demographic and clinical presentation

Items	Values (no=460) No.	%
Sociodemographic characteristics		
Age (Years) **Mean±SD** **Median (min-max)**	 36.9±18 35 (6-80)	
Sex **Female** **Male**	 285 175	 62.0 38.0
Risk factors		
Medication for UTI (previously)	307	66.7
catheterization	285	62.0
Diabetes mellitus	245	53.3
History of UTI	202	43.9
Clinical presentation		
Frequency	265	57.6
Dysuria	191	41.5
Urgency	174	37.8
Abdominal pain	198	43.0
Fever	121	26.3
Hematuria	55	12.0

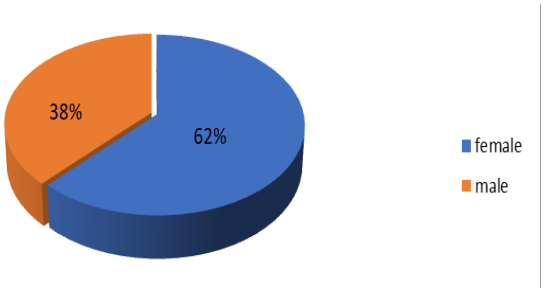

Fig. 1: percentage of male and female among participants

Out of the 460 studied samples, the commonly isolated organism was *E. coli* (155=33.7%), followed by *K. pneumoniae* (92= 20%), and other organisms: *S. aureus* (50=10.9%), *Pseudomonas* (43=9.3%), *Proteus* (24=5.2%), *Enterococci* (6=1.3%), while 90 samples (19.6%) showed sterile pyuria (no growth) **(Table 3) (Figure 2).**

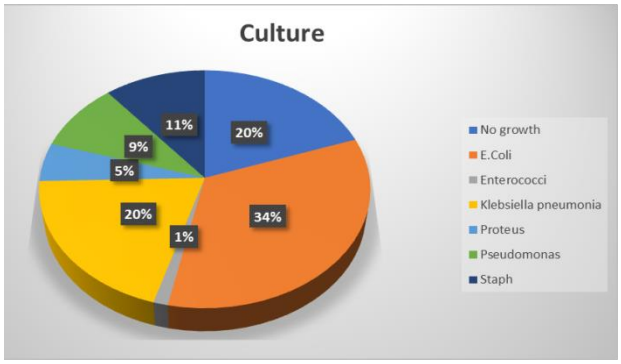

Fig. 2: Culture results in the studied urine samples.

Table 3: Results of bacterial growth of urine samples:

Type of microorganism	Values (no=460) No.	%
E. Coli	155	33.7
Klebsiella K. pneumoniae	92	20.0
S. aureus	50	10.9
Pseudomonas spp.	43	9.3
Proteus spp.	24	5.2
Enterococci spp.	6	1.3
No growth	90	19.6

As regards antibiotic susceptibility, *E. coli* isolates disclosed high resistance to ceftazidime (80%), ampicillin (77.4%), cefixime (76.1%), amoxicillin (58.1%), cefotaxime (56.1%), and nalidixic acid (50.3%). They showed intermediate resistance to gentamicin (15.5%), nitrofurantoin (16.1%), amikacin (18.1%), imipenem (18.7%), and doripenem (20%) **(Table 4) (Figure 3).**

K. pneumoniae isolates were highly resistant to ampicillin (93.5%), cefixime (91.3%), ceftazidime (88.0%), amoxicillin (88%), ciprofloxacin (80.4%), cefotaxime (80.4%), nalidixic acid (70.7%), nitrofurantoin (63.0%), and levofloxacin (62.0%). They showed intermediate resistance to gentamicin (34.8%), and ciprofloxacin (47.8%), respectively **(Table 4) (Figure 3).**

Comparing the level of resistance of the 2 common isolates, *K. pneumoniae* showed significantly higher resistance than *E. coli* towards: ampicillin, cefixime, imipenem, ciprofloxacin, amikacin, nalidixic acid, amoxicillin, cefotaxime, gentamicin, doripenem, levofloxacin, and nitrofurantoin. However, they reacted indifferently to ceftazidime **(Table 4) (Figure 3)**.

Table 4: Antimicrobial susceptibility testing of *E. coli* (no=155) and *K. pneumoniae* (no=92) isolates:

Susceptibility / Antibiotic	Resistant		Intermediately resistant		Sensitive		P-value
	E.coli	***K.pneumoniae***	***E.coli***	***K.pneumoniae***	***E.coli***	***K.pneumoniae***	
Ampicillin	120(77.4%)	**86(93.5%)**	7(4.5%)	0(0.0%)	**28(18.1%)**	6(6.5%)	<0.001*
Ceftazidime	124(80.0%)	81(88.0%)	12(7.7%)	2(2.2%)	19(12.3%)	9(9.8%)	0.141
Cefixime	118(76.1%)	**84(91.3%)**	8(5.2%)	2(2.2%)	**29(18.7%)**	6(6.5%)	0.011*
Imipenem	29(18.7%)	**51(55.4%)**	15(9.7%)	10(10.9%)	**111(71.6%)**	31(33.7%)	<0.001*
Ciprofloxacin	62(40.0%)	**74(80.4%)**	13(8.4%)	8(8.7%)	**80(51.6%)**	10(10.9%)	<0.001*
Amikacin	28(18.1%)	**44(47.8%)**	13(8.4%)	9(9.8%)	**114(73.5%)**	39(42.4%)	<0.001*
Nalidixic acid	78(50.3%)	**65(70.7%)**	11(7.1%)	6(6.5%)	**66(42.6%)**	21(22.8%)	0.005*
Amoxicillin	90(58.1%)	**81(88.0%)**	14(9.0%)	2(2.2%)	**51(32.9%)**	9(9.8%)	<0.001*
Cefotaxime	87(56.1%)	**74(80.4%)**	11(7.1%)	3(3.3%)	**57(36.8%)**	15(16.3%)	<0.001*
Gentamicin	24(15.5%)	**32(34.8%)**	4(2.6%)	6(6.5%)	**127(81.9%)**	54(58.7%)	<0.00*
Doripenem	31(20.0%)	**51(55.4%)**	1(0.6%)	6(6.5%)	**123(79.4%)**	35(38.0%)	<0.001*
Levofloxacin	58(37.4%)	**57(62.0%)**	14(9.0%)	13(14.1%)	**83(53.5%)**	22(23.9%)	<0.001*
Nitrofurantoin	25(16.1%)	**58(63.0%)**	6(3.9%)	4(4.3%)	**124(80.0%)**	30(32.6%)	<0.001*

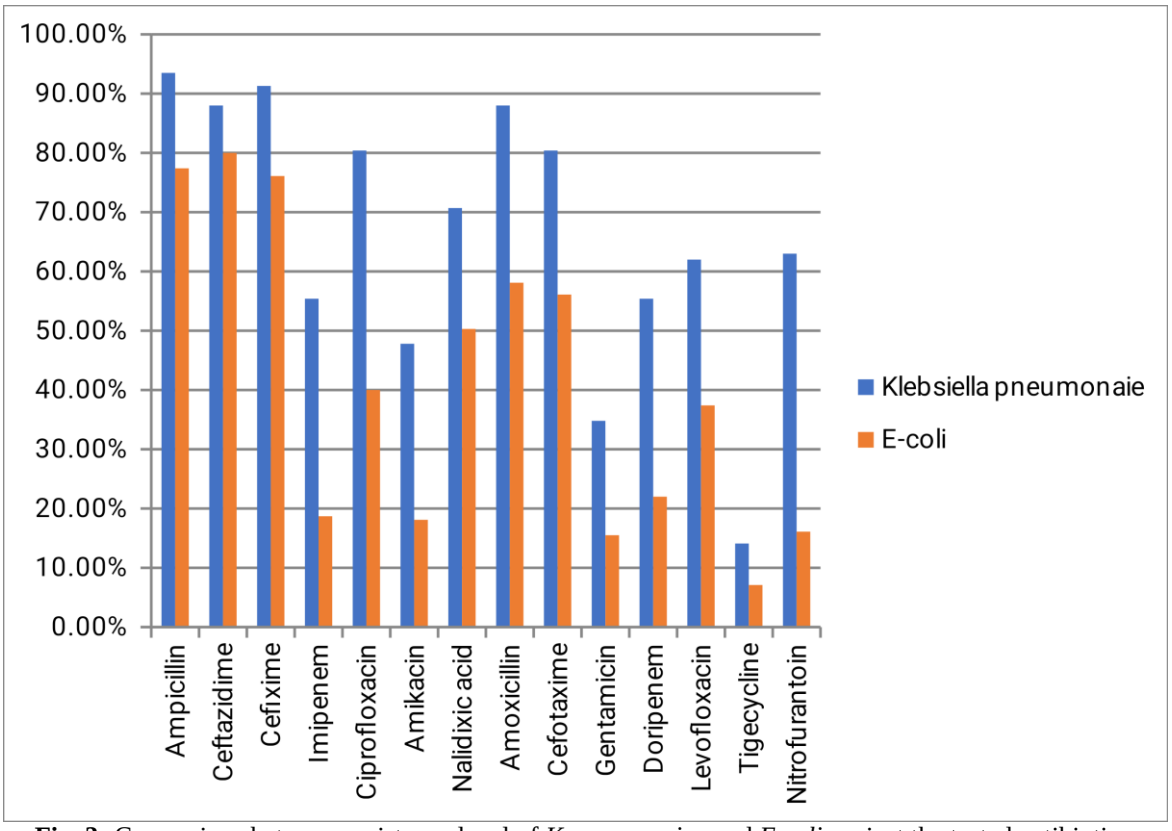

Fig. 3: Comparison between resistance level of *K. pneumoniae* and *E.coli* against the tested antibiotics.

The proportion of multidrug-resistant *E. coli* isolates was much greater than that of *K. pneumoniae*, at 63.9% and 39.1% respectively. In contrast, XDR and PDR rates were markedly elevated in *K. pneumoniae* (34.8% and 19.6%) compared to *E. coli* (8.4% and 5.3%), with a p-value of less than 0.001. Furthermore, the resistance to carbapenems was much greater in *K. pneumoniae* (60.9%) compared to *E. coli* (21.3%) (p-value <0.001) (Table 5) (Figure 4).

Of the 95 ESBL-positive isolates of *E. coli* and *K. pneumoniae*, 53.33% (32/59) of *E. coli* and 77.77% (28/36) of *K. pneumoniae* exhibited phenotypic positivity for MBL production (Table 6).

Among 60 MBL-positive *E. coli* (32) and *K. pneumoniae* (28), 44 were identified to possess the blaNDM-1 gene using PCR, with a distribution of 71.8% (23/32) in *E. coli* and 75% (21/28) in *K. pneumoniae* (Table 6).

Table 5: The classification of the resistance pattern

Items	*E. coli* (no=155)	*K. pneumoniae* (no=92)	Total	P-value
Non-MDR	35 (22.6%)	6 (6.5%)	41 (16.6%)	<0.001*
MDR	**99 (63.9%)**	36 (39.1%)	135 (54.7%)	
XDR	13 (8.4%)	**32 (34.8%)**	45 (18.2%)	
PDR	8 (5.2%)	**18 (19.6%)**	26 (10.5%)	

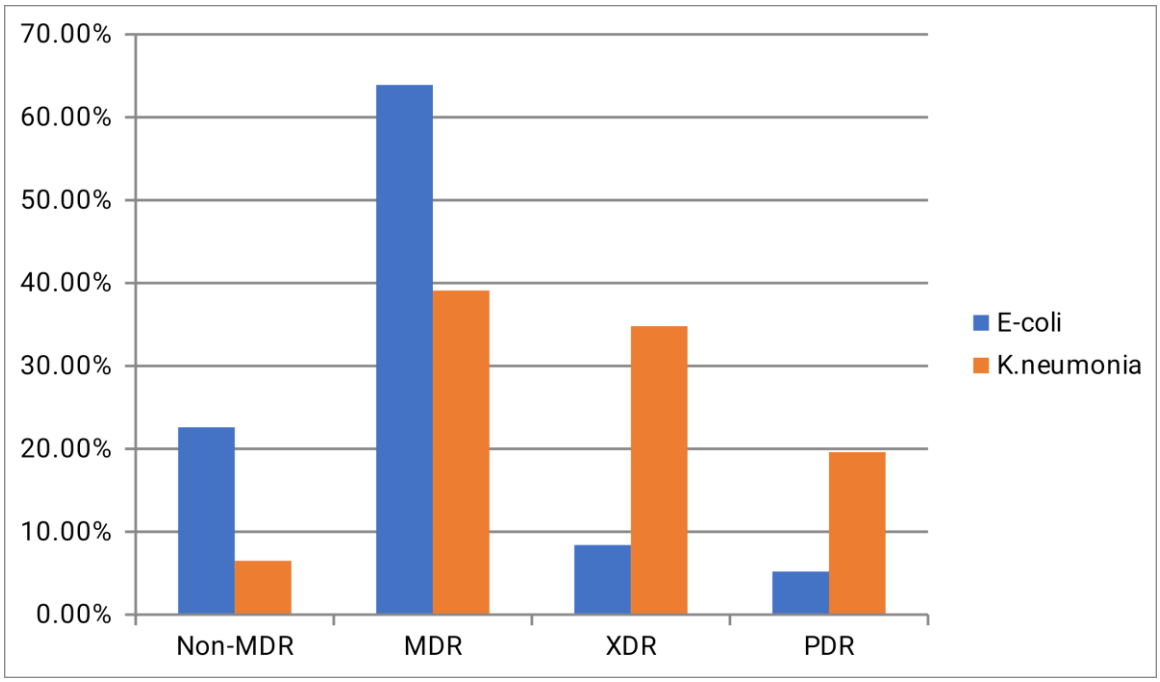

Fig. 4: Classification of *E. coli* and *K. pneumoniae* isolates regarding the type of resistance to antibiotics.

Table 6: Prevalence of ESBL, MBL-producing and blaNDM-1 positive *E. coli* and *K. pneumoniae* isolates

Bacterial species	ESBL positive\total isolates	MBL positive\ ESBL positive isolates	NDM-1 positive\ MBL positive isolates
E. coli	59\155 (38.1%)	32\59 (53.33%)	23\32 (71.8%)
K. pneumoniae	36\92 (39.1%)	28\36 (77.77%)	21\28 (75%)

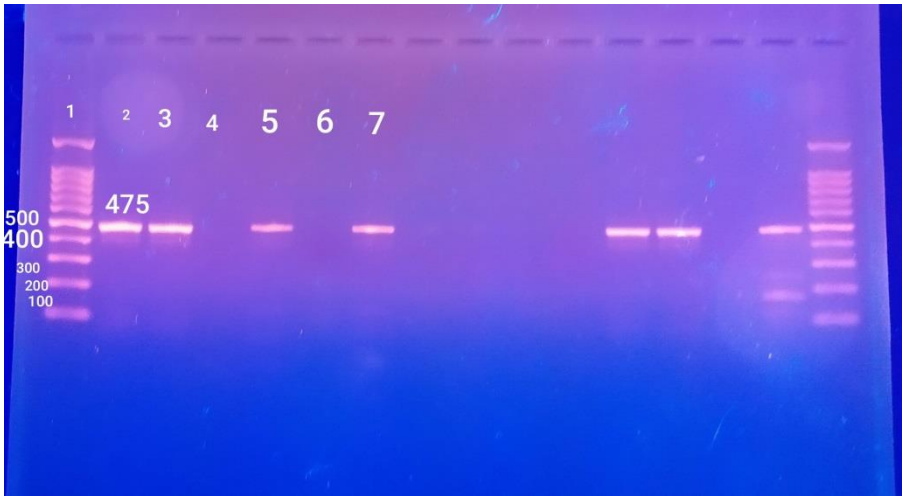

Fig. 5: Agarose gel electrophoresis image; lane 1: ladder used, lanes 2,3,5 and 7: positive samples with product size of 475 and lanes 4 and 6: negative samples.

DISCUSSION

The emergence of multi-drug resistant (MDR) Enterobacteriaceae, particularly ESBL-producing strains, is responsible for a high rate of nosocomial outbreaks leading to increased morbidity and mortality [14]. Some strains of *Klebsiella pneumoniae* and *Escherichia coli* have evolved resistance to every antibiotic now in use [15]. It is essential to regularly monitor antibiotic resistance in order to understand its spread and processes [16].

The patients investigated had an average age of 36.9±18 years, with ages ranging from six year to eighty years. This is in line with the findings of researchers in Gabon who found that 53.7% of their patients were between the ages of 18 and 49 [17].

Women made up over half of the sample (62%). Our findings are in line with those of Jalil and Al Atbee [18], who likewise found that females had a greater infection rate than men (61.7% vs. 38.3%).

Our findings indicated that 62.2% had undergone prior catheterization, 43.9% had a history of UTI or were taking medication for a UTI (66.7%), and 53.3% were diabetic. The findings were very comparable in a Turkish research where (41.6%) had a history of urinary tract infection in the last 6 months, (61.2%) had a history of antibiotic use in the last 3 months, (33.3%) had a history of urinary catheterization, and (22.5%) were diabetics [19].

Among the clinical manifestations examined in this research, frequency of micturition was the most prevalent (57.6%). However, 43% of patients reported experiencing stomach pain, which is in line with a prior research that reported that frequency (49.8%), urgency (72.0%), and abdominal discomfort (69.5%) were the most common clinical findings [20].

Among the types of bacterial isolates in this study, *E. coli* was the most prevalent, representing 155 isolates (33.7%), and the second dominant pathogen was *K. pneumoniae* (92 isolates, 20%).

Consistent with the current findings, Ndzime et al, investigation found that *E. coli* and *K. pneumoniae* were responsible for 44.9% of the pathogens involved in the development of UTIs, with 28.7% and 16.2% of the total, respectively [17].

On the contrary, Sharma et al. [21] and Johnson et al. [22] obtained different results revealed that *K. pneumoniae* is the frequently isolated among their participants in percentages of 88.3% and 37.41% respectively.

Concerning the microbial resistance to the tested antibiotics, *E. coli* isolates showed high resistance to ceftazidime (80%), ampicillin (77.4%), cefixime (76.1%), amoxicillin (58.1%), cefotaxime (56.1%) and nalidixic acid (50.3%). Though they appeared less resistant to gentamicin (15.5%), nitrofurantoin (16.1%), amikacin (18.1%), imipenem (18.7%), and doripenem (20%). Relatively similar results were reported by Malik et al.[23]. Their UPEC isolates showed a resistance pattern of (88.7%) to ampicillin, (67.9) to cefotaxime, (67.9) to ceftazidime, (24.6%) to imipenem, (9%) to amikacin and (15%) to nitrofurantoin.

K. pneumoniae isolates were highly resistant to ampicillin (93.5%), cefixime (91.3%), ceftazidime (88.0%), amoxicillin (88%), ciprofloxacin (80.4%), cefotaxime (80.4%), nalidixic acid (70.7%), nitrofurantoin (63.0%), and levofloxacin (62.0%), however, they found to be less resistant to gentamicin (34.8%), and ciprofloxacin (47.8%). Similar resistance patterns have been reported in a Nigerian study [24], where (93.3%) of the isolated *K. pneumoniae* were resistant to ampicillin, (86.8%) to ceftazidime, (86.8%) to cefotaxime, (66.6%) to ciprofloxacin, (66.6%) to gentamicin and (33.3%) to nitrofurantoin.

Recently, Al-Khfaji et al. [25] reported different resistance patterns to the tested antibiotics shown *by E. coli* isolates to cefixime and cefotaxime (90.5% and 71.4% respectively) which is slightly higher than our results, while *K. pneumoniae* resistance to imipenem, cefixime, and cefotaxime were 10%, 100%, 90% respectively.

Research population demographics and geographic location are two of the many factors that may explain why different UTI-causing bacteria exhibit different resistance patterns to the tested medications. One contributing factor to the development of bacterial resistance to antibiotics is the indiscriminate use of these drugs by individuals without medical prescription[26].

Regarding the resistance pattern of the predominately isolated strains against most of the spectrum of tested antibiotics, *K. pneumoniae* showed a statistically significant higher degree of resistance than *E. coli* did excluding ceftazidime where the difference was insignificant. Our findings are in line with those of Gebremedhin et al. [24].

Isolates of *Escherichia coli* and *Klebsiella pneumoniae* showed 63.9% and 39.1% multidrug-resistant strains, respectively. In addition, XDR and PDR prevalence rates for *K. pneumoniae* were 34.8% and 19.6%, respectively, whereas *E. coli* had rates of 8.4% and 5.2%.

In their study in Iraq, Al-Khfaji and his co-authors reported a higher rate of MDR among their isolates of *K. pneumoniae* (90%) and E. coli (71.42%) [25]. In contrast, Iqbal et al. [27] discovered lower MDR rates (7.5% for *E. coli* and 24.3% for *K. pneumoniae*), despite XDR rates of 92.06% and 75.7%, respectively.

When looking at the resistance pattern of the two most common strains, it is evidenced that *E. coli* had a much higher percentage of multidrug-resistant bacteria (63.9%) than *K. pneumoniae* (39.1%). In contrast, *K. pneumoniae* achieved higher levels of resistance as

XDR (34.8%) and PDR (19.6%), compared to *E. coli* (8.4%) and 5.2%, respectively. Our results matched the finding obtained by Fallah et al. [28] in Iran, where they found that 51.9% of *E. coli* and 28.5% of *K. pneumoniae* strains were MDR, while 3.3% and 14.2% of the same strains were found to be XDR.

Out of 247 *E. coli* and *K. pneumoniae* isolates, 95\247 (38.5%) produced ESBLs. distributed as 59/155 of *E. coli* with a ratio of 38% and 36/92 of *K. pneumoniae* with a ratio of 38.5%. This result was in line with that of an Egyptian investigation that found that among the strains examined, forty-one percent of *E. coli* and forty-eight percent of *K. pneumoniae* produced ESBLs, with a combined incidence of forty-three percent for the two [29]. On the other hand, a Nigerian study stated higher ESBL production rates among the studied isolates (50%) [30].

Out of 95 *E. coli* and *K. pneumoniae* ESBL-positive isolates, 73.73 percent of the *E. coli* and 76.7 percent of the *K. pneumoniae* showed phenotypic positivity for MBL production. Contrary to what some may have thought, other studies have shown that 13.2% of ESBL-producing isolates are really MBL producers; of these, 54.9% are ESBL-*E. coli* and 45.1% are ESBL-*K. pneumoniae* [31].

Possible explanations for the disparity between our results and theirs include variations in antibiotic policy, sample size, and infection control and prevention practices (both in terms of implementation and adherence).

A total of 44 out of 60 MBL-positive *E. coli* and *K. pneumoniae* have the blaNDM-1 gene. Of the *E. coli* and *K. pneumoniae* that tested positive, 71.8% (23/32) and 75% (21/28), respectively, had this gene. Devi et al. [7] discovered that the blaNDM-1 gene was identified in almost fifty percent of the MBL-producing isolates. A research in Egypt revealed that of 48 MBL phenotypic positive *E. coli* isolates, 15.7% were positive for BlaNDM-1 [32].

Eighty of the eighty-two MBL-positive isolates (97.6% of the total) had the blaNDM-1 gene, according to PCR results from a different Egyptian study on *K. pneumoniae* [33]. Comparable to our results, a Pakistani study on MBL-producing isolates indicated that 37% of *E. coli* and 40% of *K. pneumoniae*, taken from urine samples, tested positive for the blaNDM-1 gene.

CONCLUSION

Antibiotic resistance in *E. coli* and *K. pneumoniae* isolated from UTI patients has risen. The prevalence rate of the blaNDM-1 gene was highly elevated

Recommendation

Regular monitoring of NDM-1 gene carriers is essential to mitigate the dissemination of additional resistance genes, since the NDM-1 gene is often located on plasmids that may harbor other resistance genes, leading to the emergence of MDR, XDR, or PDR strains.

Ethics approval and consent to participate:

The present study is a cross-sectional study that was carried out at Beni-Suef University Hospital between August 2022 and April 2023. The Ethical Approval from the Faculty of Medicine, Beni-Suef University's research ethical committee, was obtained prior to the beginning of the work (Approval No: FMBSUREC/05072022/Abd Allah).

Consent for publication

The written informed consent was obtained from all study patients. All of the study participants received information about the research's protocols as well as information about their ability to decline participation or to withdraw from the study without providing a reason. Participants received a promise of anonymity, and all information was handled in confidence. The necessary administrative requirements were met. Prior to starting the study, the research ethics committee (REC) for the faculty of medicine at Beni-Suef University was consulted.

Conflicts of interests

The authors declare that they have no competing interests.

Declarations:

Availability of data and material:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

None

Author's contributions

AT: sampling, performing the experiments, data analysis, preparing the first draft of the manuscript. AG: supervision and revision of the manuscript. RI: supervision, data analysis, and revision of the manuscript. MS: conceptualization, supervision, data analysis, and preparing and revising the manuscript. All authors have read and approved the final manuscript.

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"Not applicable"

REFERENCES

1. Adugna, B, Sharew B, Jemal M. Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Factors of Community-and Hospital-Acquired Urinary Tract Infection at Dessie Referral Hospital, Dessie, Northeast Ethiopia. International journal of microbiology.2021 ;(1), p.5553356.
2. Kumar MS, Arunagirinathan N, Ravikumar M. Antibiotic susceptibility profile of extended

spectrum β-lactamase producing Escherichia coli, Klebsiella pneumoniae and Klebsiella oxytoca from Urinary tract infections. Research Journal of Pharmacy and Technology. 2021;14(8), 4425.

3. Ebrahim-Saraie HS, Nezhad NZ, Heidari H, Motamedifar A, Motamedifar M. Detection of antimicrobial susceptibility and integrons among extended-spectrum β-lactamase producing uropathogenic Escherichia coli isolates in Southwestern Iran. Oman Medical Journal. 2018; 33(3):218.
4. Mofolorunsho KC, Ocheni HO, Aminu RF, Omatola CA, Olowonibi OO. Prevalence and antimicrobial susceptibility of extended-spectrum beta lactamases-producing Escherichia coli and Klebsiella pneumoniae isolated in selected hospitals of Anyigba, Nigeria. African Health Sciences.2021; 21(2), 505-512.
5. Abdeta A, Bitew A, Fentaw S, Tsige E, Assefa D, Lejisa T, Evans M. Phenotypic characterization of carbapenem non-susceptible gram-negative bacilli isolated from clinical specimens. Plos one, 2021;16(12), e0256556.
6. Abdulall AK, Tawfick MM, El Manakhly AR, El Kholy A. Carbapenem-resistant Gram-negative bacteria associated with catheter-related bloodstream infections in three intensive care units in Egypt. European Journal of Clinical Microbiology & Infectious Diseases, 2018;37(9), 1647.
7. Devi LS, Broor S, Rautela RS, Grover SS, Chakravarti A, Chattopadhya D. Increasing prevalence of Escherichia coli and Klebsiella pneumoniae producing CTX-M-type extended-spectrum beta-lactamase, carbapenemase, and NDM-1 in patients from a rural community with community acquired infections: A 3-year study. International Journal of Applied and Basic Medical Research. 2020; 10(3), 156-163.
8. Naeem S, Bilal H, Muhammad H, Khan MA, Hameed F, Bahadur S, Rehman TU. Detection of blaNDM-1 gene in ESBL producing Escherichia coli and Klebsiella pneumoniae isolated from urine samples. The Journal of Infection in Developing Countries. 2021; 15(04), 516-522.
9. Zenati F, Barguigua A, Nayme K, Benbelaïd F, Khadir A, Bellahsene C, Timinouni M. Characterization of uropathogenic ESBL-producing Escherichia coli isolated from hospitalized patients in western Algeria. The Journal of Infection in Developing Countries. 2019; 13(04), 291.
10. Bilal H, Zhang G, Rehman T, Han J, Khan S, Shafiq M, Yang X. First report of blaNDM-1 bearing IncX3 plasmid in clinically isolated ST11 Klebsiella pneumoniae from Pakistan. Microorganisms. 2021; 9(5), 951.
11. Wikler MA. Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement. Clinical and Laboratory Standards Institute (CLSI); 2023.
12. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012; 18(3), 268-281.
13. Ejaz H, Alzahrani B, Hamad MF, Abosalif KO, Junaid K, Abualgasim A, Younas S. Molecular analysis of the antibiotic resistant NDM-1 gene in clinical isolates of Enterobacteriaceae. Clinical Laboratory. 2020; 66(3), 409-417.
14. Salama LA, Elageery SM, Alasaby NM, Abou ElKhier NT, Fawzy IM, Zeid MS, Badr Df. Molcular characterization of carbapenemases in hypervirulent klebsiella pneumoniae isolates among pediatric patients. Egyptian Journal of Medical Microbiology. 2025, 34(1), 111-118
15. Sharaf S, Ali HT, Salah MG, Kamal Z. Insight on the prevalence of clinical klebsiella isolates producing extended spectrum Beta-Lactamases. Egyptian Journal of Medical Microbiology. 2024; 33(3), 125-131.
16. Sadeghi M, Mojtahedi A, Nikokar I, Roushan ZA. The emergence of plasmid-encoded oxacillinase and carbapenemase among uropathogenic Escherichia coli (UPEC) isolated from hospitalized patients in the North of Iran. Heliyon. 2023; 9(4), e15386-e15386.
17. Ndzime YM, Onanga R, Kassa RFK, Bignoumba M, Nguema PPM, Gafou A, Bisseye C. Epidemiology of Community Origin Escherichia coli and Klebsiella pneumoniae Uropathogenic Strains Resistant to Antibiotics in Franceville, Gabon. Infection and Drug Resistance. 2021; 14, 585-594.
18. Jalil MB, Al Atbee MYN. The prevalence of multiple drug resistance Escherichia coli and Klebsiella pneumoniae isolated from patients with urinary tract infections. Journal of Clinical Laboratory Analysis. 2022; 36(9), e24619.
19. Guclu E, Halis F, Kose E, Ogutlu A, Karabay O. Risk factors of multidrug-resistant bacteria in community-acquired urinary tract infections. African health sciences. 2021; 21(1), 214-9.
20. Gebretensaie Y, Atnafu A, Girma S, Alemu Y, Desta K. Prevalence of Bacterial Urinary Tract Infection, Associated Risk Factors, and

Antimicrobial Resistance Pattern in Addis Ababa, Ethiopia: A Cross-Sectional Study. Infection and Drug Resistance. 2023; 3041-3050.

21. Sharma P, Netam AK, Singh R. Prevalence and in vitro antibiotic susceptibility pattern of bacterial strains isolated from tribal women suffering from urinary tract infections in District Anuppur, Madhya Pradesh, India. Biomedical Research and Therapy. 2020; 7(8), 3944-3953.
22. Johnson B, Stephen BM, Joseph N, Asiphas O, Musa K, Taseera K. Prevalence and bacteriology of culture-positive urinary tract infection among pregnant women with suspected urinary tract infection at Mbarara regional referral hospital, South-Western Uganda. BMC pregnancy and childbirth. 2021; 21(1), 1-9.
23. Malik S, Rana JS, Nehra K. Prevalence and antibiotic susceptibility pattern of uropathogenic Escherichia coli strains in Sonipat region of Haryana in India. Biomed Biotechnol Res J. 2021; 5(1), 80-7.
24. Gebremedhin MG, Weldu Y, Kahsay AG, Teame G, Adane K. Extended-Spectrum β-Lactamase and Carbapenemase-Producing Gram-Negative Bacteria and Associated Factors Among Patients Suspected of Community and Hospital-Acquired Urinary Tract Infections at Ayder Comprehensive Specialized Hospital, Tigrai, Ethiopia. Infection and Drug Resistance. 2023; 4025-4037.
25. Al-Khfaji ZA, Sagban SH, Al-Musawi AF. Prevalence of Drug-Resistant Strains of Escherichia coli and Klebsiella pneumoniae Isolated from Women with Urinary Tract Infections in Karbala City, Iraq. Egyptian Journal of Botany. 2023; 63(1), 295-303.
26. Yenehun Worku G, Belete Alamneh Y, Erku Abegaz W. Prevalence of bacterial urinary tract infection and antimicrobial susceptibility patterns among diabetes mellitus patients attending Zewditu memorial hospital, Addis Ababa, Ethiopia. Infection and drug resistance. 2021; 1441-1454.
27. Iqbal Z, Mumtaz MZ, Malik A. Extensive drug-resistance in strains of Escherichia coli and Klebsiella pneumoniae isolated from paediatric urinary tract infections. Journal of Taibah University Medical Sciences. 2021; 16(4), 565-574.
28. Fallah F, Parhiz S, Azimi L. Distribution and antibiotic resistance pattern of bacteria isolated from patients with community-acquired urinary tract infections in Iran: a cross-sectional study. International Journal of Health Studies. 2018; 4(2), 14-19.
29. Al Yousef SA, Younis S, Farrag E, Moussa HS, Bayoumi FS, Ali AM. Clinical and laboratory profile of urinary tract infections associated with extended-spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae. Annals of Clinical & Laboratory Science. 2016; 46(4), 393-400.
30. Mofolorunsho KC, Ocheni HO, Aminu RF, Omatola CA, Olowonibi OO. Prevalence and antimicrobial susceptibility of extended-spectrum beta lactamases-producing Escherichia coli and Klebsiella pneumoniae isolated in selected hospitals of Anyigba, Nigeria. African Health Sciences. 2021; 21(2), 505-512.
31. Devi LS, Broor S, Rautela RS, Grover SS, Chakravarti A, Chattopadhya D. infections: A 3-year study. International Journal of Applied and Basic Medical Research. 2020; 10(3), 156-163.
32. Masoud SM, Abd El-Baky RM, Aly SA, Ibrahem RA. Co-existence of certain ESBLs, MBLs and plasmid mediated quinolone resistance genes among MDR E. coli isolated from different clinical specimens in Egypt. Antibiotics. 2021; 10(7), 835.
33. Elsheshtawy N. Prevalence of New Delhi Metallo-Beta Lactamase gene among Klebsiella species isolates: An Egyptian Study. Microbes and Infectious Diseases. 2021; 2(3), 508-515.