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Multidrug-Resistant and Extended Spectrum β-Lactamase Producing Escherichia coli and Klebsiella pneumoniae Isolates from Urine Samples of Hospital Patients

Muhammad Ilyas[1], Dr Shazia Dawood[2], Eiman Syed[3], Samina Salaiha[4], Rubama Javed[5], Mah-e-noor Shamshad[6*],

[1]Primary And Secondary Healthcare Department, Punjab, Pakistan
[2]Department Of Pharmacy And Allied Health Sciences, Iqra University North Campus, Karachi
[3]Department Of Medical Laboratory Technology, Riphah International University, Lahore, Pakistan
[4]The Islamia University Of Bahawalpur, Bahawalpur, Pakistan
[5]Department Of Emerging Technologies, The Superior University, Lahore
[*6]Department Of Microbiology, University Of Central Punjab, Lahore, Pakistan

Corresponding author: Mah-e-noor
Department Of Microbiology, University Of Central Punjab, Lahore, Pakistan
mahenoorrao@gmail.com

ABSTRACT

Multi drug resistant Escherichia coli and Klebsiella pneumoniae expressing extended spectrum β-lactamase enzymes (ESBLs) has become a serious challenge to clinicians for the therapeutic management of clinical cases in urinary tract infection.

The main objective of the study was focused to determine the dominance of MDR E. coli and Klebsiella pneumoniae and the evaluation of status of β-lactamase enzyme produced by them.

The study was carried out in Holy family hospital and Khalifa gul Nawaz hospital, Pakistan, between June and November 2023. A total of 350 midstream urine samples were processed among suspected cases of urinary tract infection. The bacteria were isolated by semi quantative culture technique and identified by conventional biochemical tests. The antimicrobial susceptibility testing was performed by modified Kirby Bauer disc diffusion method following Clinical and Laboratory Standards Institute guidelines and were tested for ESBL by combination disc method. The pvalue <0.05 was considered as statistically significant.

A total of 85 samples showed significant bacteriuria with 62 E. coli and 23 Klebsiella pneumoniae. Among the isolates, 62.35% were found MDR strains. By combined disk test, 86.67% E. coli and 13.33% Klebsiella spp. were found ESBL producers. There is significant association between MDR and ESBL production as well as between age group of patients and ESBL producing organisms (P=0.01). Higher prevalence of ESBL producing E. coli and Klebsiella spp. Was observed warranting prompt need of surveillance for effective management of such MDR strains. Imipenem, Meropenem and Nitrofurantoin seemed to be drug of

choice for UTI. Amoxycillin should no longer considered as drugs for empirical treatment of clinically evident UTI, because of high resistance rates.

There is an increasing need for periodic monitoring of drug susceptibility patterns to prevent the spread and development of antimicrobial resistant strains and ESBL producers.

Keywords: Escherichia coli, Extended Spectrum β-lactamase (ESBL), Klebsiella pneumoniae, Multidrug resistance (MDR), Urinary Tract Infection (UTI)

Introduction

Urine, a sterile ultrafiltrate of blood, plays a vital role in diagnosing various physiological conditions. Urinary tract infection (UTI) is a prevalent condition, ranging from asymptomatic bacteriuria to acute pyelonephritis, potentially leading to sepsis. UTIs involve microbial invasion of any urinary tissues from the renal cortex to the urethral meatus (Klein & Hultgren, 2020). Affecting both genders, UTIs are more common in women due to the shorter urethra, lack of prostatic secretions, higher contamination risk with fecal flora, and pregnancy-related changes. Approximately 35% of healthy women experience UTI symptoms, with around 150 million cases globally each year (Öztürk & Murt, 2020).

Clinically, UTIs manifest as pyelonephritis, pyelitis, ureteritis, cystitis, prostatitis, and urethritis. Gram-negative bacteria, primarily Escherichia coli and Klebsiella pneumoniae, are common uropathogens. In Nepal, E. coli (77.5%) and Klebsiella spp. (7.1%) are prevalent among Gram-negative isolates, while Staphylococcus aureus (5.7%) and S. saprophyticus (2.3%) are common among Gram-positive isolates (Ademola, Atanda, Aji, & Abdu, 2020).

UTIs often result from rectal flora entering the urinary tract via the urethra. This ascending route is exacerbated by conditions like perineal swelling, urinary catheters, and spermicidal agents (Martins, 2021). Established cystitis can lead to pyelonephritis, with bacteria ascending from the bladder to the renal pelvis (Hudson & Mortimore, 2020).

UTIs are classified as complicated or uncomplicated based on host factors and uropathogens. Complicated UTIs, linked to age, catheterization, diabetes, and spinal cord injury, can cause significant damage due to less virulent uropathogens.

Treatment typically involves broad-spectrum antibiotics such as cephalosporins, fluoroquinolones, and aminoglycosides. However, the indiscriminate use of antibiotics has led to rising global antibiotic resistance, complicating treatment (Bhardwaj et al., 2022). Contributing factors include poor infection control, increased agricultural antibiotic use, and the availability of non-prescription antibiotics, leading to multidrug-resistant pathogens (P. Singh & Holmen, 2022).

This study aims to investigate the prevalence and resistance patterns of multidrug-resistant and extended-spectrum β-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae isolates from urine

samples of patients attending a hospital. Understanding these resistance patterns is crucial for developing effective treatment strategies and public health policies to combat the spread of resistant infections.

Materials and Methods

3.1 Materials

Key materials used in the study included essential equipment such as an autoclave, incubator, microscope, and refrigerator. Microbiological media utilized comprised Blood Agar, MacConkey Agar, and Muller Hinton Agar. Essential chemicals and reagents included Catalase Reagent (3% H_2O_2), Oxidase Reagent, and Gram's Reagent. For antibiotic susceptibility testing, antibiotic discs such as Amoxicillin (10µg), Ciprofloxacin (5µg), Gentamycin (10µg), Imipenem (10µg), and Nitrofurantoin (300µg) were employed.

3.2 Study Design and Sites

A cross-sectional study was conducted from June to November 2019 at Holy family hospital and Khalifa gul Nawaz hospital.

3.3 Study Population

Patients undergoing routine culture and antibiotic susceptibility testing at Holy family hospital and Khalifa gul Nawaz hospital were included. All age groups and both sexes suspected of UTIs were screened.

3.3.1 Inclusion Criteria

Patients attending routine culture between June and November 2019.

3.3.2 Exclusion Criteria

Patients with symptoms other than UTI or those who had taken antibiotics for UTI within the last 7 days were excluded.

3.4 Sample Size

Based on a 23.68% prevalence rate (Kayastha et al., 2020), the sample size was calculated using Fisher's formula, resulting in 280 required samples. A total of 350 urine samples were collected.

3.5 Specimen Collection and Transportation

Mid-stream urine samples were collected aseptically in sterile containers, labeled with patient demographics, and transported to the laboratory within one hour for further processing.

3.6 Processing of Specimen

3.6.1 Macroscopic Examination

Urine samples were examined for color, turbidity, appearance, and pH.

3.6.2 Culture of Specimens

Urine samples were cultured on Mac-Conkey Agar and 5% Blood Agar using the standard loop technique. Plates were incubated at 37°C overnight, and colony counts were performed.

3.6.3 Identification of Isolates

Bacterial isolates were identified using standard microbiological techniques, including colonial morphology, staining reactions, and biochemical tests.

3.6.4 Purity Plate

Purity plates ensured inoculation purity for biochemical tests.

3.6.5 Antimicrobial Susceptibility Testing

Antibiotic sensitivity was determined using the Kirby-Bauer disc diffusion method on Muller Hinton Agar. Antibiotics tested included Amoxicillin, Ciprofloxacin, Gentamicin, Nitrofurantoin, Ceftazidime, Aztreonam, Ceftriaxone, Imipenem, and Meropenem.

3.6.6 Tests for ESBL Production

Screening and confirmation of ESBL production were performed using ceftazidime and ceftriaxone disks, followed by combination disc methods involving clavulanic acid.

3.6.7 Analysis of MDR Isolates

Resistance to more than two antibiotics indicated multidrug-resistant (MDR) isolates.

3.6.8 Antibiotic Susceptibility of ESBL Producers

Susceptibility to alternative drugs (Imipenem, Meropenem) was tested using the Kirby-Bauer method.

3.6.9 Quality Control

Regular evaluation of laboratory equipment, reagents, and media was conducted. Antibiotics and media were checked for lot numbers, manufacture, expiry dates, and proper storage. Control strains of E. coli ATCC 25922 and K. pneumoniae ATCC 700603 were used for quality assurance.

3.6.10 Data Analysis

Data were analyzed using SPSS version 22.0. Descriptive analysis and Chi-square tests were used, with $p \leqslant 0.05$ considered statistically significant.

RESULTS

The study was carried out at the Microbiology Lab of university of swabi to determine the status of MDR and ESBL producing *E. coli* and *Klebsiella pneumoniae* isolated from the urine sample, from patients suspected of urinary tract infections (UTI). 350 mid-stream urine samples were collected from patients complaining of urinary tract infection.

A total of 350 urine samples fulfilled the inclusion criteria were screened for the study population. 113 (32.28%) were from male population and 237(67.72%) were from female population. Among the population, the most studied cases were obtained from age group (21-30) years with the male 34% and female 32% followed by age group (31-40) years with the female 23% and male 22%.

4.1 Growth profile of bacteria isolated in urine samples

Out of 350 urine samples processed, 85 (24%) showed significant growth while the rest of the samples 265(76%) showed no growth (Fig: 1).

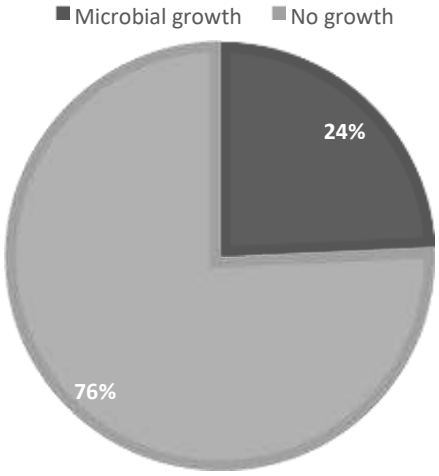

Fig 1: Microbial growth profile in urine samples

4.2. a. Growth pattern of *E.coli* and *K.pneumoniae* among inpatients and outpatients

Among the total 350 mid-stream urine samples, 277 (79.14%) were from outpatients and 73 (20.86%) were from inpatients. Among 277 outpatients 64 (23.10%) showed significant growth, while among 73 inpatients 21 (28.76%) showed significant growth (Fig: 2). The difference in growth is statistically insignificant among inpatient and outpatients (P>0.05)

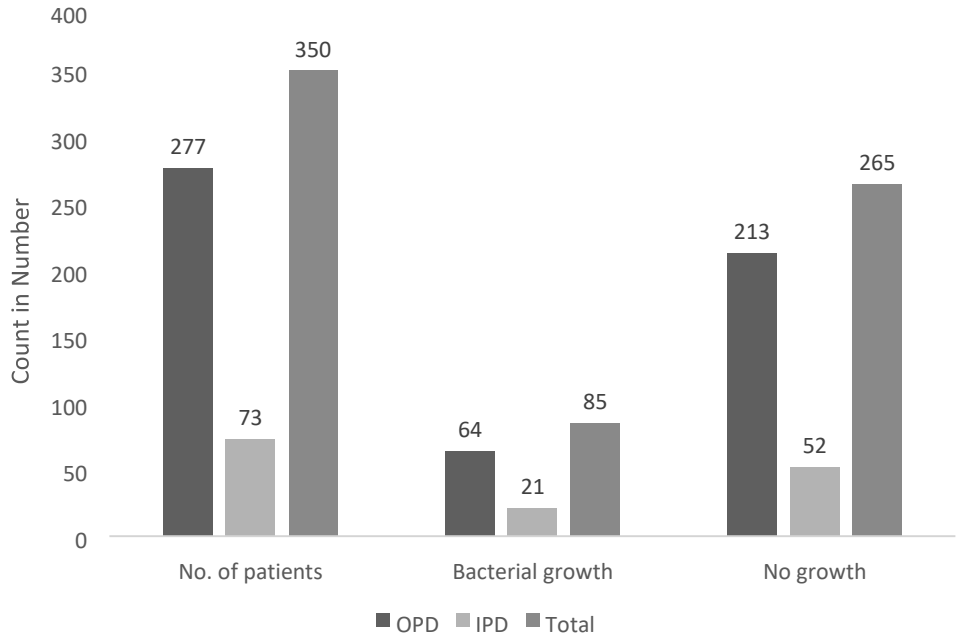

Fig 2: Growth pattern of *E.coli* and *K. pneumoniae* among inpatients and outpatients

4.2. b. Gender wise distribution of patient and growth pattern

Among the total 350 mid-stream urine samples 113(32.28%) were from male patients and 237 (67.71%) were from female patients. Out of 113 male patients, 16 (14.15%) and out of 237 female patients, 69 (29.11%) showed significant growth (Fig 4). The association of growth between male and female patient is found statistically significant (P<0.05).

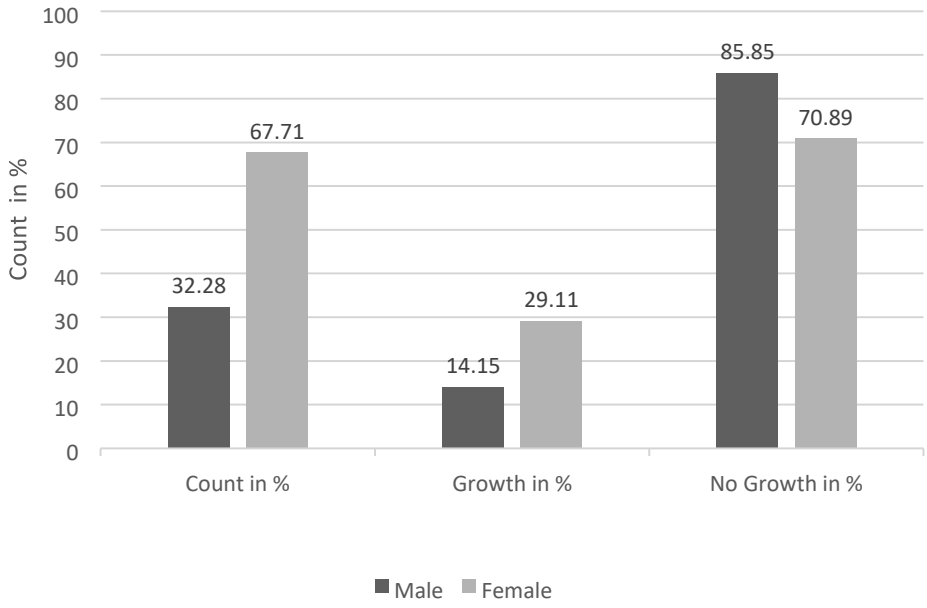

Fig 3: Gender wise distribution of patient and growth pattern

4.3 Age wise distribution of the growth positivity

Among the 85 significant bacteriuria cases, the highest percentage of bacteriuria were obtained from age group (21-30) years (31.76%) followed by age group (31-40) years (22.35%) and (41-50) years (14.11%) (Table 1). There is significant association (P<0.05) between age group of patients and growth of bacteriuria.

Table 1: Growth positivity in relation to age of patients

Age of patients in years	Growth		No gro\		Total
	Number	%	Number	%	Number
0-10	2	2.35	6	2.26	8
11-20	10	11.76	26	9.81	36
21-30	27	31.76	86	32.45	113
31-40	19	22.35	61	23.01	80
41-50	12	14.11	39	14.71	51
51-60	6	7.05	20	7.54	26
61-70	4	4.7	12	4.52	16
71-80	3	3.5	8	3.01	11
81-90	2	2.35	7	2.64	9

	85	24.28	265	75.72	350
Total					

4.4 Microbiological profiling of urine isolates

Out of 85 bacterial isolates from mid-stream urine samples in this study, 2 different genera of Enterobacteriaceae were isolated (Table 2). Among the bacterial isolates, *E.coli* (72.94%) was found to be most predominant organism and then *Klebsiella pneumoniae* (27.06%) was least in number.

Table 2: Pattern of Enterobacteriaceae isolates causing UTI

S.N.	Bacterial isolates	Number	Percentage (%)
1	*Escherichia coli*	62	72.94
2	*K. pneumoniae*	23	27.06
	Total	85	100

4.5 Antibiotic susceptibility pattern of uropathogens

4.5.1 Antimicrobial susceptibility pattern of *E.coli*

The antibiotic susceptibility pattern of *E. coli* (n=62) revealed that sensitivity was seen for aztreonam (75.8%) followed by nitrofurantoin (56.45%). The resistance was seen against amoxicillin (69.6%), followed by ceftriaxone (51.61%). (Table 3).

Table 3: Antimicrobial susceptibility pattern of *E.coli*

Antibiotics	Susceptibility pattern (n=62)					
	Sensitivity		Intermediate		Resistance	
	No	%	No	%	No	%
Amoxycillin	5	8.06	14	22.58	43	69.35
Aztreonam	40	75.8	9	14.51	13	9.67

Ciprofloxacin	19	30.64	14	22.58	29	46.77
Ceftazidime	20	32.25	18	29.03	24	38.7
Gentamycin	29	46.77	13	20.96	20	32.25
Nitrofurantoin	35	56.45	11	17.74	16	25.8
Ceftriaxone	16	25.8	14	22.58	32	51.61

4.5.2 Antimicrobial susceptibility pattern of *Klebsiella pneumoniae*

The antibiotic susceptibility pattern of *K. pneumoniae* (n=23) revealed that sensitivity was seen for gentamycin (95.65%) followed by ciprofloxacin (82.6%). The resistance was seen against amoxicillin (91.3%), followed by ceftazidime (56.52%) (Table 4).

Table 4 : Antimicrobial susceptibility pattern of *Klebsiella pneumoniae*

Antibiotics	Susceptibility pattern (n=23)					
	Sensitivity		**Intermediate**		**Resistance**	
	No	**%**	**No**	**%**	**No**	**%**
Amoxycillin	1	4.34	1	4.34	21	91.3
Aztreonam	9	39.13	3	13.04	11	47.82
Ciprofloxacin	19	82.6	1	4.34	3	13.04
Ceftazidime	6	26.08	4	17.39	13	56.52
Gentamycin	22	95.65	0	0	1	4.34
Nitrofurantoin	10	43.47	2	8.69	11	47.82
Ceftriaxone	8	34.78	3	13.04	12	52.17

4.6 Profile of MDR among uropathogens

Among 85 isolates, 53(62.35%) were found to be MDR in which maximum

MDR was found in *K. pneumoniae* (69.5 %), followed by *E.coli* (59.6%) (Table 5).

Table 5: Pattern of MDR among uropathogens

S.N	Isolated organisms	Multi drug resistant				Total
		Yes		No		
		No.	%	No.	%	
1	*E.coli*	37	59.6	25	40.3	62
2	*K. pneumoniae*	16	69.5	7	30.4	23
	Total	53	62.35	32	37.6	85

4.7 Prevalence of ESBL producing uropathogens

Among 85 isolates, 15 bacteria were found to be ESBL producer. The prevalence of ESBL production in *E.coli* (20.9%), *K. pneumoniae* (8.69%) (Table 6).

Table 6: Prevalence of ESBL producing uropathogens

S.N.	ESBL production Isolated organisms	Yes		No		Total
		No.	%	No.	%	
1	*E.coli*	13	20.9	49	79	62
2	*K. pneumoniae*	2	8.6	20	86.9	23
	Total	15	17.64	69	81.17	85

4.8 Association between MDR and ESBL production

Among 85 isolates of bacteria, 15 were ESBL producer and 53 were MDR positive. There is significant association between MDR and ESBL production at (P<0.05) (Table 7).

Table 7: Association between MDR and ESBL production

	MDR			
ESBL	**Yes (%)**	**No (%)**	**Total**	**p-value**
Positive	15(28.3)	-	15	
Negative	38(71.7)	32(60.67)	70	0.001
Total	53(100)	32(60.67)	85	

4.9 Antimicrobial susceptibility of ESBL producing organism

Fiveteen bacterial isolates were ESBL producers. All 13 ESBL producing isolates of *E. coli* were sensitive to Imipenem. 92.3% and 84.6% were resistant to Ceftriaxone and Ceftazidime, respectively. Similarly, 69.2% of *E. coli* were resistant to both Ciprofloxacin and Cotrimoxazole. Furthermore, 30.7% of *E. coli* were resistant to Meropenem. All of the 2 ESBL producing *K. pneumoniae* isolates were sensitive to Imipenem, Meropenem. All of the isolates were resistant to Amoxycillin (Table 8).

Table 8: Antimicrobial susceptibility of ESBL producing organism

Antibiotics	***E.coli*(n=13)**			
	Sensitive No.	**Sensitive %**	**Resistance No.**	**Resistance %**
Amoxycilin	-	-	13	100
Ciprofloxacin	4	30.7	9	69.2
Nitrofurantoin	12	92.3	1	7.6
Gentamycin	8	61.5	5	38.4

Ceftazidime	2		15.3	11	84.6
Ceftriaxone	1		7.6	12	92.3
Cotrimoxazole	4		30.7	9	69.2
Imipenam		13	100	-	-
Meropenam	9		69.2	4	30.7

DISCUSSION

Urinary tract infection is a serious health problem that is affecting millions of people each year. Recently, antibiotics have been used extensively and newer antibiotics are continuously being added for the treatment of UTI. A major problem has created leading to increased morbidity, mortality and health care costs due to the extensive use of β-lactam antibiotics in hospital and community. The use of proper antibiotics is very important for various reasons. The emergence of multidrug resistant isolates and rapid spread are of great concern worldwide; among them, ESBL producing Enterobacteriaceae has been major concern. During the past decades, ESBLs producing Gram negative bacilli especially *E.coli and K.pneumoniae* have emerged as serious pathogens both in hospital and community acquired infections worldwide. This study was conducted to isolate *E.coli* and *K.pneumoniae* causing UTI and determining the status of MDR and ESBL producing uropathogens from the patients suspected of urinary tract infection visiting Holy family and khalifa gul Nawaz hospitals.

This study enrolled a total of 350 mid-stream urine samples who fulfil the inclusion criteria were screened for the study population. In analyzing my study, 85 (24.28%) showed significant growth out of the screened population. Similar results were obtained by Awasthi et al (Awasthi, Pant, & Dahal, 2015), Sharma et al (Sharma, Bhatta, Shrestha, & Banjara, 2013) and Paudel et al (Paudel, 2013) showed with the percentage of growth positivity of 23.87%, 25.5%, 27.3% and 29.9% respectively. Similarly, the study carried out in India by Niranjan et al (Niranjan & Malini, 2014) obtained 18.5% significant growth. However, our result is low as compared to that reported from South Africa (51%) by Habte et al (Habte, Dube, Ismail, & Hoosen, 2009).

The majority of urine specimen showed no growth (75.72%). The possible cause of low rate might be due to relatively small sample size and differences in the study population. It might also be due to urine sample obtained from patients were on antibiotic therapy.

The samples from outpatient department were 277 which was more as compared to hospital admitted patient samples 73. In the study, 64 of samples from outdoor patients and 21 of samples from indoor patients showed significant bacterial growth. This signifies more prevalence of UTI in community.

In this study, higher rate of infections was found in female patients 69/237 (29.11%) and the rate of infections was found to be 16/113 (14.15%) in male which was statistically significant difference between them (P<0.05). This result confirms and expands the previous finding of Shakya et al (Shakya, Shrestha, Maharjan, Sharma, & Paudyal, 2017), Chander and Shrestha (Chander & Shrestha, 2013), Chhetri et al (Chhetri et al., 2001) and Rajbhandari and Shrestha (Karki, 2023), Yadav et al (Yadav, Adhikari, Khadka, Pant, & Shah, 2015) in Nepal. Significant microbial growth was higher in case of female. The patient's sex is risk factor of UTI. Even though, everyone is susceptible to UTI, there are specific subpopulations that are at increased risk of UTI, including infants, pregnant women, and elderly patients with catheters, patients with diabetes, multiple sclerosis or acquired immunodeficiency syndrome (AIDS)/ human immunodeficiency virus (HIV) and patients with underlying urologic abnormalities. Females are more frequently affected by (particularly cystitis) due to colonization of urethra with colonic Gram-negative bacteria because of its proximity to anus and short length of urethra Forbes et al (Forbes, Sahm, & Weissfeld, 2007).

In this study, the highest percentages of growth were obtained from age group (21-30) years (31.76%) followed by age group (31-40) years (22.35%) and age group (41-50) years (14.11%). The highest percentage in some group was neglected because of relatively small sample size. There was significant association (P<0.05) between age group of patients and bacterial uropathogens. This study revealed a higher occurrence of uropathogens in the adult age group of 21-30 years, which is similar to that reported in a study done by Kattel et al (2012). The female may be the reason behind the maximum growth in these age groups because this age group consist sexually active women, frequent or recent sexual activity. The most important risk factor for UTIs in young women is frequent or recent sexual activity (Yadav et al 2015). Nearly 80% of all UTIs in premenopausal women occur within 24 hour of intercourse. In celibate women, UTIs are rare. The risk of UTI can also be increased by the use of certain types of contraceptives (Yadav et al 2015). Furthermore, the use of spermicidal coated condoms dramatically alters the normal bacterial flora and has been associated with increase in vaginal colonization with *E.coli* and in the risk of UTI (Braunwald et al., 2001).

The predominant pathogens of the UTI were Enterobacteriaceae. All together 85 bacterial isolates of two different genera were isolated. *E.coli* (72.95%) was the most common uropathogens isolated followed by *Klebsiella pneumoniae* (27.06%). Higher prevalence of *E.coli* seen in this study also resembled the study done by different other researchers viz. Singh et al (V. Singh, Tuladhar, & Chaudhary, 2015) and Bawankar et al (Bawankar, Enam, Panda, & Chandi, 2016). From all the above study, *E.coli* was the major pathogen concerned with UTI. As *E.coli* is a common pathogen which is usually a commensal bacterium of humans. Intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia are caused by pathogenic variants (Von Baum & Marre, 2005). *E.coli* is a predominant isolate, because *E.coli* can bind to the glycol-conjugate receptor of the uroepithelial cells of human urinary tract so it can initiate infection itself. *E.coli* is isolated in 90% of infection and strains are characterized by presence of unique virulence determinant the pilus (Gal-Gal) receptor (Von Baum & Marre, 2005). *E. coli*, including other enterobacteria, are likely to have caused infection after colonization of the periurethral area by gastrointestinal tract flora LK and DA (2016). Also, the fact that strain of *E.coli* affecting the Urinary tract possess a variety of virulence characteristics that facilitate their intestinal

carriage, persistence in vagina and then ascension and invasion of the anatomically normal urinary tract (Karki, 2023).

Antimicrobial resistance is now accepted as a major problem in public health and patient care. It is more troublesome to developing countries. In this study, *E.coli* was highly resistant to amoxicillin (69.6%) and least resistant to aztreonam (9.6%). The high sensitivity rate was seen towards aztreonam (75.8%) which was followed by nitrofurantoin (56.4%). This is similar to the finding by Shakya et al (Shakya et al., 2017) were resistance towards amoxicillin was found to be 73.3%. In case of *Klebsiella pneumoniae* the maximum resistance was seen against amoxicillin (91.3%), followed by ceftazidime (56.52%) and Gentamycin was found to be effective drugs with the sensitivity of (95.65%), followed by ciprofloxacin (82.6%). A report by Chandar and Shrestha (Chander & Shrestha, 2013) showed among the gram-negative bacteria, highest percentage of resistance towards first line antibiotics was found for Amoxycilin. Some of the isolates were also found resistant to third generation cephalosporin i.e, cefotaxime. Also, some isolates were found resistance to Cotrimoxazole, Ciprofloxacin. The study conducted by Perez et al accounted 94% *E.coli* isolates to be resistant to ceftriaxone. This high rate of resistance may be due to the lack of antibiotic policy and the irrational use of third generation cephalosporins, mainly ceftriaxone in the hospital (Kader & Angamuthu, 2005). The cause of increasing resistance is due to outrageous and unnecessary use of antibiotics for nontherapeutic complaints and treatment of UTIs empirically (Mukherjee, BaSu, MuKherjee, & MajuMder, 2013). However, overall aztreonam, nitrofurantoin, ciprofloxacin, gentamycin showed a less resistance and can be considered as the first line therapy.

A major problem in the management of uropathogens is MDR. MDRs were classified as resistant to two or more antibiotics (CDC 2006). In this study, (62.3%) isolates were MDR. The result accords with other studies showing 64% (Shakya et al., 2017), and 64.9% (Parajuli et al., 2017) of MDR isolates. The maximum MDR was found in *K.pneumoniae* (69.5 %), followed by *E.coli* (59.6%). The fact that drugs are easily available without doctor's prescription from pharmacy is the main reason behind the high degree of resistance. In developing countries like Nepal self-medication is a common practice and this too is a major cause of antibiotic resistance in clinical isolates. The development of resistance in clinical isolates is also promoted by expired antibiotics, self-medication counterfeit drugs, inadequate hospital control measures (Parajuli et al., 2017).

The leading cause of resistance to beta lactam antibiotics in Gram-negative bacilli is the production of beta lactamases. *E.coli* and *Klebsiella pneumoniae* were subjected to phenotypic laboratory detection of ESBL production. Among 85 bacterial isolates tested for ESBL production, 15(29.5%) bacteria were found to be ESBL producer. The majority consisted of *E.coli* 13/15 (20.9%) followed by *K.pneumoniae* 2/15 (8.69%). The result was confirmed by combined disc approximation test in which 3rd generation cephalosporin were combined with β-lactamases inhibitor clavulanic acid (i.e. CTX30+Clav10 and CAZ30+Clav10) in which the structural analog of βlactam antibiotics (clavulanic acid i.e. inhibitor) inhibits the action of βlactamase and antibiotic can act on the cell wall of the bacteria, and the result confirmed by at least or more than 5 mm increase in zone of inhibition than cephalosporin alone (Rawat et al., 2009).

The prevalence of ESBL producing Enterobacteriaceae varies greatly among country and among the hospitals within the country. Less than 1% to greater than 70% ESBLs producers are reported worldwide. In this study, the prevalence of ESBL production was higher in 21 - 30 years age group. The prevalence was higher in this age group as most isolates, accounting 53.84%, were isolated from this group. Moreover, self-medication practice which is high in this age group, could have further accounted for higher prevalence (Shankar, Partha, & Shenoy, 2002). A higher prevalence of ESBL production was observed in *E.coli* (20.9%) followed by *K.pneumoniae* (8.69%). The findings are in agreement with the study by (Aminzadeh, Kashi, & SHAABANI, 2008), however, contrary to the findings of other study (Ullah, Malik, & Ahmed, 2009) of 365 *E.coli* isolates, 33 (9.0%) were ESBL producers. Likewise, of 23 *Klebsiella pneumoniae* isolates, 2 (8.69%) were ESBL producers.

Detection methods are based on a phenotypic profile that has potential to yield false positive and false negative results. In some of the isolates, additional mechanisms of resistance, such as AmpC- β-lactamases, porin changes and inhibitor resistant TEMs (IRTs) and SHV β-lactamases with reduced affinities for β-lactamase inhibitor can mask clavulanic acid (CA) inhibition. Current Clinical and Laboratory Standards Institute (CLSI 2013) recommend the use of a screening and confirmation test, in addition to standard susceptibility testing methods, to detect extended spectrum β-lactamases (ESBLs) in the routine clinical laboratory among the strains of *E.coli* and *K.pneumoniae*. This method has proven reliable over many years in detecting the great majority of conventional ESBLs, particularly of variants of TEM and SHV enzyme class. The CLSI method, however, does not address the significance of strains that are positive on the screening test but negative on the confirmation test. By default, the result of standard susceptibility test (e.g. broth micro dilution or disc diffusion) is applied to organism with this ESBL test profile. It is important for both clinical and infection control reasons to detect strains harboring transmissible resistance mechanisms to extended spectrum cephalosporin (Bell et al., 2007).

In modern medical practice, newer antimicrobial drugs have been unextensively in emergence and rapid dissemination of resistant bacterial strain. ESBL producing strains are mostly associated with UTIs. UTIs is a common bacterial disease, often contributes to a frequent cause of morbidity in outpatients as well as hospitalized patients. Clinical experience has indicated the pressure of numerous cases of antibiotic resistance to common antibiotics by uropathogens in both developed and developing countries. In many parts of pakistan, the facilities for urine culture and antimicrobial susceptibility testing are still not available, leading to improper diagnosis and irrational antibiotic treatment (e.g. self-medication) of UTI. The updated knowledge and situation of the prevailing bacterial uropathogens that are multidrug resistant (MDR) is of prime importance for the proper use of antimicrobial drugs and the policy making to combat multi drug resistance in UTIs (Baral et al., 2012).

A significant finding in this report was that for the ESBL producing Enterobacteriaceae, Nitrofurantoin was found to be effective against the isolates *E.coli* (92.3%), and impenem was found to be 100% similar to *K.pneumoniae*. Meropenem was also found to be effective against *E.coli* (69.2%). For UTIs causing isolates, Nitrofurantoin was also found to be optimal drugs. This may be due to the restricted use of these drugs in our hospital setting and nitrofurantoin is usually reserved to be prescribed only in case of UTIs since it is excerted and concentrated in urine. These indicated that imipenem, meropenem and nitrofurantoin are the drug of choice for treating serious infections caused by ESBL producing

microorganism. Imipenem, belonging to the carbapenem group, are extremely potent and broad spectrum β-lactam antibiotics as it is resistant to most β-lactamase (Baral et al., 2012).

Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today. Therefore, detection of the ESBL producing strains is of significant important for all major hospital worldwide as these strains are most likely to be even more prevalent then it is currently recognized, difficulty in their detection by current clinical methods, constitute a serious threat to current Beta lactam therapy, and institutional outbreaks are increasing because of selective pressure due to the heavy use of extended-spectrum cephalosporins and also due to lapses in effective infection control measure (Rawat et al., 2009). However, antibiotic susceptibility testing should be performed for each strain before prescribing antibiotics. Correct precaution against ESBL should be taken before the organism begins to develop resistance mechanism against antibiotics. Awareness and health problem can be effective. Although most of the outbreaks were limited to the high-risk patient care areas such as ICUs, oncology units etc.

Therefore, now a day the threat of the ESBL is not limited to only in ICUs or the tertiary care hospitals, but they are also found in OPD patients. The Clinical and Standard Laboratory Institute (CLSI) have issued recommendation for ESBL detection, for the reporting for organism other than *E.coli* and *Klebsiella pneumoniae* (Dalela, 2012).

6.1 Conclusion

The study found that the prevalence of multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae was 15.14% and 4.28%, respectively, among patients at Holy Family Hospital and Khalifa Gul Nawaz Hospital in Pakistan. Infections were significantly higher in female patients (29.16%) compared to males, particularly among the 21-30 age group. E. coli was the most common uropathogen, with a significant association between MDR and ESBL production. Imipenem showed 100% sensitivity for ESBL-producing E. coli and Klebsiella pneumoniae, while Nitrofurantoin and Meropenem also demonstrated high effectiveness. These findings highlight the need for strategic policy initiatives to address the increasing prevalence of ESBL strains and the importance of early detection methods for appropriate antibiotic use.

6.2 Recommendations

1. Regular monitoring of female patients due to higher ESBL-associated UTI prevalence.

2. Use Imipenem, Meropenem, and Nitrofurantoin for treating ESBL-producing E. coli and Klebsiella spp.

3. Consider ESBL-producing organisms and request tests if patients do not respond to third-generation cephalosporins.

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