

Multiple drug resistance and ESBL production in bacterial urine culture isolates

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Abstract: Transmission of bacterial strains between patients is a serious problem in hospitals and with the increasing rate of antibiotic resistance the problem has farther escalated. Enterobacteriaceae produced ESBLs, especially *E.coli*, are increasingly important nosocomial pathogens. These bacteria are often multiple resistant and are responsible for many intestinal infections and urinary tract infections. Urine samples [4010] were collected cultured and the bacterial isolates were identified in this study, 1000 isolates showed significant bacterial growth. Among the sample 1000 showed bacterial growth in which *E.coli* strains was most common 58.5% of the 1000 bacterial isolates from urine cultures, gram negative rods accounted for 95.30 %, while gram positive cocci accounted for the test 4.70 %. Total pathogen isolated and recovered is distributed as *K. pneumoniae* 16.7 %, *Enterobacter* spp 0.57 %, *P. aeruginosa* 14.5 %, *Proteus* spp 1.34 % *Enterococci* 1.05 %, *S. aureus* 0.76 % and *E. faecalis* 2.87 %, *A. calcoaceticus* 1.05 %, *Enterobacter* spp 0.57 % *E. agglumarance* 2.20 % *serratia* 0.1 %. In case of g negative bacteria 58 [2.45 %] were ESBL producers and 379 [47.54 %] were MDR. while in case of gram positive 2 [0.2 %] were MRSA. Resistance has arisen to all antibiotics introduced into general clinical practice and is likely to arise to any new antibiotics introduced in the future. It is therefore imperative to consider what can be done to minimize the development and transfer of antibiotics resistance gene clusters. Methods can be developed to minimize antibiotic resistance.

Keywords: MDR, ESBL, Bacteria, UTI

1. Introduction

Multiple drug resistance is a condition enabling a disease causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism. Organisms that display multidrug resistance can be pathologic cells, including bacterial and neoplastic [tumor] cells [1]. Multidrug resistant [MDR] isolates are even more likely to be associated with complications in the therapeutic management of patients with infectious diseases [2].

Extended spectrum beta lactamases [ESBLs] are one of the most important causes of resistance to penicillin and cephalosporins in *E.coli* and *Klebsiella* spp. ESBLproducing bacteria are also frequently resistant to aminoglycosides, trimethoprim sulfamethoxazole and

quinolones. This situation restricts the treatment choices [3]. Antimicrobial resistance among the Enterobacteriaceae, a group of highly pathogenic Gram negative organisms, has increased dramatically in recent years against commonly used antimicrobials such as the tetracyclines, β lactams, fluoroquinolones, aminoglycosides and co trimoxazole [4]. This is particularly true among pathogens such as *Escherichia coli* and *Klebsiella pneumoniae* that produce extended spectrum β lactamases [ESBLs] [5], a class of enzymes that has been strongly correlated with multidrug resistance among the *Enterobacteriaceae* [6]. However, study during the last two decades found that bacterial resistance mediated by plasmids which carry resistance gene to a large number of antibiotics, which are rapidly transferred has worsened the scenario [7].

Urinary Tract Infections [UTIs] are one of the most

common bacterial infections in humans, both in the community and the hospital settings [8, 9]. UTIs are amongst the most prevalent infectious diseases affecting approximately 150 million people worldwide annually which results in more than 6 billion US dollars loss to the global economy [9, 10]. The lifetime risk for UTI in females is greater than 50% [11]. In the United States, about 8 million physician visits and more than 100,000 hospital admissions per year are due to UTIs [12]. UTIs are mostly caused by *E. coli* accounting for more than 70% of uncomplicated cases both in outpatients and inpatients [13]. Other gram negative bacteria include *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa*, *Proteus* spp. Gram positive bacteria account for 5 to 15% of UTIs and include *Enterococcus* spp., *Staphylococci*, and *Streptococci* [14, 15].

The aim of the present study is to explore the possibility of developing “MDR and ESBL Production in Bacterial Urine Culture Isolates, to check the ratio and frequency of MDR and ESBL bacteria, to analyze their susceptibility profile to commonly used antibiotics for uropathogen and Age wise Prevalence of MDR and ESBL.

2. Materials and Methods

2.1. Human Urine Samples

Urine samples [4010] of male and female of UTI [urinary tract infection] patients [child, adults, old age] of PIMS hospital were preceded for MDR and ESBL detection.

2.2. Bacterial Strains

Three bacterial strains used in the tests [used for antibacterial activity of blood] were kindly provided by Mr. Sardar Atiq Fawad, Head, Diagnostic Division, Qarshi Industries [Pvt.] Ltd, District Haripur, Pakistan, under Material Transfer Agreement [MTA]. These bacterial strains included; *Staphylococcus aureus* ATCC®6538, *Escherichia coli* ATCC®25922 and *Pseudomonas aeruginosa* ATCC®27853

2.3. Collection of Urine Sample [Clean Catch Midstream Method]

Samples were taken from different wards and OPDs of Pakistan Institute of Medical Sciences, Islamabad. These pathological samples were preceded for isolation of pathogens. While collecting urine for culture, care should be taken to avoid contamination with normal flora of the anterior urethra or perineal skin. The common method of collection is midstream clean catch [“urine, clean catch midstream,” The Jewish Hospital, Inc.8/1/95].

2.3.1. Urine Sample Processing

All urine specimens were inoculated on CLED agar using bacteriuria strips [meditest, UK]. And these plates were incubated aerobically at 35±2 °C for 18 hours. Identification of culture isolates. After overnight incubation,

established microbiological methods, which include colonial morphology, Gram's stain reaction and biochemical characteristics, were used for identification.

2.3.1.1. Grams Staining

2.3.1.2. Reagents

1] Violet dye Crystal violet 10g; Absolute alcohol 100 ml; Distilled water 1000 ml.

2] Iodine solution Iodine crystals 10g; Potassium iodide 20 g; Distilled water 1000 ml.

3] Decolorizer Absolute alcohol.

4] Counter stain Safranin 0.5% in diluted ethanol.

2.3.1.3. Procedure

The direct smear from the thick and tenacious part of clinical samples and the culture smear from the suspected Enterobacteriaceae colony was prepared, air dried and heat fixed. Smear was flooded with 1% Crystal violet [Primary stain] for 1 minute, and washed with water. Smear was decolorized with absolute alcohol till no colour was seen to flow out of the preparation. Smear was counterstained with 0.5% safranin for 1 minute. The smear was washed with water, air dried and observed under oil immersion objective for the presence of inflammatory cells and organisms. Gram negative organism's tissue elements take up pink [red] colour.

2.3.2. Turbidity Standard Solution [0.5 McFarland Standard]

1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulfuric acid to 99ml of water. 1.75% w/v solution of barium chloride was prepared by dissolving 2.35g of dehydrated barium chloride in 200ml of distilled water. 0.5% turbidity standard was prepared by adding 0.5ml of BaCl₂.2H₂O solution to 99.5ml of sulphuric acid solution and constantly stirred. This was stored in dark at room temperature.

2.3.3. Biochemical Identification of Isolates

Isolates were identified by API 20E kit [Biomérieux, USA] is a standardized identification system for Enterobacteriaceae. It has 20 miniaturized biochemical tests. A strip contains 20 micro tubes containing dehydrated substrates. These tests were inoculated with bacterial suspension. During incubation, metabolism produced color changes that were either spontaneous or revealed by the addition of the reagents.

2.4. β Galactosidase Test

Ortho NitroPhenyl β D Galactopyranosidase was identified by using the substrate 2 nitrophenyl β D galactopyranoside [ONPG]. If β galactosidase was produced by the isolate, it turned the colorless solution to yellow. Bacteria require this enzyme to hydrolyze lactose.

2.4.1. Arginine Dihydrolase [ADH] Test

Bacteria require ADH to breakdown Arginine, an amino acid. Yellow color of the solution was turned red or orange due to its activity.

2.4.2. Lysine Decarboxylase [LDC] Test

L-lysine, an amino acid is hydrolyzed by lysine Decarboxylase. It turned yellow color of the solution to red or orange.

2.4.3. Ornithine Decarboxylase [ODC] Test

L-ornithine is broken down by ornithine Decarboxylase. Positive test was indicated by red or orange color of the yellow colored solution.

2.4.4. Citrate Utilization Test

Bacteria use trisodium citrate as an energy and carbon source. Green color was turned blue if bacteria used citrate.

2.5. H₂S Production Test

H₂S production was detected by the blackening of the media. Media contained sodium thiosulphate and ferric citrate.

2.5.1. Urea Test

Urea is broken down by urease enzyme. Yellow color of the solution was turned red or orange in case of positive test.

2.5.2. Tryptophane Deaminase [TDA] Test

L-Tryptophane, an amino acid is hydrolyzed by tryptophane deaminase enzyme. Immediate color change was noted after adding TDA reagent. Positive result was indicated by reddish brown color of the yellow colored solution. TDA reagent contained ferric chloride.

2.5.3. Indole Production Test

L-Tryptophane is degraded by enzymatic action to Indole, by the bacteria. Ehrlich's reagent was added and change in color from colorless to pink was noted. Ehrlich's reagent contained benzaldehyde.

2.6. Voges Proskauer [VP] Test

If Pyruvate was converted to acetone its production was determined by addition of reagents containing alpha-naphthol and potassium hydroxide. Pink or red color was considered positive after 10 minutes of addition of solution. No appearance of color was considered as negative result.

2.6.1. Gelatin Hydrolysis Test

Gelatinase broke down the gelatin. Black color throughout the tube indicated the positive test.

2.6.2. Carbohydrate Test

Fermentation or oxidation of different sugars was detected by production of acid. Acid turned the blue/blue-green color of solution to yellow. D glucose, D mannitol, inositol, D sorbitol, L rhamnose, D sucrose, D melibiose, amygdalin, and L arabinose were added in the panel.

2.6.3. Motility Test

The motility of test organisms was determined by stabbing the semisolid motility media with sterile wire loop. Growth was observed along with the line [non-motile] or

growth spreading away from the line in media [motile].

2.6.4. Oxidase Test

Cytochrome oxidase enzyme activity was determined by using freshly prepared oxidase reagent and poured on filter paper. Colonies of isolates were spread on the paper and blue color within 10 seconds was observed.

2.6.5. Preparation of Inoculum

Growth method was used to make bacterial suspension. Bacterial suspension was used within 30 minutes. Four colonies of isolate were transferred to nutrient broth. Broth was then placed in incubator shaker at $36 \pm 1^\circ\text{C}$ if required until the visible turbidity was equal to or greater than the 0.5McFarland standard.

2.7. CLED Media

CLED media are non inhibitory differential media used for the cultivation and presumptive identification of urinary tract pathogens. Same with 58.0 ml of Andrade's Indicator a Final pH 7.5 ± 0.2 at 25°C

2.8. Preparation

Suspend 36 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C , mix well and dispense into plates. When the medium is solidified, invert the plates to avoid excess moisture. Store at $2-8^\circ\text{C}$. The color of the prepared medium is green. The dehydrated medium should be homogeneous, free-flowing and greenish beige in color. If there are any physical changes, discard the medium.

2.9. Uses

CLED Agar is a non-selective differential plating medium for the growth and enumeration of urinary tract microorganisms. Omitting sodium chloride inhibits the Proteus swarming and supports the growth of the vast majority of bacteria causing urinary tract infections, and is used to differentiate and identify them. The presence of bacterial contaminants like Diphtheroids, Lactobacilli and other microbes indicate the degree of care taken with the handling of the urine specimen.

2.9.1. Urine Sample Inoculation

Prior to inoculation, the medium should be brought to room temperature. Using a calibrated loop, inoculate the urine specimen onto the surface using standard microbiological procedures to obtain isolated colonies. Incubate aerobically at 35°C for 18-24 hour.

2.10. Mueller Hinton Agar

Mueller Hinton agar [MHA] [Mueller and Hinton, 1941] was used to perform antibacterial susceptibility testing and control antibiotics as it is an antimicrobial susceptibility testing medium which may be used in

internationally recognized standard procedures [CLSI, 2000].

2.10.1. Composition of Mueller Hinton Agar

Dehydrated beef infusion 300.0 g/l, Casein hydrolysate 17.5 g/l, Starch 1.5 g/l, Agar 17 g/l and PH 7.3 ± 0.1 @ 25°C .

2.10.2. Disk Agar Diffusion Method

Overnight fresh cultures were used to make lawns on Mueller-Hinton agar [MHA]. Suspension was made as turbid as 0.5% McFarland standard. 6 disks were placed on the 90mm Petri plates as using modified Kirby-Bauer method. Each bacterial suspension with the help of cotton swab was used to make lawn. The inoculums were spread evenly on the entire surface of MHA plate in three directions. The plate was allowed to dry for 5 minutes. Antibiotic disks were aseptically placed at reasonable equidistance on the prepared lawn [16]. The plates were incubated at $36 \pm 1^{\circ}\text{C}$ for 16 to 18 hours in ambient air. *E. coli* ATCC 25922 was used as control strain in every test run. Zone of inhibitions in millimeter were measured, recorded and the isolates were classified as “resistant”, “intermediate”, “sensitive” according to clinical laboratory standard institutes criteria [CLSI, 2006].

2.11. Antibiotics

Aztreonam ATM[30 μg], ceftazidime CAZ[30 μg], cefpodoxime CPD[10 μg], ceftriaxone CRO[30 μg], cefepime FEP[30 μg], amoxicillin/clavulanic acid AMC[20/10 μg], cefoxitin FOX[30 μg], imipenem IPM[10 μg], trimethoprim-sulfamethoxazole SXT[1.25/23.75 μg], chloramphenicol C[30 μg], tetracycline TE[30 μg], ciprofloxacin CIP[5 μg], amikacin AK[30 μg], Tigecycline TGE[15 μg], piperacillin PRL[100 μg], nitrofurantoin F/M[300 μg], cefoperazone/sulbactam SCF[95/10 μg], piperacillin/tazobactam TZP[100/10 μg], were used [Oxoid, UK].

2.12. Detection of Extended Spectrum Beta Lactamase [ESBL] Production

Evaluation of isolates for ESBL production was done by double disk [potentiation] test.

2.13. B Lactamase Investigations

Extended spectrum β lactamase production of clinical isolates were investigated by Double Disk Diffusion Method using aztreonam, ceftazidime, cefpodoxime and ceftriaxone in close proximity of 20 to 30 mm center to center from amoxicillin/clavulanic acid disk. Cefepime is placed 25mm away center to center from piperacillin/tazobactam disk.

3. Results

The present study was conducted on different urine culture isolates from PIMS, Islamabad from Nov 2009 to May 2010 to determine the frequency of MDR [multiple drug resistance] & ESBL [extended spectrum beta lactamase] producers.

Total 4010 urine samples were collected from different units of PIMS Islamabad, out of these, 24.93% [n = 1000] were positive isolates and rest of all 75.6% [n = 3010] were contaminants. Out of these positive isolates 37.9% [n = 379] were MDR producers and 5.8% [n = 58] were ESBL producers.

3.1. Colonial Morphology

All the isolates were identified by their growth on Cled agar. Isolated colonies of all the organisms on this agar were used to study colony characteristics. All the positive isolates were preceded further for standard identification and susceptibility [Table 1].

Table 1. Characteristics of different organisms on Cled agar.

Organisms	Cled Agar	Cled Agar with Andrade's indicator
<i>E. coli</i>	Yellow, clear, translucent ,bright pink colonies	Clear colonies with pink halogranular surface in surrounding media
<i>Proteus spp</i>	Blue-green, small translucent colonies	Blue translucent colonies
<i>Klebsiela</i>	Grey-green mucoid colonies	Grey-green mucoid colonies
<i>Enterobacter spp</i>	Yellow to white translucent colonies.	Yellow to white translucent colonies.
<i>Pseudomonas spp</i>	Green to blue -green colonies with blue green matted surface and rough periphery	Green to blue -green colonies with blue green matted surface and rough periphery
<i>S. aureus</i>	Yellow opaque colonies	White to pale pink colonies opaque
<i>S. epidermidis</i>	White opaque colonies	White to pale pink colonies
<i>E. faecalis</i>	White opaque raised colonies	Deep yellow orange colonies

3.2. Criteria Used for MDR

Criteria used for MDR in this study were “Any organism that was resistant to at least 3 or more than three groups of antibiotics was said to be MDR [multiple drug resistant]”.

3.3. Susceptibility Testing

All the isolates were tested against a panel of 20 different antibiotics i.e. AMC [30 μg], CAZ [30 μg], CRO [30 μg], IPM [10 μg], AK [30 μg], ATM [30 μg], LEV and SCF

[95/10µg], FEP [30µg], TZP [100/10µg], FOX [30µg], CIP [5µg], SXT [1.25/23.75µg], VA, FD, CXM, CN [10µg], E, CTX, MEM [10µg]. 58 isolates were presumed to be MDR and they have proceeded further for ESBL production.

3.4. ESBL Detection Test

In this test the organism was swabbed onto a Mueller Hinton agar plate. A susceptibility disk containing Amoxicillin-clavulanic acid is placed in the centre of the plate and disks containing one of the oxyimino beta lactam antibiotics are placed 30mm [centre to centre] from the Amoxicillin clavulanate disk. Enhancement of the zone of inhibition of the oxyimino β lactam caused by the synergy of the clavulanate in the Amoxicillin clavulanate disk is a positive result as shown in Figure 1.

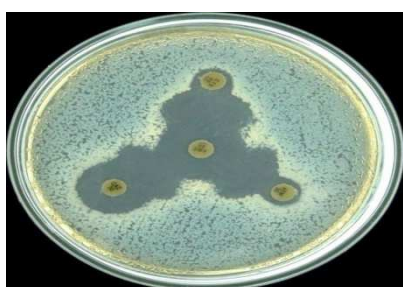


Figure 1. Double disc synergy test [DDST]

3.5. The Prevalence of ESBL Producers

Out of 1000 positive isolates, 37.9% [n=379] were MDR [Multiple drug resistant] ESBL production was detected in 5.8% [n=58] isolates by phenotypic method, while the remaining were ESBL negative 32.1% [n=321] out of 379 in total MDR.

3.6. Gender Distribution

Of the 1000 isolates, MDR [multiple drug resistance] isolates, 203 [20.3%] were obtained from male patients, of which 26 [12.8%] were ESBL positive and rest of all were ESBL negative and 172 [84.7%] from female patients, of which only 32 [18.6%] was ESBL positive and remaining were ESBL negative. In this study the prevalence of ESBL producers was higher in female than in male. Overall, 55.1%

[32/58] of ESBLs were from females comparing to those from males; 44.8% [26/58] Figure 2 and 3.

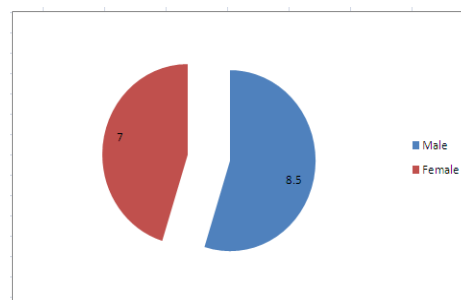


Fig 2. Male to female ratio in MDR producers

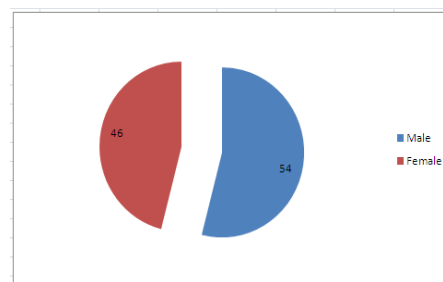


Fig 3. Male to female ratio in ESBL producers

3.7. Sample Origin Distribution

MDR [Multiple drug resistant] isolates were more prevalent at Emergency, 14.7% [n =147]; followed by urology 9 % [n =90], Nephrology 5.2% [n =52], and Med. Wards 9.2 % [n =92], MICU 2.5 % [n =25], PWGF 2.5 % [n =25] A&E 1 % [n =10] respectively. Out of 147 isolates from Emergency; 10.20% [15/147] were ESBL producers. Out of 10 isolates from A&E; 10 % [1/10] were ESBL producers and out of 92 isolates from Medical wards; 10.86% [10/92] was ESBL positive and out of 52 isolates from Nephro;9.61% [5/52] were ESBL producers and out of 25 isolates from MICU;20% [5/25] ,out of 90 isolates from Urology;14.4% [13/90] were ESBL producers and out of 25 isolates from PWGF;12% [3/25] were ESBL producers based on phenotypic detection [Figure 4].

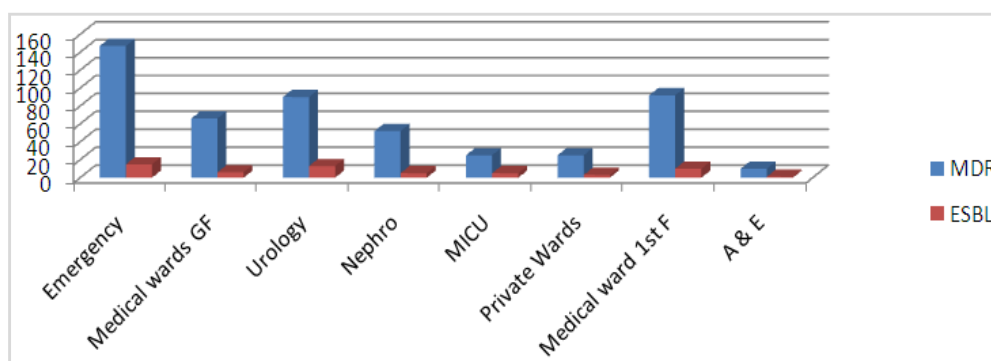


Figure 4. Hospital wise distribution of MDR and ESBL producers

3.8. Age Distribution

The age of patients was categorized into four groups. Common age group for sample isolation was 21-40 years from which 88 were MDR and only 18 was ESBL producer. From age group 0-20 year 75 were MDR and 9 were ESBL producers. From 41-60 age group 157 were MDR and 16 were ESBL producer and from 61+ age group 95 were MDR producers and 15 were ESBL producer Figure 5.

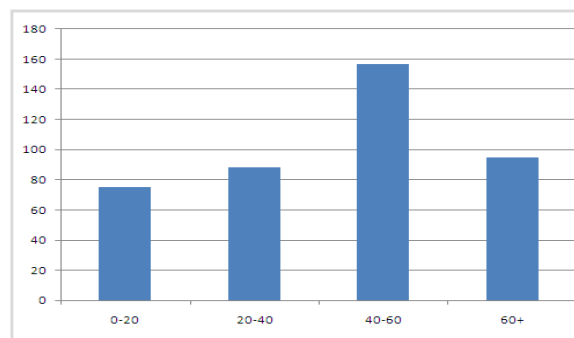


Figure 5. MDR producing isolates related to age

Table 2. Percentage resistance of antibiotics to different organism

	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Pseudomonas ssp</i>	<i>Proteious spp</i>	<i>Enterococcus cloacae</i>	<i>Acinobactor ssp</i>	<i>Step. ssp</i>	<i>Enterococci ssp</i>
Ampicillin	NT	NT	NT	NT	NT	NT	NT	NT
Amoxicillin	40.37	73.43	70.43	35.71	48.27	90.90	NT	NT
Nalidixic Acid	69.28	54.84	86.95	73.33	68.96	81.81	NT	NT
Nitrofurantoin	16.58	36.53	50	56.4	41.32	54.54	NT	NT
Co-trimoxazole	87.37	59.61	51.17	21.41	54.54	54.54	NT	NT
Norfloxacin	60.97	57.29	65.21	36.36	24.37	77.21	NT	NT
Piperacillin	4.39	5.76	15.21	28.57	6.86	81.81	NT	NT
Amikacin	8.78	9.61	21.73	7.14	55.17	36.36	NT	NT
Ceftazidime	41.46	42.3	39.13	7.14	13.79	90.9	NT	NT
Imipenem	0.97	3.84	13.04	35.71	82.71	19.9	NT	NT
Cefixim	53.65	65.38	58.69	0	51.73	90.9	NT	NT
Ceftriaxone	45.8	46.15	69.59	53	65.95	81.81	NT	NT
Cefepime	42.96	42.3	36.95	42.83	68.95	90.9	NT	NT
Carbenicillin	63.41	71.15	65.21	71.42	48.27	81.21	NT	NT
Levofloxacin	41.95	46.15	41.36	28.57	34.43	81.2	NT	NT

4. Discussion

Multiple antimicrobial resistances among gram negative organisms have been a long term and well recognized problem with urinary tract infections. This study tried to determine frequency of extended spectrum β lactamase [ESBL] positive and multidrug resistance pattern in uropathogen bacteria. Antibiotic resistance in uropathogens is increasing worldwide. Multi drug resistance is a major problem in the management of uropathogens [15].

A prospective study was carried out in PIMS Islamabad in order to determine the frequency of multidrug resistance [MDR] and extended spectrum β lactamases [ESBL] producing uropathogen from community acquired urinary tract infection [UTI].

Urine is the most common sample to be received in a microbiology laboratory. UTI is the most common bacterial infection. It is one of the common causes of morbidity in

the general population and is the second most important cause of hospital acquired infection [17]. There are estimated 150 million urinary tract infections per annum worldwide [10]. In the community, women are more prone to develop UTI. About 20% of women experience a single episode of UTI during their life time and 3% of women have more than one episode of UTI per annum [18]. In children approximately 5% of girls and 1% boys have UTI by 11 year of age. Pregnancy also makes more susceptible towards infection. *Escherichia coli* are the most frequently isolated bacteria in both communities acquired and hospitalized patients [19].

Out of 4010 urine samples, 1000 [24.9%] showed the growth of significant bacteriuria also observed such a low rate of growth positive for UTI 20-23. In our study gram negative bacilli showed 90.30% growth and gram positive cocci showed 4.70% growth which is in contrast to the study done. Their study also showed the growth of 90.7% gram negative bacilli and 9.3% gram positive cocci. The 13

pathogens were isolated by 24 pathogens and 21 pathogens [20]. According to the study, the commonest bacterial species isolated was *E. coli* [77.5%] followed by *Klebsiella* spp. [7.1%], *A. calcoaceticus* [2.3%], *P. aeruginosa* [1.3%] among gram negative isolates whereas *S. aureus* [5.7%] was the commonest one followed by *S. saprophyticus* [2.3%] and *E. faecalis* 1.2%] among gram positive isolates, whereas in our study the commonest bacterial species isolated was *E. coli* [58.5%] followed by *Klebsiella* spp. [17.2%], *P. aeruginosa* [15.2%] *A. calcoaceticus* [1.1%] among gram negative isolates whereas the commonest one followed by *S. saprophyticus* [3%] and *E. faecalis* 1.3%] *S. aureus* [0.8%] among gram positive isolates. Gram positive cocci accounted for 4.70% of the uropathogens tested in this study, with *Staphylococcus* spp. being predominant. It was found that the antibiotic resistant pattern of the gram positive cocci is less than that of gram negative pathogens. Resistance to Penicillin was most common in *Staphylococcus* spp. *E. coli* isolates were most commonly resistant to Ampicillin, Co-trimoxazole, and Nalidixic acid followed by the Norfloxacin, Tetracyclin, and Cefotaxime. On the other hand Imipenem [100%], Levofloxacin [41.%], and Amikacin [8.7%] have a high potency against *E. coli* isolates as well as other gram negative uropathogens were tested. Similar antibiotic susceptibility pattern was observed with other gram negative microorganisms including *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., and *P. aeruginosa* which go consistently with other reports [21, 22, 23, and 24]. β lactam drugs are commonly included in the empirical antibiotics treatment of gram negative pathogens; however ESBL producing bacteria may not be susceptible to such treatment. These findings showed that 5.8% of UTI pathogens tested were ESBL producing strains out of 17.24% were *Klebsiella* spp., 80% were *E. coli* and 3.44% were *Proteus* spp. The high prevalence rate of ESBL producing strains have been previously reported in a number of studies [13, 15]. A 30.3% of *E. coli* isolates ESBL producers, which is higher than reported figures in Canada [2.7 - 6.2%] and USA [2.2 - 6.6%] and is lower than those reported in India [41 - 40%]. ESBL prevalence varies in different countries. ESBL prevalence of 67, 42 and 43% has been reported in *E. coli* from Iran, India and Bangladesh respectively. While less than 1% of *E. coli* isolates produce ESBL in the Scandinavian countries. In this study, 5.8% isolates were ESBL producers. Carbapenems have better activity against ESBL producers [25].

5. Conclusion

Resistance has arisen to all antibiotics introduced into general clinical practice and is likely to arise to any new antibiotics introduced in the future. It is therefore imperative to consider what can be done to minimize the development and transfer of antibiotics resistance gene clusters. Methods can be developed to minimize antibiotic resistance.

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