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ORIGINAL ARTICLE

Extended-spectrum beta-lactamase and Antibiotic susceptibility pattern in uropathogens.

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ABSTRACT… Objective: To identify rapidly changing antibiotic susceptibility of ESBL & Uropathogens. **Study Design:** Comparative Cross-sectional prospective study. **Setting:** Allied Hospitals of Peshawar Medical College. **Period:** 8th November 2021 to 5th March 2022. **Material & Methods:** 10-15ml of midstream urine was collected in a sterile container from 158 patients Pus cells, red blood cells, and bacteria were examined using microscopy. The standard loop technique was used to inoculate urine specimen on MacConkey and Blood agar. Plates were incubated for 24 hours at 37°C. A colony count of 10^5 cfu/ml was thought to be significant. Gram staining of the colonies was performed. The biochemical tests were conducted on API 10S for the identification of organisms. Extended-spectrum beta-lactamase organisms were identified by the double-disc synergy method. The Kirby-Bauer disc diffusion method was used to test antibiotic susceptibility on Mueller-Hinton agar according to CLSI guidelines 2021. A statistical package for the social sciences (SPSS) version 20.0 was used for statistical analysis. **Results:** Out of 158 urine samples 135 (85.5%) had positive culture growth with 35 (25.9%) ESBL confirmed. Antibiotic susceptibility was Nitrofurantoin (70.1%), Trimethoprim-sulfamethoxazole (26.8%), Ciprofloxacin (51.5%), Levofloxacin (51.5%), Ceftriaxone (25.77%) Cefotaxime (23.7%) Ceftazidime (19.5%) Cefepime (27.8%) Aztreonam (2.1%) Meropenem (86.6%) Amoxicillin/clavulanic (37.1%) Gentamycin (73%) Penicillin (0%). **Conclusion:** Surprisingly, only nitrofurantoin was found to be advised orally as a suitable drug for the treatment of UTIs among the 13 commonly used antibiotics.

Key words: Antibiotic Susceptibility, ESBL, MDR, UTI.

INTRODUCTION

Extended-spectrum beta-lactamase (ESBLs) production is emerging as a challenge with a higher incidence of resistance for the treatment of hospital as well as community-acquired infections.[1] Long-term use of antibiotics, prolonged stay in hospitals, especially intensive care units (ICUs), nursing homes, compromised immune system, chronic illness, and instrumentation, such as catheterization, are major risk factors for increasing the incidence of extended-spectrum beta-lactamase-producing bacteria and MDR.[2]

Gram-negative bacteria that produce ESBLs act in three ways: first, -lactam cannot access penicillin-binding proteins (PBP); second, decreased affinity for PBP; and third, hydrolysis of antimicrobials' -lactam ring. Bacterial resistance to routinely prescribed antibiotics such as penicillins, 1st, 2nd, and 3rd generation cephalosporins, and aztreonam is conferred as a result of this mutation.[3,4] The presence of extended-spectrum beta-lactamase producing Gram-negative bacteria significantly increases antimicrobial resistance globally, leading to increased morbidity and mortality. Carbapenems are the only effective drugs available for the treatment of ESBL-producing organisms. However, bacteria resistant to carbapenem are emerging due to their inadvertent use. An increase in resistance to currently available oral antibiotics is a significant challenge for physicians to treat patients outside the hospital setting.[5]

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Uropathogen identification is important for the successful treatment of the patient suffering from UTIs, and blindly starting the empirical treatment should be avoided. This will help in the successful completion of treatment and reduce the chances of bacterial resistance due to inappropriate use of antibiotics. Annually around 700,000 deaths, due to antimicrobial resistance (AMR) are recorded. If proper monitoring and prevention measures are not done, AMR will become one of the leading causes of death among hospitalized and non-hospitalized patients in developing nations.[6,7]

In our country essay access and lack of restrictions against the use of antibiotics have led to an increase in resistance to the commonly prescribed antibiotics. This has given rise to MRD and ESBL producing uropahtogens which show regional variations. Therefore, it is important to conduct research so that we can acquire knowledge of the frequency of different uropathogens and their antibiotic susceptibility pattern. This will allow us to develop effective treatment options and help us make policies that can stop the spread of MDR and ESBL producing uropathogens. This study is planned to identify rapidly changing antibiotic susceptibility patterns in ESBL uropathogens.

MATERIAL & METHODS

This Comparative Cross-sectional prospective study was conducted from November 2021 to March 2022. Samples were collected from allied hospitals of Peshawar medical college and were processed at the Microbiology laboratory of Peshawar medical college.

Inclusion Criteria

Urine samples of outpatients with symptoms of UTI or inpatients having indwelling urinary catheter in place for at least 48 hrs were collected from allied hospitals of Peshawar medical college.

Sample size has been calculated with open epi info sample calculation (95%CL) based on 11.6 % prevalence of UTI in symptomatic patients of our population margin of error 5% and absolute precision 0.05% sample size will be 158.[8] Non-probability convenience sampling technique was used.

10-15ml of midstream urine was collected in a labeled sterile container while in catheterized patients samples were collected directly in the sterile container from the catheter while separating the nozzle attached from the bag and then cleaned with an alcohol swab.

Ethical approval was taken from the Institutional Review Board (Prime/IRB/2021-385) of the Prime Foundation.

Uropathogen Isolation & Identification

After centrifuging the urine, a drop of sediment was put on a clean slide and microscopy was done for pus cells, red blood cells, and bacteria using the high-power field. On MacConkey and Blood agar, the conventional loop approach was utilized for inoculation. For 24 hours, plates were incubated at 37°C. It was determined that a colony count of 10^5 cfu/ml was significant. The colonies were stained with Gram staining. Biochemical assays were performed, including oxidase, citrate, urease, indole, methyl red, and Voges Proskauer tests on API 10S.

Extended-spectrum Beta-lactamase

In accordance with CLSI recommendations, a modified double-disc synergy test was utilized to determine ESBL activity using a combination of ceftriaxone (30 g), cefotaxime (30 g), ceftazidime (30 g), aztreonam (30 g), and amoxicillin/clavulanic acid (20/10 g). The four antibiotics were spaced 20 mm apart in the center of the plate, from end to end of the amoxicillin/clavulanic acid disc. After 24 hours of incubation, the test is declared ESBL positive if the zone of inhibition towards the amoxicillin/clavulanic acid increases.

Antibiotic Susceptibility

Antibiotic susceptibility was done on Mueller-Hinton agar by the Kirby-Bauer disc diffusion method. Nitrofurantoin, ciprofloxacin, levofloxacin, ceftriaxone, ceftazidime, cefepime, gentamycin, meropenem, trimethoprim sulfamethazine was used for the determination of antibiotic susceptibility according to Clinical and Laboratory Standards (CLSI) protocol.

Statistical Analysis

Analysis was done by using a statistical package for the social sciences (SPSS) version 20.0.

RESULTS

Results show that among 158 UTI patients: 53 were males with mean age 46.9 ± 16.5 and 105 were female with mean age 42.4 ± 13.8 (Table-I). The culture was done on MacConkey and blood agar. The identification of bacteria was done on API 10s. Out of 135 positive cultures, 97 (71.9%) were E. coli, 4 (3%) were Enterobacter, 7 (5.2%) were Klebsiella oxytoca, 15 (11%) were Klebsiella pneumoniae, 9 (6.7%) were Pseudomonas aeruginosa, 2 (1.5%) were Serratia marcescens and 1 (0.5%) was Serratia odorifera (Table-II). The antibiotic sensitivity and resistance in ESBL positive and ESBL negative E. coli and their association was recorded. A significant association was seen between extended-spectrum beta-lactamase E. coli positive and negative ($p<0.005$) except in Aztreonam ($p=0.413$) and Gentamycin ($p=0.818$) (Table-III). Table-IV shows the antibiotic sensitivity and resistance in ESBL positive and ESBL negative Klebsiella oxytoca and Klebsiella pneumoniae. No significant association was found in Klebsiella oxytoca and Klebsiella pneumoniae with all the above antibiotics except a significant association was seen with Meropenem ($p=0.047$) only in Klebsiella oxytoca.

Gender	Mean Age (years)	Frequency
Male	46.9 ± 16.5	53 (34%)
Female	42.4 ± 13.8	105 (66%)
Total	43.9 ± 14.8	158 (100%)

Table-I. Demographic characteristics of UTI patients according to gender and age.

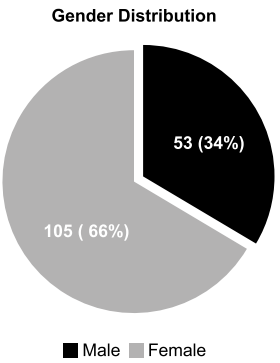

Figure-1

DISCUSSION

In the present study, it was found that the frequency of UTIs was more in female patients 66% (almost twice) than those of the male patients 34% (refer Table-I) this concurs with prior reports from Iran and other countries. According to a Columbian study, acute UTIs affect 23.3 percent of women and 6.8 percent of men, respectively, and recurring UTIs affect 54.2 percent and 15.7 percent of women and men, respectively.[9]

This confirmed our study that UTIs are more prevalent in females than males. This could be related to the structural differences between the female and male genital tracts, as the female's urethra is shorter, allowing infectious organisms (such as Escherichia coli) to conveniently enter the urinary bladder. Furthermore, the location of the female urethral aperture near the anus and vagina contributes to the bacteria's easier access into the urinary tract system.[10-12]

Isolated Organisms	*ESBL Positive	*ESBL Negative
	Number (%)	Number (%)
E. coli n=97	24 (17.7%)	73 (54.1%)
Klebsiella pneumoniae n=15	8 (6.0%)	7 (5.1%)
Klebsiella oxytoca n=7	3 (2.2%)	4 (3.0%)
Pseudomonas aeruginosa n=9	0 (0.00%)	9 (6.7%)
Enterobacter n=4	0 (0.00%)	4 (3.0%)
Serratia marcescens n=2	0 (0.00%)	2 (1.5%)
Serratia odorifera n=1	0 (0.00%)	1 (0.7%)
Total = 135	35 (25.9%)	100 (74.1%)

Table-II. ESBL status in the isolated organisms
***p-value ESBL positive vs ESBL negative is 0.043 which is < 0.05 considered statistically significant**

Drugs		ESBL Positive n=24	ESBL Negative n=73	P-Value
Nitrofurantoin	Sensitive	12 (50%)	63 (86.3%)	0.001
	Resistance	12 (50%)	10 (13.7%)	
Trimethoprim sulfamethoxazole	Sensitive	1 (4.1%)	32 (43.8%)	0.002
	Resistance	23 (95.9%)	41 (56.2%)	
Ciprofloxacin	Sensitive	6 (25%)	44 (60.3%)	0.003
	Resistance	18 (75%)	29 (39.7%)	
Levofloxacin	Sensitive	7 (29.1%)	43 (58.9%)	0.011
	Resistance	17 (70.9%)	30 (41.1%)	
Ceftriaxone	Sensitive	0 (0%)	28 (38.3%)	0.001
	Resistance	24 (100%)	45 (61.7%)	
Cefotaxime	Sensitive	0 (0%)	30 (41.1%)	0.001
	Resistance	24 (100%)	43 (58.9%)	
Ceftazidime	Sensitive	0 (0%)	23 (31.5%)	0.004
	Resistance	24 (100%)	50 (68.5%)	
Cefepime	Sensitive	0 (0%)	27 (36.9%)	0.00
	Resistance	24 (100%)	46 (63.1%)	
Aztreonam	Sensitive	0 (0%)	2 (2.7%)	0.413
	Resistance	24 (100%)	71 (97.3%)	
Meropenem	Sensitive	24 (100%)	60 (82.1%)	0.026
	Resistance	0 (0%)	13 (17.9%)	
Amoxacillin/calvanic	Sensitive	1 (4.1%)	42 (57.5%)	0.00
	Resistance	23 (95.9%)	31 (42.5%)	
Gentamycin	Sensitive	18 (75%)	53 (72.6%)	0.818
	Resistance	6 (25%)	20 (27.4%)	
Pencillin	Sensitive	0 (0%)	0 (0%)	-
	Resistance	24 (100%)	73 (100%)	

Table-III. Antibiotic sensitivity and resistance in ESBL positive and ESBL negative E. coli

Drugs		Klebsiella oxytoca (n=7)			Klebsiella Pneumoniae (n=15)		
		ESBL Positive n=3	ESBL Negative n=4	P-Value	ESBL Positive n=8	ESBL Negative n=7	P-Value
Nitrofurantoin	Sensitive	0 (0%)	2 (50%)	0.147	3 (37.5 %)	6 (85.7%)	0.057
	Resistance	3 (100%)	2 (50%)		5 (62.5%)	1 (14.3%)	
Trimethoprim sulfamethoxazole	Sensitive	0 (0%)	1 (25%)	0.35	0 (0%)	1 (14.3%)	0.268
	Resistance	3 (100%)	3 (75%)		8 (100%)	6 (85.7%)	
Ciprofloxacin	Sensitive	2 (66.7%)	3 (75%)	0.809	2 (25%)	5 (71.4%)	0.072
	Resistance	1 (33.3%)	1 (25%)		6 (75%)	2 (28.6%)	
Levofloxacin	Sensitive	2 (66.7%)	3 (75%)	0.809	2 (25%)	5 (71.4%)	0.072
	Resistance	1 (33.3%)	1 (25%)		6 (75%)	2 (28.6%)	
Ceftriaxone	Sensitive	0 (0%)	0 (0%)	-	0 (0%)	1 (14.3%)	0.268
	Resistance	3 (100%)	4 (100%)		8 (100%)	6 (85.7%)	
Cefotaxime	Sensitive	0 (0%)	0 (0%)	-	0 (0%)	1 (14.3%)	0.268
	Resistance	3 (100%)	4 (100%)		8 (100%)	6 (85.7%)	
Ceftazidime	Sensitive	0 (0%)	0 (0%)	-	0 (0%)	0 (0%)	-
	Resistance	3 (100%)	4 (100%)		8 (100%)	7 (100%)	
Cefepime	Sensitive	0 (0%)	0 (0%)	-	0 (0%)	1 (14.3%)	0.268
	Resistance	3 (100%)	4 (100%)		8 (100%)	6 (85.7%)	
Aztreonam	Sensitive	0 (0%)	0 (0%)	-	0 (0%)	0 (0%)	-
	Resistance	3 (100%)	4 (100%)		8 (100%)	7 (100%)	
Meropenem	Sensitive	3 (100%)	1 (25%)	0.047	8 (100%)	5 (71.4%)	0.104
	Resistance	0 (0%)	3 (75%)		0 (0%)	2 (28.6%)	
Amoxacillin/calvanic	Sensitive	0 (0%)	2 (50%)	0.147	0 (0%)	1 (14.3%)	0.268
	Resistance	3 (100%)	2 (50%)		8 (100%)	6 (85.7%)	
Gentamycin	Sensitive	2 (66.7%)	2 (50%)	0.659	5 (62.5%)	4 (57.1%)	0.833
	Resistance	1 (33.3%)	2 (50%)		3 (37.5 %)	3 (42.9%)	
Pencillin	Sensitive	0 (0%)	0 (0%)	-	0 (0%)	0 (0%)	-
	Resistance	3 (100%)	4 (100%)		8 (100%)	7 (100%)	

Table-IV. Antibiotic sensitivity and resistance in ESBL positive and ESBL negative Klebsiella spp.

ESBL-associated infections vary in occurrence based on local epidemiology and antibiotic dosing policies, although they have been on the rise in recent years. The prevalence of ESBL-producing Enterobacteriaceae was found to be 25.9% in this investigation (ref Table-II). The incidence of extended-spectrum beta-lactamase producing Enterobacteriaceae isolated from urine cultures in hospitalized patients has been estimated to range from 4% to 38% in studies conducted in Spain and France, and our findings are consistent with previous findings. Between 1997 and 2000, 7% of Klebsiella isolates from urine cultures in 30 US hospitals were positive for ESBL production, according to the Antimicrobial Surveillance Program. In 79 US hospitals between 2011 and 2013, the prevalence of ESBL-producing Klebsiella isolates climbed to 15%. In our study, ESBL in Klebsiella oxytoca 3/135 (2.2%) and Klebsiella pneumoniae 8/135 (6%) were isolated from 135 urine culture cases (refer Table-II). If we combine both the species of Klebsiella together, this value becomes 11/135 (8.2%), which is comparable to the above study. Due to its epidemiological behaviour, ESBL-producing Klebsiella pneumoniae has been regarded almost entirely as a nosocomial pathogen, but new evidence suggests that it is also a significant player in community-based processes.[13]

Statistics from a multicenter investigation done in 11 Spanish hospitals from 2011 to 2016 found an overall rise in ESBL-producing Klebsiella pneumoniae, reaching a frequency of more than 18 percent in 2016, compared to a comparable study conducted from 2002 to 2010. ESBLs were shown to be more common in Klebsiella pneumoniae (16.3 percent) and Escherichia coli (13.3 percent) in that investigation. Community-acquired K.pneumoniae was followed by nosocomial E. coli isolates (9.5 percent).[14] In our study *Enterobacteriaceae* was reported at 17.7% of ESBL-E. coli while it was reported in another study to be10.5 percent, the prevalence varies by location. Rates in the United States have ranged from 4.5 to 12.2 percent. The rates in other countries have ranged from 8.2 to 34.7 percent.[15,16]

Since multiple investigations demonstrated that ESBL producers also have a high proportion of non-lactam antibiotic resistance, global concern over ESBL-producing E. coli has grown. In our investigation antimicrobial resistance against nitrofurantoin, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, cephalosporin, meropenem, and amoxicillin/clavulanic, in ESBL-producer isolates, resistance was higher than in non-ESBL producers (p-value < 0.05 which was statistically significant), while in gentamycin the resistance in both ESBL positive and negative group showed non-significant results (p-value was 0.818) (refer Table-III). Similarly, resistance to ciprofloxacin and trimethoprim-sulfamethoxazole was higher in extended-spectrum beta-lactamase producers than in non-extended-spectrum beta-lactamase-producing UPEC isolates from community-acquired UTIs in Turkey, Azap et al. Furthermore, in research from Spain, the prevalence of ciprofloxacin resistance in extended-spectrum beta-lactamase producer and non-extended-spectrum beta-lactamase producer Escherichia coli isolated from community-acquired UTIs was 31.5 percent vs 9.1 percent.[12] Our study confirmed the above statement by showing results that antimicrobial resistance is more in ESBL producer Escherichia coli than non-ESBL producer E. coli (refer Table-III)

The overall rates of antibiotic resistance in this investigation were relatively high 111/135 (82.2%) of the tested antibiotics. In addition, all of the isolates (100%) were penicillin-resistant. (see Table-III), with aminoglycosides, gentamycin showing the lowest frequency of resistance (39/135=28.9% of all isolates). Gentamycin resistance was found in 26/97=27 percent of E.coli isolates. These findings are in line with those of a similar study conducted in Mexico[17], which found that 97.4 percent of E. coli isolates were resistant to penicillin, with gentamycin resistance being the lowest (14.4 percent). In addition, in research from Saudi Arabia[18], the resistance of penicillin in Escherichia Coli was 99.5 percent, with gentamycin resistance being the lowest. As a result of the rising prevalence of penicillin resistance, this antibiotic is no longer

indicated for empirical treatment of UTIs.

In our study resistance to non-β-lactam antibiotics in ESBL producers (E. coli) had a significant resistance as compared to non-ESBL producers E. coli. In one study, ESBL-producer isolates had increased antimicrobial resistance to cephalosporins (p=0.001), quinolones (p=0.05), and trimethoprim-sulfamethoxazole (p=0.02) than non-ESBL producers (refer Table-III & IV). In a study of uropathogenic Escherichia coli isolates from community-acquired UTIs in Turkey, ESBL producers showed higher resistance to ciprofloxacin and trimethoprim-sulfamethoxazole than non-ESBL producers (p=0.001). According to their findings, gentamicin resistance was much greater in ESBL-producing isolates (57 percent vs. 9%). (p=0.001). However, while ESBL-producers had a slightly higher proportion of gentamicin resistance than non-ESBL producers (25 percent vs. 27.4%), the difference was not statistically significant (p>0.05) in our analysis. Furthermore, in Spanish research, the prevalence of resistance to ciprofloxacin in extended-spectrum beta-lactamase-producing and non-extended-spectrum beta-lactamase-producing Escherichia coli isolated from community-acquired UTIs was 31.5 percent vs 9.1 percent.[12]

According to our susceptibility patterns, ESBL producing K. pneumoniae was highly sensitive to meropenem 100% followed by gentamycin 62.5%, while it showed 62.5% resistance to nitrofurantoin, 75% to levofloxacin, 75% to ciprofloxacin, and 100% to trimethoprim-sulfamethoxazole, ceftriaxone, cefotaxime, ceftazidime, cefepime, amoxicillin/clavulanic acid, and penicillin (refer table-IV). Comparatively in an Iranian study, ESBL producing K. pneumoniae was 90% sensitive to both meropenem and gentamycin.[19] In another investigation, ESBL-producing Klebsiella pneumoniae demonstrated 100% resistance to amoxicillin/clavulanic acid, trimethoprim-sulfamethoxazole, ciprofloxacin, gentamycin, nitrofurantoin, cefoxitin, cefotaxime, ceftazidime, and meropenem (20 percent).[20]

Penicillin, cephalosporins, trimethoprim-sulfamethoxazole, quinolones, carbapenems, aminoglycosides, 2nd, and 3rd generation cephalosporins were the most active compounds against these uropathogens, according to an analysis of non-ESBL generating strains' susceptibility to the antibiotics utilised (E. coli and K.pneumoniae).[21] The finding in our study is similar to the above study.

CONCLUSION

Alarmingly, 35.9% of inpatients (hospitalized) and 16.9% of outpatients (community) in the current investigation were ESBL-positive, with all extended-spectrum beta-lactamase-producing strains being multi-drug resistant. These strains are a major epidemiological concern because they are spreading antimicrobial resistance across the community, making most routinely used medicines for the treatment of urinary tract infections ineffective in the near future in our area. Meropenem is the 100% effective parenteral medicine against ESBL-producing uropathogens and Nitrofurantoin is the only effective oral medication observed in our study. Regular terrestrial screening of ESBL producers is required for the suitable antibiotic or for designing new therapeutic methods for UTIs, particularly in developing countries.

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No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Faiza Asghar	Principal Author.	
2	Abdul Shaheed Asghar	Supervised and helped in writing the manuscript.	
3	Sardar Muhammad	Supervisor.