Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[8] July 2020 : 72-79

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Antibiotic Resistance Patterns and Prevalence of ESBL producing microbes in community associated Urinary Tract Infections in different Hospitals of Abbottabad

Kainat Saeed[1],Saifullah Khan[*1], Palwasha Khan[1],Muhammad Farooq[2], Hajra Bibi[1], Atia Ijaz[1], Zahid Ahmed[1].
[1]Department of Microbiology Government Postgraduate College Mandian, Abbottabad
[2]Department of Pathology, CMH Abbottabad
Corresponding Author: saifullahkha@gmail.com, saifkhan62000@yahoo.com

ABSTRACT
Formidable boost in resistance is making Urinary Tract Infections (UTIs) a more challenging ailment. Extended-Spectrum-β-Lactam (ESBL) producing bacteria cannot be appropriately detected by conventional disc diffusion methods which can ultimately lead to treatment failures. The aim of this study was to determine the distribution and antibiotic susceptibility patterns of bacterial strains isolated from patients with community acquired urinary tract infections and to identify ESBL producers among different uropathogens.79.6% patients had E coli positive cultures and 4.1% bacteria were Extended Spectrum β Lactam (ESBL) producers among total positive cultures.E.coliwas most resistant to ampicillin (99%) and least resistant to cefoperazone+sulbactam, tazobactam and imipenem (6.45, 7.09 & 7.09% respectively). Other human pathogens such as Klebsiella (4.10%) Proteus spp. (1.02%), Pseudomonas spp. (1.02%), Enterobacter spp. (2.56%), Staphylococcus aureus (3.08%), Citrobacterspp (2.56) were also identified during this study. Prevalence rates of UTI varied by age, gender and region. Increase in antibiotic resistance is mainly occur due to misuse and over use of antibiotics and also due to without diagnostic test prescription. Therefore it is now essential to use these antibiotics with extreme caution and also develop new antimicrobials having high efficiency with slight/ no side effects, easily available and less costly.
Keywords: *UTI, ESBL producers, Antibiotic Resistance, In-vitro*

Received 21.04.2020 Revised 05.05.2020 Accepted 19.07.2020

INTRODUCTION

Urinary tract infections (UTI) are one of most frequently reported infectious disease across the globe that affect millions of people annually [1].Worldwide about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars[2].Women are more prone to catch UTI because of their urinary tract morphology [3]. Few observational studies reveal the fact that around 50-60% of women at least once in their lifetime will catch urinary tract infection [4]. After an uncomplicated UTI, around 25% of women experience a recurrent infection within 6–12 months, and around 5% have several episodes within a year [5]. Most common organisms that cause UTI are *Escherichia coli, Staphylooccus saprophyticus* and less common organisms are *Proteus sp., Klebsiella pneumonia, Pseudomonas aeruginosa, Enterococci sp.* and *Candida albicans* [6].*Escherichia coli* remain the most frequently reported culprit microbe with 75%-85% prevalence in uncomplicated UTI[7]. UTI causing microorganisms usually come from skin, near or at urethral opening. Gram negative bacteria cause 80-85% whereas; gram positive bacteria cause 15-20% cases of UTI[8].Gram negative bacteria that cause UTI are *E. coli, Klebsiella, Proteus, Enterobacter, Pseudomonas* and *Serratia spp*. Gram positive bacteria include group B *Streptococci, Enterococcus spp, Staphylococcus aureus and Staphylococcus saprophyticus* [9].

For the sake of better understanding UTIs are categorized as lower tract infections including infection of bladder or urethra also called as cystitis and urethritis and upper tract infection, i.e. infection of ureter, collecting ducts, and parenchyma also called as pyelonephritis[10].

Lack of rationale and prudence in the use of antibiotics in under-developed and most of developing countries, resistance against antibiotics is increasing at a rapid pace and treatment of UTI is becoming gradually more difficult.[11] Resistance to antibiotics is due to three common mechanisms adopted by microbes including, alteration in drug targets, increased extracellular efflux of antimicrobial agents thus reducing intracellular concentrations of drugs and production of enzymes responsible for destruction of drugs rendering them ineffective[12].Frightening rise in resistance is observed against frequently used drugs like Norfloxacin, Ciprofloxacin, Ofloxacin, Cotrimoxazole and Cephalexin [13]. Mostly, treatment failure in UTI is due to increasing antibiotic resistance among organisms.[14] This resistance pattern is different in different countries, states, hospitals etc but in our subcontinent region; antibiotic resistance is majorly due to misuse of antibiotics [15].

Extended spectrum β-Lactamase (ESBL) producing bacteria are a global emerging threat as their treatment is intricate due to resistance over β-lactam and non β-lactam drugs[16] First ever ESBL producer isolate was reported in Germany and England in 1983 and in USA in 1988. ESBL are enzymes that produce resistance against beta lactam drugs such as Penicillins, Cephalosporins, Monobactams. ESBL are commonly produced by *E. coli, Klebsiellasp,* and up to extent Pseudomonadaceae and other Enterobacteriaceae [17].Types of ESBL include Temoniera (TEM) most commonly produced by gram negative bacteria, sulphydryl variable (SHV) most commonly found in *K. pneumonae*, Cefotaxime-M (CTX-M), Oxacillinase (OXA) [18].

The aim of current study was to conclude bacterial etiologic agents that cause UTIs and to assess their*in vitro* susceptibility and resistance patterns against routinely used antimicrobial drugs. This study was further aimed to study the prevalence difficult to treat superbugs i.e. ESBL producing bacteria and their types. This study is vital to make possible the successful management and treatment of patient with urinary tract infection referred to the Combined Military Hospital Abbottabad.

MATERIAL AND METHODS

This study was carried out in both inpatient and outpatient departments of Abbottabad hospitals for oneyear time period from June 2017 to July 2018. During study total 1200 urine samples of patients with age 10-70 years were collected. Verbal consent was taken from the patient and relevant guidelines were followed to collect mid-stream urine in sterile container provided to them. Samples were transported to the Microbiology lab without any delay and further processed in the laboratory.

Sampling and bacteriological analysis:

Specimen collection:

Midstream specimen of urine (MSU):

Mid-stream urine was collected in a sterile plastic container by clean catch technique.

Transport of specimen:

Collected specimen was transported to the Microbiology lab without any delay.

Processing of specimen:

Semi-quantitative culturing:

All the collected specimens were inoculated on Blood agar and cysteine lactose electrolyte deficient (CLED) agar by calibrated loop method and were incubated overnight at 37°C. Culture positive cases (semi-quantitative colony count $>1x10^5$ CFU/ml) were included for further processing. Growth of organisms were evaluated by biochemical tests including Motility indole urea (MIU), Triple sugar iron (TSI), Simon citrate and by their colony characters according to Borrow's guidelines. To obtain pure growth isolated colonies were sub cultured on MacConkey and Blood agar.

Antibacterial susceptibility testing:

Antibiotic susceptibility testing was determined by Kirby Bauer disk diffusion method using commercially available discs [19]. Muller Hinton agar (MHA) media was used for the evaluation of resistance and sensitive patterns. Bacterial colonies were spread on MHA plates and were incubated at 37°C for 24 hours. Common 13 antibiotics used for susceptibility test are Tazobactum (TZP), Imipenem (IPM), Ceftriaxone (CRO), Ceftazidime (CAZ), Ciprofloxacin (CIP), Ampicillin (AMP), Amikacin (AMI), Gentamicin (GEN), Doxycycline (DOX), Sulbactam (SUL), Trimethoprim Sulfamethoxazole (COT), Nitrofurantoin (NIT) and Amoxicillin (AUG). After incubation zone of inhibition for bacterial growth were measured and compared with CLSI guidelines[20]. For sensitivity both gram negative and positive isolates were tested against different group of antibiotics. For antibiotic sensitivity pattern a total of 195 uropathogens were subjected against 17 different types of antibiotics.

Detection of ESBL producers by NCCLS Phenotypic Method

Detection of ESBL producerswas carried out by inoculating bacterial suspension of the isolate with a turbidity equivalent to 0.5 McFarland standards on Muller Hinton agar. Commercial discs containing Cefotaxime (CTX) and Ceftazidime (CAZ) alone with Clavulanic acid were used. According to test

antibiotic discs were placed on the lawn culture. According to CLSI; a zone of ≤ 27mm for Cefotaxime and ≤22mm for Ceftazidime indicate ESBL production as positive.

Statistical Analysis

To find significant difference between various antibiotics all data were analyzed by IBM SPSS 20 package for one-way analysis of variance (ANOVA) testing; post hoc Tukey test was used with P-value 0.05 as a measure of significance.

RESULT

1200 urine samples were studied during time of one year. Out of 1200 sample 196 (15.86%) were positive (Fig 1). There were 40% females and 60% males (Fig 2).The age range of the Patient was 1-80years (Fig 3). The predominant organism was *E. coli* which was responsible for 79.48% of the infection. *Klebsiella* was responsible for 4.10% of the infections while the rest were accounted for by *Proteus spp*. (1.02%), *Pseudomonas spp*. (1.02%), *Enterobacter spp*. (2.56%), *Staphylococcus aureus* (3.08%),*Citrobacterspp* (2.56%) and ESBL (4.08%). ESBL %age is shown in fig 4.The urinalysis of the patients is as shown in Fig 2.

The sensitivity of the isolates to seventeen antimicrobial agents is shown in (Table 2). Most of the isolates were susceptible to TZP, SUL, IPM, GEN AMI and NIT. The percentage resistance of all the isolates to the different antimicrobial agents is also shown in Table 1. The highest resistance was recorded with COT, AMP, TET, MIN, MXF and CEPH. while the lowest resistance was with SUL.

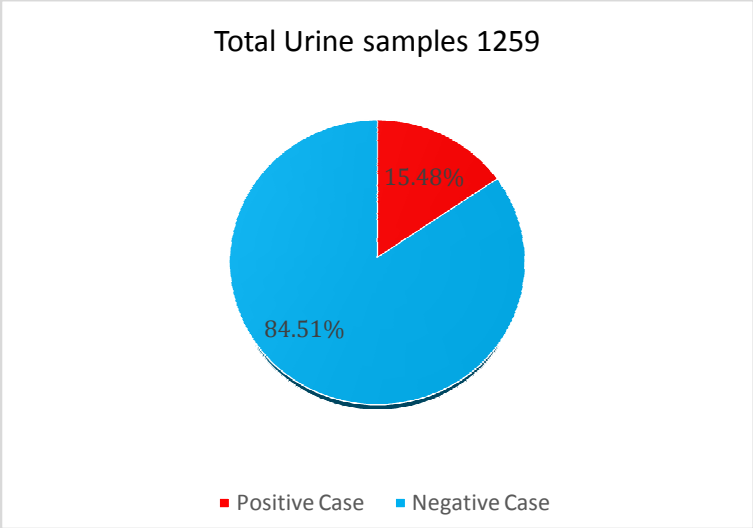

Figure 1 shows total urine samples

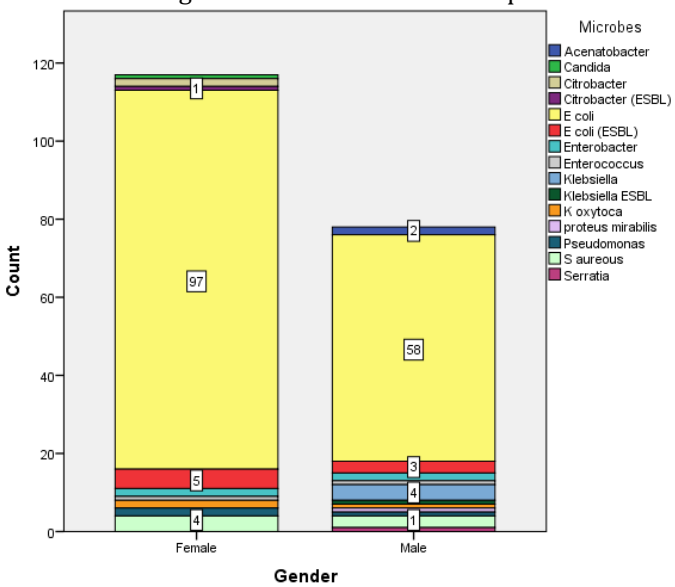

Figure 2: Shows the urinalysis of the male and female patients

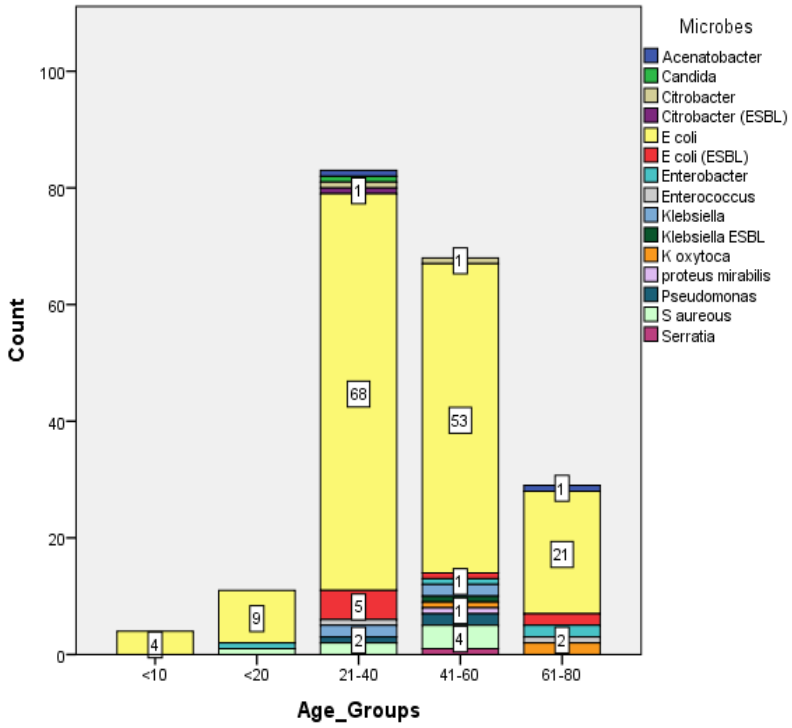

Figure 3 shows age range of the Patients

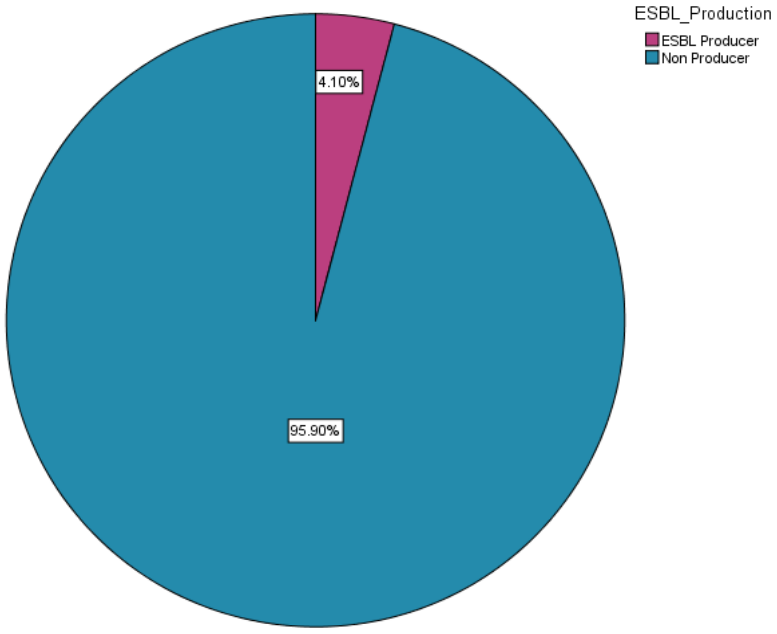

Figure 4: Shows ESBL Production

Table 1 shows Percentage Antibiotic Resistance

		Percentage Antibiotic Resistance																			
Organisms	**%Age of Isolate Prevalence**	**AMP**	**COT**	**CEC**	**CIP**	**GEN**	**AMI**	**NIT**	**AUG**	**CRO**	**CAZ**	**TZP**	**SUL**	**IPM**	**TET**	**MIN**
E.coli	79	99	46	20	62	37	15.5	13.5	43.2	24	19.4	7.09	6.45	7.09	31	23
Pseudomonas	1.54	100	100	33.3	67	33.3	33.3	33.3	67	33.3	33.3	33.3	67	33.3	33.3	33.3
Klebsiella	2.05	100	100	17	17	33.3	17	17	33.3	17	17	17	33.3	17	33.3	33.3
Acenatobacter	1.03	100	100	67	33.3	33.3	67	67	33.3	33.3	48	67	62	75	100	33.3
Enterobacter	2.05	75	75	25	100	25	75	75	50	75	25	25	25	50	25	25
Sertia	0.51	66.6	33	33.3	66.6	33.3	33.3	33.3	66.7	33.3	33.3	33.3	33.3	66.7	66.7	33.3
S aureous	3.59	71.4	29	29	71.4	43	43	14.3	43	14.3	43	14.3	14.3	14.3	43	14.3
Citrobacter	1.00	100	33	33.3	66.6	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	100
ESBL Producers	4.00	75	50	50	50	50	50	25	25	75	50	25	50	75	75	50
Enterococcus	1.03	50	50	75	75	50	50	50	50	75	50	75	50	75	50	50
P Value		0.001**	0.032*		0.021*											

** Statistically highly significant values (P value<0.01); *Statistically significant values (P value <0.05)

Table 2: Resistance patterns of uropathogens isolated in this study

Uropathogens	Resistance Patterns	No. of isolates
E. coli	AMP,CIP,TET, MIN	06
	AMP, COT, CIP, AUG, TET, MIN	04
	AMP,COT,CIP,GEN,AMI,AUG,MXF	20
	AMP,CIP,GEN,CRO,TET,MIN,CAZ	09
	AMP,COT,CIP,NIT,CAZ,TET,MIN,TZP	08
	AMP,CIP,GEN,AMI,NIT,AUG,CRO,SUL	02
	AMP,COT,GEN	07
	AMP	10
	AMP,AUG	03
	AMP,COT,TET,MIN	04
	AMP,GEN	02
	AMP,CIP,GEN,AUG	04
	AMP,CIP	04
	AMP,CEC,CIP,NIT	07
	AMP,CIP,GEN,NIT,AUG,CRO,CAZ,TZP,IPM	09
	AMP,COT,CIP,NIT,AUG,CRO,CAZ	08
	AMP,CIP,GEN,NIT	03
	AMP,COT,CRO,IPM,CIP	04
	AMP,COT,CIP,GEN,AMI,CRO	07
	AMP,CIP,AMI,AUG,TZP,SUL,IPM	10
	AMP,COT,CEC,CIP,CRO	05
	AMP, CIP, AUG	04
	AMP, AUG, TET	02
	AMP, CRO	01
	AMP, AUG, CRO, CAZ	01
	AMP, CIP, GEN, CEPH	01
	AMP, COT, CIP, GEN, CEPH	05
	AMP, CEC, CIP, AMI, AUG, SUL, CEPH	07
	AMP, GEN, TET	04
	CEPH	02
Acenatobacter	**AMP,COT,GEN,AMI,CRO,TET,MIN**	01
	AMP,COT,CIP,NIT,AUG,TZP,TET	01
Citrobacter	**AMP,COT,CIP,AUG**	01
	AMP,CEC,CIP	02
Enterobacter	**AMP,COT,CIP,NIT,IPM,TET,MIN**	01
	AMP,COT,CEC,CIP,GEN,NIT,AUG,IPM	01
	AMP,COT,CIP,AMI,NIT,AUG,CAZ,TZP	01
	CIP,AMI	01
Klebsiella	**AMP,COT,CIP,CRO,CAZ,TET,MIN**	01
	AMP,COT,GEN,TET,MIN	01
	COT,AMP	01
	AMP	01
	AMP,COT,NIT	01
	AMP,COT,GEN	01
	AMP,AUG,MIN	02
Proteous	**AMP,AUG**	01
Pseudomonas	**AMP,COT,CIP,AMI,CRO,IPM,MXF**	01
	AMP,CIP	01
	AMP,COT,CIP,GEN,NIT,TET,MIN	01
Serratia	**AMP,COT,AUG,CAZ,SUL,TET,MIN,MXF**	01
	AMP, CIP, CRO,CAZ,IPM	01
	AMP,COT,GEN, AUG,CRO	01

DISCUSSION

Identification of the uropathogens and their susceptibility pattern is very important in treating the cases of Urinary Tract Infections (UTI). In the present study urine specimens were cultured to see pattern of uropathogens and some 196 (15.48%) of the urine showed significant growth of bacteria. So majority (84.51%) of the cases remaining showed either insignificant bacteriuria or no growth with urine from the suspected cases of UTI. Previous use of antibiotic before submitting the urine samples and clinical circumstances like non-gonococcal urethritis or others that mimic UTI could be the factors responsible

for non significant bacteriuria or no growth. This indicates the need for educating the patients about the method of collection of clean catch mid-steam urine specimens. There was a majority of young and middle aged females, while in the children and younger stage groups, nearlyequivalentmagnitudes of male and females had UTI. In the current study, the most common pathogens isolate was *Escherichia coli*-79.48%, followed by *Klebsiella* 4.10&*Pseudomonous* species-1.02%, *Staphylococcus aureus* (3.58%), *Proteus* species (1.02%), *Acenatobacter* (1.02%)&*Citrobacter* (2.56%) The isolation rate of urinary pathogens of the present study is consistent with reports of the studies published elsewhere recently[Bauer *et al*., 1996]*E. coli* was the principal pathogen isolated.This is consistent with reports from different countries who have reported an increasing resistance to Amoxicillin, Ciprofloxacin, and Ceftrixone[21] . Another study from Bangladesh reported and increases resistance of the uropathogens to Ciprofloxacin[22]. The results of the present study showed that sensitivity rate of the uropathogens were low for AMP and TET. This little sensitivity might be due to extensive use of the antibiotics in the community. It is probable that the low sensitivity is existing among uropathogens of the nosocomial as well as community-acquired UTI. In the present study, community acquired UTI and nosocomial UTI were not been distinguished. This was the main limitation of the study. A high isolation rate of pathogens from urine samples of clinically suspected UTI shows a good association between clinical findings and microbiological methods. Gram-negative bacteria were the commonest organism isolated, among which *E.coli* was the principal urinary.

REFERNECES

1. Al-Assil B, Mahfoud M, Hamzeh AR.(2013). Resistance trends and risk factors of extended spectrum β-lactamases in Escherichia coli infections in Aleppo, Syria. American journal of infection control. Jul 1;41(7):597-600.
2. Araj GF, Avedissian AZ, Ayyash N.(2012). A reflection on bacterial resistance to antimicrobial agents at a major tertiary care center in Lebanon over a decade. Lebanese Medical Journal. Jan;103(368):1-1.
3. Rameshkannan, S, Nileshraj, G, Rameshprabu, S., Mangaiarkkarasi, A, MeherAli, R. (2014). Pattern of pathogens and their sensitivity isolated from pus culture reports in a tertiary care hospital, puducherry. *Indian Journal of Basic and Applied Medical Research*, *4*(1), 243-248.
4. Foxman B.2003. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Disease-a-month. Feb 1;49(2):53-70.
5. Fihn SD.2003. Acute uncomplicated urinary tract infection in women. New England Journal of Medicine. Jul 17;349(3):259-66.
6. GalateBangdeLB, S. 2015. Urinary tract infection: study of microbiological profile and its antibiotic susceptibility pattern. Int J CurrMicrobiolAppl Sci.;4(9):592-7.
7. Grabe M, Bishop MC, Bjerklund-Johansen TE, Botto H, Çek M, Lobel B, Naber KG, Palou J, Tenke P.2008. Management of urinary and male genital tract infections. Update. Mar.
8. Khalid, M. Al Naimi, M. K, Saleem, H. G. M, Ullah, M. M, AlBaloshi, A. Y. M. D.2017. Antibiotics Resistance Profile of Uropathogens Isolated from Al Buraimi Hospital, Sultanate of Oman. *Global Journal of Health Science*, *10*(1), 98.
9. Kontiokari T, Sundqvist K, Nuutinen M, Pokka T, Koskela M, Uhari M.2001. Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. Bmj. Jun 30;322(7302):1571.
10. Hanna-Wakim RH, Ghanem ST, El Helou MW, Khafaja SA, Shaker RA, Hassan SA, Saad RK, Hedari CP, Khinkarly RW, Hajar FM, Bakhash M.2015. Epidemiology and characteristics of urinary tract infections in children and adolescents. Frontiers in cellular and infection microbiology. May 26;5:45.
11. Daoud Z, Salem Sokhn E, Masri K, Cheaito K, Haidar-Ahmad N, Matar GM, Doron S.2015. Escherichia coli isolated from urinary tract infections of Lebanese patients between 2005 and 2012: epidemiology and profiles of resistance. Frontiers in medicine. Apr 28;2:26.
12. Akram M, Shahid M, Khan AU.2007. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. Annals of clinical microbiology and antimicrobials. Jan;6(1):4.
13. Gupta K, Hooton TM, Stamm WE.2001. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Annals of internal medicine. Jul 3;135(1):41-50.
14. Linhares I, Raposo T, Rodrigues A, Almeida A.2013. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000–2009). BMC infectious diseases. Dec;13(1):19.
15. Stamm WE, Norrby SR.2001. Urinary tract infections: disease panorama and challenges. The Journal of infectious diseases. Mar 1;183(Supplement_1):S1-4.
16. Pullukcu H, Tasbakan M, SipahiOR, Yamazhan T, Aydemir S, Ulusoy S.(2007). Fosfomycin in the treatment of extended spectrum beta-lactamase-producing Escherichia coli-related lower urinary tract infections. International journal of antimicrobial agents. Jan 1;29(1):62-5.
17. Asna SM, Akter S, Rahman MM, Mohammad N, Hafez MA.(2015). Frequency and Sensitivity of Extended Spectrum Beta-Lactamase Positive Organisms in a Secondary and Tertiary Level Hospital Netwark in Dhaka. J Enam Med Coll. Jun 29;5(2):80-7.
18. Pitout JD, Nordmann P, Laupland KB, Poirel L. (2005). Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. Journal of Antimicrobial Chemotherapy. May 25;56(1):52-9.

19. Boyle, V. J., Fancher, M. E., & Ross, R. W. (1973). Rapid, modified Kirby-Bauer susceptibility test with single, high-concentration antimicrobial disks. *Antimicrobial agents and chemotherapy*, *3*(3), 418-424.
20. Mikulska, M., Viscoli, C., Orasch, C., Livermore, D. M., Averbuch, D., Cordonnier, C., &Akova, M. (2014). Aetiology and resistance in bacteraemias among adult and paediatrichaematology and cancer patients. *Journal of infection*, *68*(4), 321-331.
21. Bauer AW, Kirby WMM, Sherris JC, Turch M.1996. Antibiotic susceptibility testing by a standardized single disk method. Am J ClinPathol ; 45: 493-499.
22. National Committee for Clinical Laboratory Standards.2000. Approved standard M2A7: Performance standards for antimicrobial susceptibility testing. Eighth informational supplement. Villanova, PA: NCCLS

CITATION OF THIS ARTICLE

K Saeed, S Khan, P Khan, M Farooq, H Bibi, A Ijaz, Z Ahmed. Antibiotic Resistance Patterns and Prevalence of ESBL producing microbes in community associated Urinary Tract Infections in different Hospitals of Abbottabad .Bull. Env. Pharmacol. Life Sci., Vol 9[8] July 2020 : 72-79