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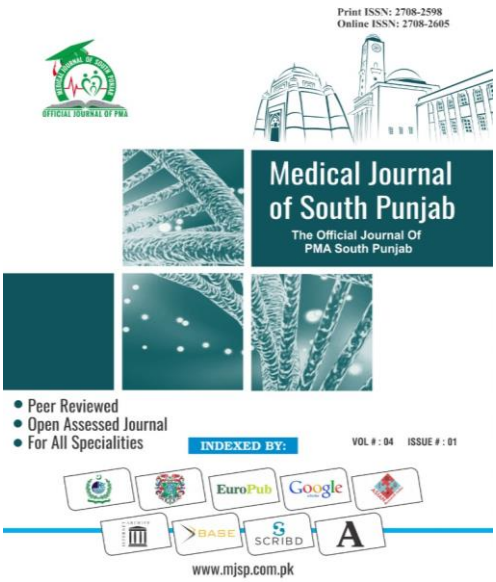

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ABSTRACT

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Synergetic effect of *Azadirachta indica* and *Ocimum tenuiflorum* leaves on esbl producing uropathogenic e. Coli in urine samples of children at Khairpur, Sindh, Pakistan

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ABSTRACT

Objective: *To compare the antibacterial activity between commercial antibiotics versus Azadirachta indica (Neem) and Ocimum tenuiflorum (Tulsi) against clinical isolates of E. coli in urine sample of children.*

Methods: *This study was comparative analytical conducted at pediatric department, talukas hospitals of district Khairpur (Mir's), Sindh, from January 2020 to February 2022. Midstream urine samples of children (n=596) were collected from Taluka hospitals of District Khairpur (Mir's) such as Khairpur, Gambat and Taluka Hospital Kotdiji. All samples were inoculated on different laboratory media and incubated for 24-48 hours. The determination of ESBL producing bacteria were tested by E-test. Double disk synergy method and antibiotic susceptibility test of ESBL producing E. coli isolates was performed by agar gel diffusion method.*

Results: *Out of 596 urine samples, ESBL producing E. coli 76.1% were isolated from urine samples of children. The mean age was* 9.50±5.48 years (ranged between 2 – 17 years). *In this study 42.6% were male while majority 57.4% were female. The greater resistance among the E.coli isolates was noted in Aztreonam 35.1% followed by Ampicillin 28.3%, Amoxicillin/clavulanate 23.7% and others antibiotics.*

Conclusion: *Urinary tract infection caused by multidrug resistant E. coli and ESBL producing E. coli was greatly prevailing all age groups of both genders particularly in 13-17 years of children. The antibiotic susceptibility test revealed multidrug resistant while Azadirachta indica and Ocimum tenuiflorum extracts revealed the greater antibacterial effects.*

Keywords: *ESBL-E.coli, Urinary Tract Infection, Antibiogram, Effects of Azadirachta indica and Ocimum tenuiflorum*

1. INTRODUCTION

Urinary tract infection (UTI) a common childhood infection and affect greatly the females throughout adolescence than boys in the early life, making urinary tract infection (UTI) a common childhood infection that can impact both community people and hospitalized patients[1]. Most frequent causes of UTI is *Escherichia coli* (*E. coli)* followed by Proteus species, K. pneumoniae, Enterobacter, Enterococcus, Acinetobacter species, and Pseudomonas aeruginosa[2]. The UTI may manifest in either the lower system bladder and urethra or the upper system kidneys and ureters. Both the upper and lower tracts are impacted by UTI and can quickly progress to a bactaraemia state in newborns with a developing renal system, more often in infants under the age of one year[1].

Extended spectrum beta-lactamase (ESBL) producers, which pose a serious threat to the community, have been known to produce antibiotic resistance in gram negative bacteria for more than 20 years. Both sexes experience an increase in UTI frequency with age, with females being more susceptible in younger populations, while older populations over the age of 70 are reported to experience 10% more UTIs on an annual basis[3]. The main danger to human health is from infections contracted during surgery. Within 72 hours of being admitted to the hospital, this typically occurs. The majority of these illnesses, urinary tract infections, are caused by bacteria, particularly ESBL and because of the increased use of antibiotic therapy, these bacteria have become resistant, making nosocomial UTI prevention and management challenging[3]. The ESBL-producing bacteria may swiftly disperse and cause a larger variety of UTIs. They often infect patients who don't receive proper medical care more frequently. The members of enterobacteriaceae family, which include Pseudomonas aeruginosa, produces ESBLs by hydrolyzing penicillin and extended spectrum cephalosporin. The TEM-1, TEM-2, SHV-1, and OXA are the four most prevalent types. These enzymes are plasmid-dependent and contain resistance genes to the antibiotics aminoglycoside and fluoroquinolone, which have a high incidence and mortality rate[4].

The treatment of patients, particularly children, has become problematic due to the antibiotic resistance among the uropathogens that produce ESBLs. Because of the active ingredients in herbs, which can be used as a treatment for a variety of complications, medical practitioners now advocate using herbal therapy[5]. The plants are the primary source of herbal therapy, including Ayurvedic and Unani medicines, and they have some degree of therapeutic properties[6]. Plants are frequently employed for therapeutic purposes all over the world because of their numerous advantages, particularly in underdeveloped countries[7]. This work aims to study the epidemiology of ESBL-producing bacteria, to investigate the ESBL-producing UTI pathogens from hospitalized children in various parts of the Khairpur District, and the synergetic effects of Azadirachta indica and Ocimum tenuiflorum on clinical isolates.

2. METHODOLOGY

This comparative analytical study was conducted at pediatric department of three hospitals of District Khairpur (Mir's), Sindh, Pakistan, such as Khairpur, Gambat and Taluka hospital Kotdiji, from January 2020 to February 2022. After getting informed consent from parents of children, all information like sex, history of prior

infection, medications and current hospital stay were noted. Total 596 admitted children in the hospital with suspected nosocomial UTI with age ranged between 2-17 years were included in our study and immunocompromised children were excluded from study. Midstream urine samples of children (n=596) were collected and transported with standard protocol to laboratory. After centrifuging the samples for two minutes at 3000 rpm, the deposit was inoculated on different plates of CLED agar (BDTM), TCBS agar, and MacConkey's agar with antibiotic ceftazidime added at a rate of 2 mg/liter (Oxoid-UK). The plates were kept incubated at 35°C for overnight. One group was incubated for 24 hours at 37°C, while the other was incubated for 48 hours. The isolated *E. coli* colony was chosen and a new batch of BHI broth and then separately emulsified for 24 and 48 hours in BHI broth. Later 0.1 mL of the broth culture was streaked on CHROM agar and incubated overnight to analyzing the results. Following incubation, 105 (CFU/mL) bacteria were used for further investigation of cultural, morphological, and biochemical studies using the colony counter, Gram staining, and API system, respectively.

The anti-microbial affectability design of *E. coli* by agar gel diffusion method where ampicillin (AMC) 10µg, amoxicillin/clavulanate (AMC) 30µg, aztreonam (ATM) 30 µg, ceftazidime (CTZ) 30µg, ceftriaxone (CRO) 30µg, cefotaxime (CTX) 30µg, ciprofloxacin (CPFX) 5µg and nitrofurantoin (NIT) discs were applied on the culture of test *E. coli* and ATCC 25922 as control over the MHA plates, which displayed the zone of growth inhibition in changing sizes compared with the CLSI standard zones.

The ESBL-E-Test strips (Biomerieux) were used, which contains a concentration of ceftazidime at one end and the clavulanic with cefotaxime. The plates were incubated for 24 hours at 37°C. After incubation, the growth was emulsified in BHI broth to create a turbid suspension of the 0.5 McFarland standard. This liquid culture was applied as an inoculum to a Muller Hinton agar (MHA Oxoid) plate and left to grow overnight. Double disc synergy method involved the independent placement of 30µg discs of ceftazidime, ceftriaxone, cefotaxime, and aztreonam on the test culture of *E. coli* over the surface of MHA plates, along with a 30µg disc of amoxicillin/clavulanic acid, and the MHA plates were incubated for 24 hours at 37°C.

The Neem and Tulsi leaves were used for preparation of extract. The dried leaves of test plants were individually ground into a fine powder with an electric grinder and kept until use in an airtight container. Solvents with a purity of 95–98% were used in these procedures, including methanol and ethanol (Merck). By centrifuging 10 ml of an aqueous sample at 1500 rpm for 10 minutes in a centrifuge machine (Gyrozen-416) the sediment was analyzed. Eventually, the methanol dissolvable was dissipated totally by rotating vacuum evaporator (BUCHI R-215, Germany). The extricated material was processed twice to wipe out methanol and ethanol at 50ºC, 70ºC, and 90ºC for 2 hr. Whatman channel paper was utilized for extricate extraction. The extricates were centrifuged for sediment assurance at 3000 rpm and tried for their rack life for 40 days at room temperature with characteristic highlights that incorporate appearance in methanol and ethanol extricates at room temperature. Other characters were dim brown color, a solid scent in refined water. The sleek extricates appeared moo thickness. After longer term of capacity, the color got to be light brown, and decreased smells less. The pH gotten from all extricates.

For alkaloids and flavonoids identification; the extracts were first dissolved in diluted hydrochloric acid, then clarified, and the alkaloids were identified using Hager's Test and Mayer's test, while the flavonoids were identified using the alkaline reagent test and the Bate-Smith and Metcalf tests, respectively. In this trial, Hager's reagent was used, which is a concentrated solution of picric acid, is used to react with the filtrate and for Mayer's test, filtrate is treated with Mayer's reagent; a potassium iodide and mercuric chloride solution. For alkaline test 10% ammonium hydroxide solution is used to assess the extract's aqueous solution. For the test of Bate-Smith and Metcalf; 0.5 mL of strong hydrochloric was added to the extract for pre-treatment. After being heated for 20 minutes in a water bath, it is then cooled and soaked for 60 minutes.

Agar gel diffusion method were used to see synergetic effect of the aqueous extracts of neem and tulsi leaves in which equal volume of both ethanolic and methanolic extracts *Azadirachta indica* and *Ocimum tenuiflorum* were mixed separately with magnetic stirrer. The broth culture of the test bacteria was maintained at 10^6 cells/ml by hemocytometer (Qujing-China) separately in sterile test tube. Whatman filter paper discs of 6 mm size were immersed in each microliter dilution per milliliter (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 µl/100 mL.) volume by volume (v/v) for 5 seconds and placed over the MHA plates containing the test inoculum. The plates were incubated at 37°C for 24 h to observe the colonies. Amoxicillin antibiotic disc of 30µg was placed on separate plate of inoculated test culture for positive control and 5% DMSO as negative control.

In agar well diffusion method three 6 mm wells were bored by a sterile cork borer in the MHA plates containing ESBL producing test culture of *E. coli*. Each bore of each agar plate was filled with 1 microliter (µL) of melted nutrient agar and allowed to solidify. Later, 1-25 µL of test extracts of Neem and Tulsi were poured in each well of the plates containing test extract dissolved in methanol and ethanol respectively. The plates were then left at room temperature for a short while before being incubated for 24 hours at 37°C.

3. RESULTS

Total 596 urine samples were randomly collected from three Talukas hospitals of Khairpur, Gambat and Kotdiji of the District Khairpur (Mir's) were investigated for the prevalence of urinary tract infection caused by ESBL producing *E. coli*. Overall results for *ESBL producing E. coli* 456 (76.1%) *were noted in urine samples of children. Our study results showed* higher percentages of ESBL-producing *E. coli* in UTI patients at hospitals of Taluka Kotdiji, Khairpur and Gambat indicated 133 (83.1%), 152 (76%) and 171 (72.4%) respectively. *The mean age was* 9.50±5.48 years (ranged between 2 – 17 years). *In this study* 254 (42.6%) were male while majority 342 (57.4%) were female. Urine samples were collected from different hospitals with both sex and different age groups of 2-6, 7-12 and 13-17 years respectively. ESBL producing *E. coli* 271 (79.3%) was predominantly observed in female gender and 277 (84.2%) in age group 13-17 years. (Table I - II).

The greater resistance among the *E. coli* isolates was noted in Aztreonam 160 (35.1%) followed by Ampicillin 129 (28.3%), Amoxicillin/clavulanate 108 (23.7%) and others antibiotics. (Table III). The observation of *Azadirachta indica* and *Ocimum tenuiflorum* extracts revealed the greater antibacterial effects on ESBL producing test isolates at the concentration of 01 to 25 µL/100 mL whereas the synergetic

effect of both extracts was greatly observed at 14 µL/100 mL concentration by agar well diffusion method. (Table IV).

Table I: Gender wise distribution of ESBL producing E. coli isolates

Talukas		Gender	Samples	*E. coli* isolates N (%)
Khairpur	KHP N=200	Male	96	64 (66.7%)
		Female	104	88 (84.6%)
Gambat	GMB N=236	Male	91	67 (73.6%)
		Female	145	104 (71.7%)
Kotdiji	KD N=160	Male	67	54 (80.6%)
		Female	93	79 (84.9%)
Total N=596		Male	254	185 (72.8%)
		Female	342	271 (79.3%)

Table II: Determination of ESBL producing *E. coli* isolates in different age groups

Talukas	Age group (Years)	Number of samples	*E. coli* isolates N (%)
Khairpur N=200	2-6	32	18 (56.2%)
	7-12	57	41 (71.9%)
	13-17	111	89 (80.2%)
Gambat N=236	2-6	34	20 (58.8%)
	7-12	71	52 (73.2%)
	13-17	131	112 (85.5%)
Kotdiji N=160	2-6	17	10 (58.8%)
	7-12	56	38 (67.8%)
	13-17	87	76 (87.3%)
Total N= 596	2-6	83	48 (57.8%)
	7-12	184	131 (71.2%)
	13-17	329	277 (84.2%)

Table III: The antibiotic resistant of ESBL producing *E. coli* isolates

Antibiotics	Name of Talukas			Total resistant *E. coli* N (%)
	Khairpur N=200	Gumbat N=236	Kotdiji N=160	
Ceftriaxone	22	18	31	71 (15.6%)
Cefotaxime	29	37	32	98 (21.5%)
Ciprofloxacin	19	17	22	58 (12.7%)
Ceftazidime	23	21	28	72 (15.8%)
Nitrofurantoin	20	23	26	69 (15.1%)
Aztreonam	67	54	39	160 (35.1%)
Amoxicillin/Clavulanate	39	31	38	108 (23.7%)
Ampicillin	42	51	36	129 (28.3%)

Table IV: Determination of the synergetic effect of different dilutions of aqueous extract of *Azadirachta indica* (Neem-a) and *Ocimum tenuiflorum* (Tulsi-b) on the growth of ESBL producing *E. coli* isolates.

Test concentration (µL/100 mL)	Zone of inhibition (mm) by agar well diffusion method		
	Neem (a)	Tulsi (b)	(a) and (b)
1.0	9.2	7.6	5.5
2.0	10.5	8.4	10.6
3.0	12.5	9.2	12.8
4.0	13.4	10.5	15.0
5.0	14.5	11.8	17.0
6.0	15.5	12.5	19.0
7.0	16.7	13	21.2
8.0	17.6	15.2	24.0
9.0	18.7	16.5	26.5
10	19.5	17.5	28.7
11	20.5	18.4	30.5
12	22.3	19.5	32.0
13	23.0	20.7	34.0
14	24.2	21.2	**35.5**
15	25.4	21.9	35.5
16	26.5	23.7	35.5
17	27.8	24.4	35.5
18	29.0	25.3	35.5
19	30.2	26.5	35.5
20	31.5	27.5	35.5
21	32.5	28.4	35.5
22	31.5	29.5	35.5
23	**33.0**	30.7	35.5
24	33.0	**32.5**	35.5
25	33.0	32.5	35.5

4. DISCUSSION

Urine tract infections (UTIs) are more frequent infection that may arise in any part

of UTI where pathogen entered inside the bladder and multiply there and start the process of infection. The UTI is brought on by *E. coli* at a high circumstance rate of about 90% [8], which are typically found in the colon and may enter the urinary tract (UT). This may be caused by patient's surgical procedures, heaved bladder intubations, increased blood sugar , which modifies the physiological functions of the body that include altered body's immunity, renal inhibition, increased prostate glands, neonatal infection and the excessive sex especially in the young females. These findings are consistent with[9-10].

The ESBL-producing *E. coli* produces a serious type of UTI with higher death rates[11]. According to our findings, there were higher percentages of ESBL-producing *E. coli* in UTI patients at the hospitals in Taluka Khairpur, Gambat, and Kotdiji, indicated 152 (76%), 171 (72.4%) and 133 (83.1%) respectively due to the poor hygiene and carelessness in both the hospital setting and the OPDs as reported by Naushad VA et al[11]. Our study revealed greater incidence rate of ESBL producing *E. coli* among the hospitalized children as reported by Peerayeh SN et al[12]. The emergence of antibiotic resistance due to extended spectrum β-lactamase (ESBL) production limited the use of β-lactam antibiotics against *Escherichia coli* ESBL producing[13]. *E. coli* showed greater resistance to Aztreonam 160 (35.1%) followed by Ampicillin 129 (28.3%), Amoxicillin/clavulanate 108 (23.7%) and others antibiotics. The resistance pattern of ESBL producing *E. coli* in different region showed lesser resistance against nitrofurantoin, which is similar to the studies of Ejaz H et al [14].

In this study the prevalence of ESBL-UTI in children was higher in the age group of 13-17 followed by 7-12 and 2-6 years. The ESBL-producing *E. coli* resistance pattern, which is similar to the study of Kumar A et al[15] and Erol B et al[16]. Lower nitrofurantoin resistance was found in *E. coli* from different geographical areas. The study found that children with ESBL-UTI were more likely to have them between the ages of 15 and 21 than they were between the ages of 7-12, then 2-6, respectively due to the inappropriate and excessive uses of antibiotics that could resulted in multidrug resistant bacteria. In such type of resistance the multidrug resistant genes can spread rapidly among bacterial population[17]. Alternative therapies other than antibiotics need to be developed to stop emergence of MDR bacteria. Having therapeutic qualities of the bioactive ingredients the herbal extracts have recently used in the treatment of various diseases and the struggle against the burden of antibiotic resistance in pathogenic bacteria[18].

In this study, we found that hospitalized individuals had a higher incidence of *E. coli* that produces ESBL. The synthesis of extended spectrum beta-lactamases (ESBL) led to the formation of antibiotic resistance, which restricted the use of β-lactam antibiotics against *E. coli*. In the course of our research, we discovered that patients in hospitals had a higher incidence of ESBL-producing *E. coli*. Antibiotic resistance developed as a result of the production of extended spectrum β-lactamases (ESBL), which limited the effectiveness of β-lactam antibiotics against *Escherichia coli*. In this study the aqueous extracts of *Azadirachta indica* (Neem) and *Ocimum tenuiflorum* (Tulsi) leaves dissolved in ethanol and methanol were used. Neem leaves are the natural source of flavonoids, polyphenols, isoprenoids, sulphurous and polysaccharides that play important role in scavenging the free radical and subsequently arresting disease pathogenesis[18]. The solvents have vital role in the emulsification of dried

extracts to form aqueous extracts such as methanol acts as both polar and non-polar substances whereas ethanol is a polar solvent that readily mixes with water and breaking down water-soluble molecules[19-21].

This is due to the fact that the cell wall contain more the cell wall contained more lipopolysaccharide and lipoprotein but less peptidoglycan, which gives the bacterial wall stiffness and also because it was more concentrated having less water, which aids in the diffusion and uptake of potent substances[18]. Increasing prevalence of ESBL- *E. coli* is a global problem with higher prevalence rate. In fact, the differences in results may be due to the difference in the risk factors include the un-prescribed therapy, use of corticosteroids and prolonged hospitalization[22].

Numerous studies have described the broad antibacterial activity of *Azadirachta indica* and *Ocimum tenuiflorum L.* leaves and linked this activity to the presence of essential oils. The previous studies revealed the antimicrobial potency of various *Azadirachta indica* and *Ocimum tenuiflorum L.* parts and compared that only the leaf extracts have been shown to be more effective against potent bacterial pathogens including the drug-resistant ESBL- *E. coli*[23]. Our findings have great potentials of synergetic effects of both methanolic extracts (in equal volume v/v) on the growth of test ESBL- *E. coli* (35.5 mm zone) at 14 µL/100 mL concentration.

In this study, *Azadirachta indica* compared to *Ocimum tenuiflorum* leaves when dissolved in methanol has greater bactericidal effect on ESBL-producing *E. coli*. Our work demonstrates that Aztreonam is the drug that ESBL-producing *E. coli* are most resistant followed by Ampicillin, Amoxicillin/Clavulanate and Cefotaxime. It is also found that acquired resistance to beta lactams is predominantly mediated by ESBLs, which were mutants of the plasmid borne TEM and SHV penicillinases[24]. It is also known that ESBL production is due to the multi-resistant genes carried by plasmids, transposons and integrons, which have the potentials to transfer to other bacteria. The reason behind this is that the test isolates could have produced CTX gene that had encoded ESBL, which is more frequent in *E. coli* because CTX enzyme has a water loving capability against certain antibiotics[25].

5. CONCLUSION

It is concluded that a rise in urinary tract infection due to ESBL-positive *E. coli* exists. UTI infection predominantly was observed in 13-17 years of children. The greater resistance among the *E. coli* isolates was noted in Aztreonam followed by Ampicillin, Amoxicillin/clavulanate and others antibiotics. On contrary, both neem and tulsi aqueous extracts have antibacterial activity (33 mm) and (32.5 mm) zones at 23 and 24 µL/100 mL concentration when dissolved in methanol. When mixed in equal volume (v/v), these extracts showed greater synergetic effect (35.5 mm) zone against the test ESBL- *E. coli* at 14 µL/100 mL concentration. These findings are concluded with the emergence of MDR strains of ESBL-*E. coli* could be treated with test extracts as alternate and inexpensive therapy for UTI caused by ESBL producing MDR strains of *E. coli*.

REFERENCES

1. Kaufman J, Temple-Smith M, Sanci L. Urinary tract infections in children: an overview of diagnosis and management. BMJ Paediatr Open. 2019 Sep 24;3(1):e000487.
2. Salman HA, Alsallameh SM, Muhamad G, Taha Z. Prevalence of multi-antibiotic resistant bacteria isolated from children with urinary tract infection from Baghdad, Iraq.

Microbiol Biotechnol Letters. 2022;50(1):147-56.
3. Hanna-Wakim RH, Ghanem ST, El Helou MW, Khafaja SA, Shaker RA, Hassan SA, et al. Epidemiology and characteristics of urinary tract infections in children and adolescents. Frontiers Cellular Infect Microbiol. 2015 May 26;5(45):1-8.
4. Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. Avicenna J Med. 2017 Jan-Mar;7(1):12-16.
5. Ghafourian S, Sadeghifard N, Soheili S, Sekawi Z. Extended spectrum beta-lactamases: definition, classification and epidemiology. Curr Issues Mol Biol. 2015 Jan;17(1):11-22.
6. Shinwari ZK. Medicinal plants research in Pakistan. J Med Plants Res. 2010;4:161-76.
7. Aslam MS, Ahmad MS. Worldwide importance of medicinal plants: Current and historical perspectives. Recent Adv Biol Med. 2016 Sep 24;2(2016):909.
8. Tullus K, Shaikh N. Urinary tract infections in children. The Lancet. 2020 May 23;395(10237):1659-68.
9. Kadigi DM, Mosha F, Moyo S, Matee MI. Etiology and antimicrobial susceptibility patterns of bacterial agents causing urinary tract infection in children under five years, dar es salaam. J Biotechnol Immunol. 2020;2(1):2.
10. Bonkat G, Pickard R, Bartoletti R, Bruyère F, Geerlings S, Wagenlehner F, et al. Urological infections. Eur Association of Urol. 2018;1-65.
11. Naushad VA, Purayil NK, Wilson GJ, Chandra P, Joseph P, Khalil Z, et al. Epidemiology of urinary tract infection in adults caused by extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae–a case–control study from Qatar. IJID Reg. 2022 Jun 1;3:278-86.
12. Peerayeh SN, Navidinia M, Fallah F, Bakhshi B, Jamali J. Pathogenicity determinants and epidemiology of uropathogenic E. coli (UPEC) strains isolated from children with urinary tract infection (UTI) to define distinct pathotypes. Biomed Res. 2018;29(10):2035-43.
13. Harris PN, Tambyah PA, Paterson DL. β-lactam and β-lactamase inhibitor combinations in the treatment of extended-spectrum β-lactamase producing Enterobacteriaceae: time for a reappraisal in the era of few antibiotic options?. Lancet Infect Dis. 2015 Apr;15(4):475-85.
14. Ejaz H, Zafa A, Mahmood S, Javed MM. Urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae. African J Biotechnol. 2011;10(73):16661-6.
15. Kumar A, Gupta R, Parveen R, Kumari P, Afzal M. Aging and UTI: are Teenagers more prone?. Asian J Multidisciplin Studies. 2020 Feb;8:2.
16. Erol B, Culpan M, Caskurlu H, Sari U, Cag Y, Vahaboglu H, et al. Changes in antimicrobial resistance and demographics of UTIs in pediatric patients in a single institution over a 6-year period. J Pediatr Urol. 2018 Apr;14(2):176.e1-e5.
17. Ali E, Islam MS, Hossen MI, Khatun MM, Islam MA. Extract of neem (Azadirachta indica) leaf exhibits bactericidal effect against multidrug resistant pathogenic bacteria of

poultry. Vet Med Sci. 2021 Sep;7(5):1921-7.
18. Yadav DK, Bharitkar YP, Chatterjee K, Ghosh M, Mondal NB, Swarnakar S. Importance of Neem Leaf: An insight into its role in combating diseases. Indian J Exp Biol. 2016 Nov;54(11):708-18.
19. Kibret M, Abera B. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. Afr Health Sci. 2011 Aug;11(1):S40-5.
20. Hashim N, Abdullah S, Hassan LS, Ghazali SR, Jalil R. A study of neem leaves: Identification of method and solvent in extraction. Materials Today: Proceedings. 2021 Jan 1;42:217-21.
21. Osmić S, Begić S, Mićić V. The effect of concentration of methanol as a solvent on the antioxidative activity of sage extract. InNew Technologies, Development and Application 4. Springer Int Publishing. 2019;42:480-90.
22. Rajabnia M, Forghani MS, Hasani S, Bahadoram M, Mohammadi M, Barahman M. Prevalence and antibiotic resistance pattern of extended spectrum beta lactamase producing Escherichia coli isolated from urinary tract infection. J Renal Inj Prev. 2019; 8(2):78-81.
23. Ghandour A, Abdel-Rahim M, Bayoumi SA, Sayed HM, El-Badawy O. Evaluation of antimicrobial and synergistic effects of some medicinal plant extracts on antimicrobial resistant organisms. Microbes Infectious Dis. 2021 Nov 1;2(4):807-18.
24. Picozzi SC, Casellato S, Rossini M, Paola G, Tejada M, Costa E, et al. Extended-spectrum beta-lactamase-positive Escherichia coli causing complicated upper urinary tract infection: Urologist should act in time. Urol Ann. 2014 Apr;6(2):107-12.
25. Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. CTX gene of extended spectrum beta-Lactamase (ESBL) producing Escherichia coli on broilers in blitar, Indonesia. Sys Rev Pharm. 2020 July 1;11(7):396-403.