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Original Article

Phenotypic Detection of Antibiotic Resistance and Production of Extended-Spectrum Beta-Lactamases in E. coli isolated from Urinary Tract Infected patients at Hayatabad Medical Complex

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ABSTRACT

This study aimed at the isolation of antibiotic resistance and ESBL producing *E. coli* from urinary tract infection patients visiting HMC hospital Peshawar. **Method:** A total of 200 samples were collected and cultured on selective media for bacterial isolation. Two hundred isolates were subjected to different morphological and biochemical tests for the isolation and identification of *E. coli*. A total of 121 *E. coli* isolations were recovered. These *E. coli* isolates were subjected to antibiotic sensitivity testing. Eight antibiotics (Meropenem, Tazocin, Ciprofloxacin, Fosfomycin, Cefotaxime, Augmentin, Ceftazidime, Cefepime) were used for this purpose. **Result:** The isolates showed high resistance to these antibiotics such as meropenem having percent resistance of 1.85 while sensitivity is 98.14%, Tazocin showed percent resistance of 9.25 and sensitivity is 85.18%, Ciprofloxacin showed resistance of 87.96 percent, sensitivity is 12.03%, Fosfomycin showed resistance of 3.70 percent and sensitivity is 95.37%, Cefotaxime, Augmentin, Ceftazidime, Cefepime showed resistance of 100 percent. The isolates were then investigated for the existence of ESBL enzymes production through double disc diffusion test. Among the 121 isolates, 108 were positive for ESBL enzymes production. **Conclusion:** Current findings highlight the high prevalence of multi drug resistant and ESBL positive isolates of *E. coli* in clinical samples. Proper management and control measures are needed to control the spread of these highly pathogenic *E. coli* isolates to ensure public health safety.

INTRODUCTION

Escherichia coli are commonly rod-shaped Gram-negative bacteria with a range from spherical, elongated and filamentous having rounded ends. They were first discovered by Theodor Escherich in an infant stool in 1885 [1]. They lack spore formation and their motility exist through the action of flagella. These bacteria are naturally facultative anaerobic but also exist as aerobic in fresh water and due to fecal nature are considered to be part of warm-blooded animals, especially humans in their digestive system. This helps to determine coliform index due to their exposure in fresh water which could be used as an indicator of human or animal excreta contamination [2].

Many E. coli groups are non-pathogenic while some groups are pathogenic. The E. coli O157:H7 strain is reflected to be very critical and pathogenic as vast literature exist about their pathogenicity [3]. Escherichia coli was first named as Bacterium coli till 1919, and then later on Castellani and Chalmers defined Escherichia as genus and introduced other types of the species E.coli [4].There are at least six main diarrheagenic pathovars of E.coli and two other pathovars are linked with neonatal meningitis and urinary tract infections and each type associated with some form of initial attachment to the host cell with following adverse effects, which could be through direct action or in the form

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of some toxin [5]. Each specific type is responsible for causing disease like diarrhea with different clinical symptoms and mechanisms. The watery diarrhea accompanied with abdominal cramps is mostly caused by enterotoxigenic E. coli. Blood, pus and mucus are generally absent from the diarrhea, and vomiting and fever are rarely evident. ETEC induce illness by the production of two types of enterotoxins, a heat-labile and stable toxin, which both affect electrolyte transport resulting in excessive fluid loss. This illness varies, from mild to life-threatening. Children under age of 5 are more prone to these infections caused by ETEC and is a potential source of their death in developing countries. Traveler's diarrhea infects adults and older children as they are asymptomatic carriers of ETEC which manifests as with nausea, vomiting and abdominal pains and with loose stools. low quality drinking water and antibodies to ETEC are statistically linked with each other in children between 7 to 10 years [6]. EPEC, are found in water and these, like ETEC, can cause traveler's diarrhea. In addition, EPEC are responsible for causing infant diarrhea under age of 2 years old children in the developing world, where it is estimated they are responsible for 5–10% of pediatric diarrheal cases. Significantly, mortality rates can be high, and in several outbreaks have exceeded 30%.Evidence suggests that transmission typically occurs directly between people and waterborne outbreaks have not been documented, although in one study by Regua et al suggest that EPEC infected children admitted to hospital with diarrhea in Rio de Janeiro, were related to limited water supply[7].

METHODS

The study was conducted at microbiology laboratory, Hayatabad Medical Complex(HMC), Peshawar. UTI samples were collected using urine samples from 200 patients. After the collection of UTI samples, the required E. coli and the ESBL producing E. coli bacteria were isolated using the following steps.

Media and Plates preparation: CLED (Cystine-Lactose-Electrolyte-Deficient Agar) Agar and MacConkey Agar media were prepared. The medium was sterilized through autoclave at required temperature 121°C and time 20min and then were poured 20 ml media in each petri plate. Each sample was inoculated using a sterile swab on CLED Agar and MacConkey Agar media plates. These plates were covered with aluminum foil and were kept for 24 hours at 37°C in aerobic conditions. After every 4 hours the plates were checked for any formation of colonies. Standard microbiological techniques were used for the identification of isolates, which were consisted of the study of colonial morphology and staining reactions Clinical Laboratory Standards Institute (CLSI) (2012). Standard biochemical tests were performed for identification of the isolated colonies of the pure culture including the: Catalase test, Oxidase test, Indole test, Triple sugar iron test, Urease test, Motility test, Citrate test

MacConkey Agar:A differential and selective medium prepared for isolation and differentiation enterics constructed on their capability to lactose fermentation for fermentable carbohydrates.The media was prepared by adding 50 gram of agar in 1 liter of distilled water and heated to dissolved it completely. The media was autoclaved for 15 minutes at 121 °C and 15ibs and then cooled to 50 °C before pouring it into sterile plate.

Gram/Liter	Formula
17.2	Gelatin digest via Pancreatic.
1.51	Peptone (Meat)
1.51	Peptone (Casein)
10.3	$C_{12}H_{22}O_{11}$
1.4	Bile (Salt)
14.0	Agar
0.04	Neutral Red
4.0	NaCl

Table1: Protocol for MacConkey Agar media preparation

MacConkey agar was used for the separation of enteric gram-negative bacteria. It was used in the distinction of fermenting lactose from non-fermenting lactose gram-negative bacteria. It was used for the isolation of intestinal pathogens and coliforms in biological specimens, water, and dairy products. MacConkey agar was generally used medium for additional bacterial cultures, such as cerebrospinal fluid and pus in UTI's patients.

Property of CLED Agar: The CLED ager consist of different vitamins, amino acids, nitrogen, and minerals for basic growth due to enzymatic digest of gelatin, Casein, and beef extract. For the growth of cysteine dependent dwarf coliform L-cystine support. For energy source lactose is fermentable carbohydrate. Color changes (green to yellow) and lowering of PH is due to lactose fermentable organisms. Bromothymol blue presence reflect the color changes capacity.

The CLED media was prepared as follows:

Gram/liter	Components
10.00	$C_{12}H_{22}O_{11}$
4.00	Peptone
128.00	Cystine (L)
20.00	Bromothymol (B)
4.00	Tryptone

Table 2: Protocol for CLED media preparation

The CLED media is using for the detection of urinary bacterial pathogen. Which help and support the growth of microorganism specifically gram positive and negative

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bacteria. It was also used for isolation of urinary pathogen which provide different colonial morphology. It also provides clearly differentiation of contaminants, microorganisms and pathogens in urine. The CLED media was prepared by adding 36 g of premix media in 1 L distilled water. The mixture was boiled for one minute in order to dissolve it completely. The media was autoclave for 15 minutes at 121 °C and 15ibs. Before pouring it into plate the prepared media were cool to 50 °C and then pour into the plates.

Gram Staining and its Microscopy: After Appling primary strain (crystal violet Gram-positive bacteria) to a heated seamer of bacterial cultural and let it incubate for one minute. To remove unbound crystal violet seamer slide was gently washed for approximately 5 seconds. For fixing the crystal violet to the bacterial cell wall gram iodine was added for one minute. Then with tap water slowly and gently washed the slide. Ad acetone or alcohol as decolorizer for three seconds. Then immediately wash it with distilled water. For counter straining need safranin to add and let them rest for 45 seconds. Repeat the step, wash the slide with distilled water gently. Dry it with bibulous paper. Now it ready to examine under light microscope.

Biochemical Tests: For identification of bacterial species by distinguishing them on the origin of biochemical activities Biochemical tests are implied. The change in fat and protein metabolism, carbohydrate uptake, compound consumption ability, enzyme invention, resistance to inhibitory substances etc. are some influences that help in bacterial identification and documentation. Some biochemical tests are given below:

Oxidase Test: In first step the plastic ampoule holds in between two fingers while protecting face from hazardous. Apply pressure against the ampoule until it breaks. The first involved in Oxidase test is in 1% solution of Kovasc oxidase reagent a small piece of filter paper should be soaked and then it let free for dry. From a fresh bacterial plate (18-24 hours) culture pick a well isolated colony in loop. And rub against the treated filter paper. In third step detect/ notice for color changes. Within five to ten seconds if color change from dark to purple so it indicates the concern organism is oxidase positive. If the color changes from dark to purple with in sixty to ninety seconds it indicates delayed oxidase positive. For oxidase negative criteria when the color does not change at all. Or it takes more than two minutes.

Indole test: The tryptophane broth was inoculated with a pure culture and incubated for 24-48 hours at 35 °C. Five drops of Kovac's reagent were added directly to the tube for observing indole production. After adding the reagent, the formation of pink to red color within seconds, is the indication of positive test while yellow or slightly cloudy layer of the regent showing negative result.

Citrate Test: In bacterial culture agar was inoculated in test tube having citrate medium. For one day at temperature of 37 oC culture was incubated. As no growth detected in citrate medium with no color change represent E. coli negative. If the reaction is positive the color change from green to blue will be possible.

Catalase Test: To identify the E. coli the literature also suggests catalase test. The catalase test was performed in agar slant tube instead of conventional slide-based method. The bacteria were inoculated on agar slant tube and incubated for 24 h at 35 °C. Then Hydrogen peroxide was added into the tube and the formation of bubbles within a minute is the indication of positive catalase test.

Triple sugar iron test: Triple Sugar Iron test is a microbiological test produce hydrogen sulfide due to fermenting sugars having pH-sensitive constituents' phenol red, 1% sucrose, 1% lactose, 0.1% glucose, sodium thiosulfate and ferrous ammonium sulfate or ferrous sulfate for the determination of fermentation glucose, sucrose, and lactose. Based on differential hydrogen sulfide production and carbohydrate fermentation patterns indicated by color change from red to yellow, gas production

Urease test: The urease test recognizes those bacteria that are capable of breakdown of urea to yield carbon dioxide and ammonia. It is mostly used to differentiate urease-positive Protease from other Enterobacteriaceae

Positive Reaction: Growth of concentrated magenta to bright pink color in 15 min to 24 h while the phenol red is used as indicator. Examples: Proteus spp, pylori Cryptococcus spp, Brucella spp, Corynebacterium spp, Yersinia spp, Helicobacter, etc.

Negative Reaction: No color change. Examples: Escherichia, Salmonella, Shigella, etc.

Negative: Escherichia coli, Weak positive: Klebsiella pneumoniae, Positive: Proteus vulgaris

Motility test: The motility test is recognizing to regulate whether an organism is non-motile or motile. This test helps us to distinguish between species and genera of bacteria. A semisolid agar medium containing 1% glucose with motility nitrate in a test tube can be inoculate with a straight wire making a single stab down, in the test tube center, and incubate at 37°C. Observe stab line in the test tube by looking up to the light to determine motility at intervals 6 h, 1 day and 2 days. Growth pattern of non-motile bacteria is clear limited to the stab line, with clear borders and translucent surrounding, while motile bacteria give hazy, rambling and scattered growths.

Determination of Antibiotics Resistance bacteria: Antimicrobial resistant pattern of E. coli isolates was determined towards various antibiotics following Kirby-

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Bauer Method to determine the antibiotic sensitivity pattern by using MH agar for testing of 24 hrs old liquid culture of bacteria. For streaking on MH agar culture, the cotton swab was streaked in three directions and in complete circle edges for purpose of regular growth. Plates were allowed to dry for 5-10 minutes. In next step fixed concentration paper disks were placed at equal distance on MH agar and were placed in incubator at 36-37 o C for 24 hrs. zone of inhibition were measured in mm around each disk. Cefotaxime (CTX), Augmentin (AMC), Ceftazidime (CAZ), Tazocin (TZP), Meropenem (MEM), Ciprofloxacin (CIP), Fosfomycin(FOS), Cefepime(FEP)

Phenotypic Detection of ESBLs: Phenotypic detection of ESBLs was performed through double disc diffusion test. Plate of Mueller-Hinton agar medium was inoculated with bacterial broth culture. A disc of amoxicillin-clavulanic acid [AMC](30 µg) in the center of the disc. Discs of ceftazidime [CAZ](30 µg), CEFOTOXIME[CTX](30 µg), CEPIFIME[FEP] (30 µg) and cefpodoxime (10 µg) were placed around the central disc with an edge-to-edge distance of about 15 mm. the plate was placed at 37 ◦C overnight. The next day, zones of inhibition were recorded and increase in zone inhibition around the outer discs and towards the central disc was taken as EBSL positive result. Statistical analysis was conducted to estimate percent resistance to antibiotics, number and distribution of phenotypically ESBL positive isolate, and gender wise distribution of antibiotics resistant isolate. Data was presented in tabulated and graphical form.

RESULTS

The current study was consisted of testing of 200 urine samples collected from the UTI patients. The samples were cultured on CLED agar, and MacConkey agar in order to identify gram positive and gram-negative bacteria based on colonial morphology. Further confirmation was carried out by different biochemical tests such as oxidase, catalyze and indole biochemical test. Antimicrobial susceptibility testing of the E. coli based UTI was performed on CLED and MacConkey agar media. A total of 121 E. coli isolations were recovered. The isolates were then investigated for the existence of ESBL enzymes production through double disc diffusion test. Among the 121 isolates, 108 were positive for ESBL enzymes production. The total number of the ESBL E. coli isolates were 108 comprising of 78 female and 30 males. The female to male ration were 73% and 27% respectivel.

Occurrence of ESBLs positive isolates of E. coli: Among the 121 isolates of E. coli, 108 were ESBLs positive based on double diffusion disc test Table 1). Among these ESBL positive isolates 30 were isolated from male patient and 78 were isolated female patients.

Gender	Frequency	Percentage
Male	30	27.7
Female	78	72.2

Table1: Gender wise frequency and percentage of UTI patients

In total 108 samples studied, 10 samples were showed resistance and 92 were sensitive to TZP and 6 samples showed intermediate result. The percentage of resistant samples were 9.25% while 85% sensitive to TZP antibiotic and 6% were in intermediate range as shown in the table 2.

Antibiotic	% Resistance	% Intermediate Resistance	% Sensitivity
TZP	9.25	6	85.18

Table 2: Antibiotic susceptibility of Tazocin

The samples were also tested for Meropenem antibiotic sensitivity. Among all the samples tested 02 samples were showed resistance and 106 were sensitive to Meropenem antibiotic. The percentage of Meropenem antibiotic resistant and sensitive were, 98% and 2% respectively as shown in the table 3.

Antibiotic	% Resistance	% Intermediate Resistance	% Sensitivity
Meropenem	1.85	0	98.14

Table 3: Antibiotic susceptibility of MEM(Meropenem)

In total 108 samples studied, 95 samples were resistant and 13 were sensitive to ciprofloxacin. The percentage of resistant samples were 88% % while 12% sensitive to ciprofloxacin antibiotic as shown in the table 4.

Antibiotic	% Resistance	% Intermediate Resistance	% Sensitivity
Ciprofloxacin	88	0	12

Table 4: Antibiotic susceptibility of CIP(Ciprofloxacin)

The samples were also tested for Fosfomycin antibiotic sensitivity. Among all the samples tested 05 samples were resistant and 103 were sensitive to Fosfomycin antibiotic. The percentage of Fosfomycin antibiotic resistant and sensitive were, 5% and 95% respectively as shown in the table 5. Bacterial isolates with infection rate are shown in table 6.

Antibiotic	% Resistance	% Intermediate Resistance	% Sensitivity
Fosfomycin	5	0	95

Table 5: Antibiotic susceptibility of FOS(Fosfomycin)

Isolated Culture	Number
E. coli ESBL	108
S. aureus	17
Proteus volgarus	13
Pseudomonas spp	10
Provedentia	5
Proteus mirabilis	6
E. coli	13

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Mixed growth	16
No growth	12
Total	200

Table 6: Bacterial isolates with number of infection rate

DISCUSSION

This study was conducted to find out the antimicrobial resistence in E.coli realated UTI in patients who visited department of Microbiology, Hayatabad Medical complex (HMC), Peshawar. UTI is one of most frequent infection for which medical attention and sometime hospital admission are necessary. While the effective treatment and consideration is based on identification of actual strain of UTI and drug that are less resistant to that particular strain. In previous literature it reported that UTI is one of the most communicable disease which affect every age of population from neonate to geriatric patients [8]. In world wide community acquird diseases UTI listed on topic of that category. Both in nosomal infection (hospital acquired diseases) and community based Currently antibiotic resistance is one of the most important public health concerns. Failure in treatment may be the cause of antibiotic resistance. In our study a urine sample from suspected patient of UTI were processed on CLED agar, and MacConkey agar for the identification of gram positive and gram-negative bacteria based on colony morphology. Further confirmation was carried out through biochemical tests that include oxidase, catalase indole tests, Catalase test, Triple sugar iron test, Urease test, and Motility test [9]. Antimicrobial resistance identification was done through CLED and MHA media. Across the world, E. coli acts as a virulent pathogen and its resistance intensity is increasing with time. The occurrence of E. coli is not merely related to countries having low hygiene protocol., intercourse with multiple partners, urethritis infection of urethra urine flow blocked, problems with urine bladder emptying, kidney infections pyelonephritis and stones, geriatric population, recurrent UTI, Diabetes, and pregnancy [10]. In Pakistan E. coli is one of the main causing agents of UTI as confirmed by some of the main studies. In Pakistan the study conducted by [11] reported that E. coli is the major pathogen responsible for UTI [12]. Similarly, Rawalpindi armed force at department of microbiology reported 63% of incidence of E. coli related UTI [13]. A hospital-based study conducted in Lahore, Pakistan reported 83% of UTI patients were infected with E. coli. A study conducted in Peshawar Pakistan report 77% of the UTI who presented to the hospital was due to E. coli. In our study more female patients 78(73%) were infected with E. coli related UTI compared to the male patients 30(27%). A study conducted in 2018 revlead that while investigating uropathogen large number of female patients were infected with UTI compared with male [14]. A study conducted in north America reported high incidence of E. coli related UTI in female compared to the male[15]. Similarly, the study conducted in Pakistan stated that 70% of the UTI patients were female as compared to our study the same percentage were of female patients. This study found UTI as common in both genders but the previous literature suggested that it is most prevelent in female. The reason may be anotmy of the female urinary tract in which there is shorten urethera, vigina and anus are nearly located . A study concludes that in the last ten years antimicrobial resistance does not increase but there are some exceptional cases like ciprofloxacin and extend spectrum beta lactamases for which more resistance was found in E. coli [16]. In our study 95% patients were sensitive to Fosfomycin while 5% population were resistant to Fosfomycin. A study also revealed that in patients with urinary tract infection associated with E. coli were resistant to fluroquinolone, co-amoxiclav and co-trimoxazole and less resistant and more prescribed antibiotic was Fosfomycin [17]. In our study the rate of resistant to ceprofloxacine was 88 % . Similar results can be concluded from the litrature that the effectiveness of ciprofloxacin is reduced against gram negative bacteria that causes UTI. A study conducted in Turkey reported that resistant against ciprofloxcin was 81% [18]. Although the study also reported 58% resistant against ciprofloxcin [19]. In contrast to our finding of ciprofloxcin resitence in E.coli realted UTI, a study also concluded 32% resistence and 67 % suciptibility[20].

CONCLUSION

A high prevalence of ESBL positive E. coli was observed in the current study. These findings are alarming from the public health point of view. The choice of antibiotics becomes limited due to the occurrence of these resistant bacteria.

REFERENCES

[1] Yang X, Wang H. Pathogenic E. coli (introduction), p 695–701. Encyclopedia of food microbiology, 2nd ed. Academic Press, Oxford, UK. 2014. doi.org/10.1016/B978-0-12-384730-0.00383-9

[2] Leclerc HD, Mossel DA, Edberg SC, Struijk CB. Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. Annual Reviews in Microbiology. 2001,55(1): 201-34.doi.org/10.1146/annurev.micro.55.1.201

[3] Bavaro MF. E. coli O157: H7 and other toxigenic strains: the curse of global food distribution. Current gastroenterology reports. 2012,14(4):317-23. doi.org/10.1007/s11894-012-0264-6

[4] Castellani A, Chalmers AJ. Manual of tropical

DOI: https://doi.org/10.54393/pbmj.v5i4.360

medicine. Baillière, Tindall and Cox; 1919.doi.org/10.5962/bhl.title.84653
[5] Croxen MA, Finlay BB. Molecular mechanisms of Escherichia coli pathogenicity. Nature Reviews Microbiology. 2010,8(1): 26-38.doi.org/10.1038/nrmicro2265
[6] Brüssow H, Rahim H, Freire W. Epidemiological analysis of serologically determined rotavirus and enterotoxigenic Escherichia coli infections in Ecuadorian children. Journal of clinical microbiology. 1992,30(6):1585-7. doi.org/10.1128/jcm.30.6.1585-1587.1992
[7] Regua AH, Bravo VL, Leal MD, Lobo Leite ME. Epidemiological survey of the enteropathogenic Escherichia coli isolated from children with diarrhea. Journal of tropical pediatrics. 1990,36(4): 176-9.doi.org/10.1093/tropej/36.4.176
[8] Khan IU, Mirza IA, Ikram A, Ali S, Hussain A, et al. In vitro activity of fosfomycin tromethamine against extended spectrum beta-lactamase producing urinary tract bacteria. J Coll Physicians Surg Pak. 2014,24(12):914-17.
[9] Liu HY, Lin HC, Lin YC, Yu SH, Wu WH, et al. Antimicrobial susceptibilities of urinary extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae to fosfomycin and nitrofurantoin in a teaching hospital in Taiwan. Journal of Microbiology, Immunology and Infection. 2011,44(5): 364-8.doi.org/10.1016/j.jmii.2010.08.012
[10] Shahzad K A, Ullah F, Muhammad K, Khatoon F, Hassan Qazi M, et al. "Multiple Drug Resistance Pattern in Urinary Tract Infection Short Communication ARTICLE HISTORY ABSTRACT". J Infect Mol Biol, 2013,4(1):67–70.
[11] Gupta K, Hooton TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Annals of internal medicine. 2001,135(1): 41-50.doi.org/10.7326/0003-4819-135-1-200107030-00012
[12] Jafri SA, Qasim M, Masoud MS. Antibiotic resistance of E. coli isolates from urine samples of Urinary Tract Infection (UTI) patients in Pakistan. Bioinformation. 2014,10(7):419. doi.org/10.6026/97320630010419
[13] Kibret M, Abera B. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. African health sciences. 2011,11: 40-5.doi.org/10.4314/ahs.v11i3.70069
[14] Scharff AZ, Rousseau M, Mariano LL, Canton T, Albert ML, Fontes M, Duffy D, Ingersoll MA. Sex differences in IL-17 determine chronicity in male versus female urinary tract infection. bioRxiv. 2018: 449124.doi.org/10.1101/449124
[15] Dielubanza EJ, Schaeffer AJ. Urinary tract infections in women. Medical clinics. 2011,95(1): 27-41.doi.org/10.1016/j.mcna.2010.08.023
[16] Linhares I, Raposo T, Rodrigues A, Almeida A. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000–2009). BMC infectious diseases. 2013,13(1): 1-4.doi.org/10.1186/1471-2334-13-19
[17] van Driel AA, Notermans DW, Meima A, Mulder M, Donker GA, et al. Antibiotic resistance of Escherichia coli isolated from uncomplicated UTI in general practice patients over a 10-year period. European Journal of Clinical Microbiology & Infectious Diseases. 2019,38(11):2151-8. doi.org/10.1007/s10096-019-03655-3
[18] Kumar S, Dave A, Wolf B, Lerma EV. Urinary tract infections. Dis Mon. 2015,61:45–59. doi: 10.1016/j.disamonth.2014.12.002.
[19] Turpin C, Minkah B, Danso K, Frimpong E. Asymptomatic Bacteriuria in Pregnant Women Attending Antenatal Clinic at Komfo Anokye Teaching Hospital, Kumasi, Ghana. Ghana Med J. 2007,41(1):26–29.
[20] Kazemier BM, Schneeberger C, De Miranda E, Wassenaer AV, Bossuyt PM, et al. Costs and effects of screening and treating low risk women with a singleton pregnancy for asymptomatic bacteriuria, the ASB study. BMC Pregnancy Childbirth. 2012,12:52. doi:10.1186/1471-2393-12-