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ORIGINAL ARTICLE

Multi-Drug Resistance of Escherichia coli (E.coli) Isolated from Clinical Isolates in District Peshawar Kp Pakistan

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ABSTRACT

Multidrug-resistant Escherichia coli have become a main public health distress in Pakistan and many countries, causing failure in treatment with the resultingenormous health burden. The current study was aimed to find the prevalence of E. coli among clinical isolates and their antibiotics susceptibility pattern using standard procedures. The Purpose of the present study to investigate the Prevalence and antibiotic susceptibility pattern of E. coli detected from clinical patients visiting Khyber teaching hospital Peshawar. In this study a total 415 sample were isolated from the patient of Khyber teaching hospital (KTH) Peshawar. The collected samples were processed for identification including Gram staining and biochemical test. Furthermore, the antibiotic susceptibility pattern of all the positive strains of E. coli was tested using selected antibiotic discs by disc diffusion method as per CLSI guidelines 2019.A total of 415 samples, 112 clinical isolates yielded the growth of E. coli using standard procedures. Out of 112 isolates, 52% were recovered from male patients while 60% were females. The positive samples were obtained from urine (58%), pus (14.3%), swab (8.9%), sputum (10.7%), and others 8.0% respectively. The highest sensitive drugs are FOS (99.1%), AK (98.2%), TZP (97.3%), MEM (93.8%), TGC (91.1%), CN (89.3%), SCF (78.6%), DO (65.2%), CT (60.7%), F (49.1%), ATM (27.7%) AMP (23.2%), CAZ (23.2%), CPM (22.3%) , CRO (19.6%) ,CTX (13.4%) and the lowest sensitive drug is CIP which is (7.1%) . The drugs which show high resistance are CIP (92.9%), CTX (86.6%), CRO (80.4%), CPM (77.7%), CAZ (76.8%), AMP (76.8%) , ATM (72.3%) , CT(39.3%) , DO (34.8%), SCF (21.4%), CN(10.7%),TGC(8.9%), F(8.9%),MEM(6.3%) ,TZP(2.7%),AK (1.8%) ,and the lowest resistance drug is FOS (0.9%).Drug resistance monitoring and the epidemiological analysis of patient data are needed regularly and can be useful for the adequate management of antimicrobial resistance.

Key words: Multidrug-resistant, Antimicrobial Resistance, Clinical Isolates, Bacteria, Peshawar

INTRODUCTION

Escherichia coli (E. coli) is a general pathogen associated with community-associated as well as nosocomial infections. From the last few years the spreading ratio of E.coli is very high and show resistance to different broad-spectrum antimicrobial agent [1] Escherichia coli has a major Contribution and prime member of the family Enterobacteriaceae and isolated in urine samples causing urinary tract infections. It also showed significant resistance to antibiotics and created therapeutic problems [2]. Mostly Escherichia coli are facultative anaerobe and gram-negative bacillus, motile present either in single form or pairs [3]. In 1885 Escherichia coli is mostly derived from the Latin word coli which means colon and has been invented by Theodor Escherichia, a German bacteriologist and pediatrician [4]. As we know that E.coli is the genus species of Escherichia which engage usually motile gram-negative bacilli within the family Enterobacteriaceae and the caste Escherichia [5]. Escherichia coli is the major prevailing facultative anaerobe of the human colonic flora. The gastrointestinal tract of the infant is found generally by the organism within hours of human life, E. coli and the host derives mutual benefits. [6]. E. coli normally remains restricted harmless to the intestinal lumen; however, when gastrointestinal barriers are violated, in the debilitated or immunosuppressed host, or even normal "nonpathogenic" Mostly E.coli strains are responsible for different kind of infection. Moreover, even the most strong members of our species may be sensitive to infection by one of a few high modified copies of E.coli which enlarge mutually to have the ability to effect a broad spectrum of human disease. Infection due to pathogenic E. coli may be limited to the mucosal surfaces or can distribute throughout the body. The most important three clinical diseases occur due to infection with naturally pathogenic E. coli isolates: (i) urinary tract infection, (ii) enteric/ diarrheal disease, and (iii) sepsis/meningitis. The same article will also highlight the E. coli strains causing serious diarrhea and which were include few promising pathogens of universal public health significance and will particularly focus on pathogens afflicting humans. We will focus particularly on the E. coli strains whose study has been most superior since the last decade, i.e., enterohemorrhagic. coli (EHEC), enteroaggregative. coli (EAEC) and enteropathogenic. coli (EPEC). because the categories of diarrheagenic E. coli are differentiate on the basis of pathogenic features, the intensity will be placed on the mechanism of sickness and

the expansion of diagnostic techniques based on virulence factors. Escherichia coli is the the majority plentiful facultative anaerobic gram-negative bacterium of the intestinal microflora, obviously colonizing the mucous layer of the colon. The commensal and pathogenic bacteria consist of the same genomic structure required for their survival in a competitive environment in the GIT and have the capability to disseminate to other parts of the host for survival (7). The presence of E. coli in the environment is a cause of involvement due to the reason that it does not completely start its commensalism with humans. The principal root cause of colitis, peritonitis, bacteremia, diarrheal diseases, infant mortality, and urinary tract infections that all over the world charge billions of dollars for its treatment and kill approximately two million humans every year is E. coli (8). Some may strain even cause cancer (9). In 2013, E. coli was considered for 32% about of all bacteremia reports, and in 2009 its increases up to 27% (10,11). Across Europe, Year-on-year increases in cases of bacteremia due to E. coli have been observed (12). Study reported from United States, China, and Austria which show that E.coli is the basic cause of community-acquired and hospital-acquired bloodstream infection (BSI) correspondingly (13). The present study aims to discover the prevalence of E. coli in patients visiting KTH, Peshawar, Isolation, and identification of E. coli isolated from patients. In clinical samples, to find the antibiotic susceptibility pattern of E. coli isolates against selected antibiotics and evaluate the MDR E. coli isolated from clinical samples.

METHODOLOGY

Study Site: This Current study was performed from September 2019 to December 2019 at the Department of Microbiology and pathology at Khyber Teaching Hospital Peshawar Pakistan.

Sample Size: Total 415 samples were collected from Four hundred and fifteen samples were collected from admitted patients and were treated for microbiological testing.

Collection of Samples: Total 415 different types of samples like urine, pus, wound, sputum, fluids, and blood were collected in sterile bottles on the basis of the Clinical Laboratory and usual Institute of guidelines from admitted patients in Khyber Teaching Hospital (KTH), Peshawar. These collected samples were properly labeled.

Processing of Sampling: The samples collected will be inoculated over three culture media, Cysteine Lactose Electrolyte Deficient Agar (CLED), , Blood Agar, and MacConkey Agar which were incubated at 37 C for 24 hours for bacterial growth. The isolated bacteria identification were performed through gram staining and biochemical tests. The bacterial isolates were furthermore processed to culture understanding by Disc diffusion technique using special different antibiotics on Nutrient Agar.

Culturing

CLED agar: The urine samples were streaked on Cysteine Lactose Electrolyte Deficient Agar (CLED) and incubated for 24 hours at 37 C.CLED Agar is a non-inhibitory growth medium used for the differentiation and isolation of bacteria that cause urinary tract infection. The growth of urinary pathogens supports the medium and provides specific colony morphology.The composition CLED agar 36.2gm was suspended in 1 liter of distilled water and then boiled to solidify completely. The solution was sterilized by autoclaving at 121C for 15 minutes. The medium was mixed well before pouring as shown in table.No.1.

Table 1: Concentration of CLED Agar Media

Peptone	4.0
Lactose	10.0
Andrade indicator	0.1
Tryptone	4.0
Bromothymol blue	0.02
L –cysteine	2.8
Agar	5.0
PH	7.5+-0.2 at 25%

Blood Agar: Blood agar medium is also called enriched medium which is mostly used for that microbes or bacteria that do not grow easily. Add or suspend 40 grams of media in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize it by autoclaving at 121 C for 15 minutes. Cool to 45 -50 C. For blood Agar 7% of sterile differentiated blood. The composition of blood agar is as shown in Table.No.2.

Table 2: Concentration of Blood Agar Media

Peptone	10.0
Agar	15.0
Sodium chloride	5.0
Lab- Lemon Powder	10.0
Ph	7.3 +-0.2 at 25 C

MacConkey Agar: MacConkey agar is a differential and selective medium planned to isolates and differentiates between lactose and Nonlactose fermenting bacteria. Required for MacConkey Agar media Distilled water, autoclaving, media. Suspend 36.2gm in one liter of distilled water. Carry to the boil to solidify entirely. Sterilize by autoclaving at 122 C for 16 minutes. Combine well before pouring and composition of MacConkey Agar Media as shown in Table.No.3.

Table 3: Composition of MacConkey Agar Media

Bile salts	31.5
Peptone	20.0
Neutral red	0.03
Sodium chloride	5.0
Agar	15.0
Crystal violet	0.001
PH	7.1+-0.2 at 25 C

Nutrient Agar Media: Nutrient agar is used for the development of microorganisms supporting the expansion of a broad range of non-fastidious organisms. Nutrient agar is special because it can cultivate a mixture of diverse types of bacteria and fungi which contain a lot of nutrients wanted for the growth of bacteria. The procedure of NAM is to Suspend 28g in 1 liter of distilled water and Bring to the boil to dissolve completely Sterilize by autoclaving at 121C for 15 minutes. Peptone and beef extract was mixed in 1 liter of distilled water autoclaved it for 121C for 15 minutes. the Composition of Nutrient Agar Media as shown in Table.No.4.

Table 4: Concentration of Nutrient Agar Media

Yeast extract	2.0
Lemon powder	1.0
Sodium chloride	5.0
Peptone	5.0
Agar	10.0
Ph	7.4+-0.2 at 25C

Nutrient Broth Media: The nutrient broth is used for the wide variety of microorganisms in cultivation. It is not planned for use in the analysis of disease and other conditions. They are used for sensitivity testing as shown in Table.N0.5.

Table 5: Concentration Nutrient Broth Media

Peptone	5gm
Beef extract	3gm
Distilled water	1 liter

Gram Staining Method: The staining process, developed in 1884 by the Danish physician Christian Gram, is the mainly significant in Microbiology. It separates the majority of bacteria into two group , the gram-negative bacteria, which stain pink and the gram-positive bacteria, which stain purple, . Gram staining is a experiment for classifying and characterize bacteria through microscopic examination following staining by the process developed by Hans Christian Gram. Take a clean slide and add a drop of saline water by taking small inoculum of bacteria from an agar plate to make a smear. Now fix the smear by heat fixation. Crystal violet dye was applied to the smear and let it dry for 1 minute. The slide was washed with tap water. Iodine was applied on the slide and let it dry for 1 minute and wash with tap water. Decolorized the slide with ethanol for 15 seconds. Wash the slide with tap water. Safranin was added for 30 seconds to give a red/pink color background. Now apply the emersion oil and observed it under the microscope.

Identification of Biochemical Test: Biochemical diagnosis is a method which is used for the detection of bacterial species on the basis of biochemical activity of different bacteria. After observing pure colonies of bacteria, they were then applied for biochemical tests which include Citrate test, urease test, and triple sugar iron test. The citrate test is used to detects the ability of organisms to use citrate as the source of carbon and nitrogen to differentiate gram-negative bacteria from other bacteria, Urease test is used for the identification of microorganism's ability to produce urease and catalyzes the conversion of urea to ammonia and carbon dioxide. It is also used to find the capability of microorganisms to split urea through the manufacture of the enzyme urease. In urease positive red color was seen in urease negative yellow or orange color was seen and Triple sugar iron test is a type of biochemical test which contains three types of sugars like glucose lactose and sucrose. Based on the ability to metabolize the three sugars they are used to distinguish the species of the Enterobacteriaceae family from other gram-negative bacteria.

RESULTS

Prevalence of E. coli in clinical samples: A total of 415 samples were collected from both males and female patients visiting Khyber teaching hospital. Out of 415 samples, 112 (27 %) of the samples showed the growth ofE. Coli. And as well asdifferent types of Samples collected from infected patientsas shown in Table.No5 and Table.No.6.

Table 6: Prevalence of E. coli in the clinical sample

Parameters	Total cases	Total negative cases	Total positive cases	Percentage
E coli	415	303	112	27%

Table 7: Distribution of different types of samples collected from infected patients

Different Types of Samples	Frequency	Percentage
Urine	65	58.0%
Pus	16	14.3%
swabs	10	8.9%
Sputum	12	10.7%
Others	9	8.0%
Total	112	100

Gender wise distribution of E. coli positive isolates: A total of 415 clinical samples were collected of which196 were male patients while 217 were female patients. out of 198 samples of males, 52 samples showed growth of E. coli while 60 of the samples were found positive in the case of females out of 217 samples as shown in Table.No.6.

Table 8: Gender wise distribution of E. coli positive isolates

Gender	Total case	Total negative case	Total positive case
Male	198	146	52
Female	217	157	60
Total	415	303	112

Different age group distribution of clinical samples isolated from infected patients: A recent study reported various age groups of clinical samples extracted from contaminated patients visiting Khyber teaching hospital Peshawar. The maximum frequency was observed in the group age of 0 – 10 years (24.1%), followed by the age groups 21-40 years (22.3%),41-60

years (22.3%), and above 61years (21.4%) while the lowest prevalence was reported in the age group of 11-20 years (9.8%) as shown in Figure.No.1.

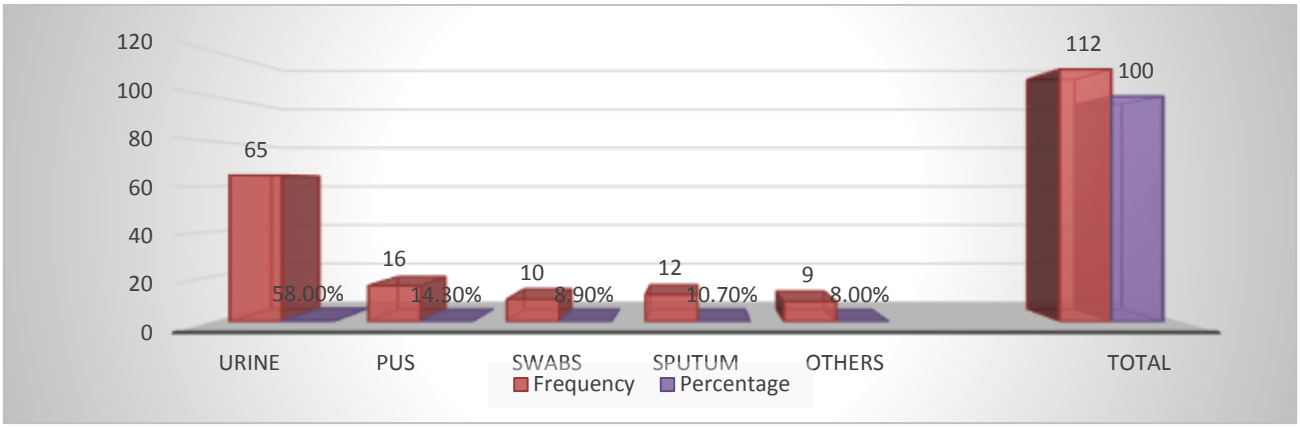

Figure 1: Different age group distribution of clinical samples isolated from infected patients

Antibiogram of E. coli in clinical isolates: A present study reported 112 of clinical samples of E. Coli from different patients. All the E. Coli isolates were tested against the antibiotics selected by the standard protocol of clinical laboratory standard institute (CLSI) of guidelines. The highest sensitive drugs are FOS (99.1%), AK (98.2%), TZP (97.3%), MEM (93.8%), TGC (91.1%), CN (89.3%), SCF (78.6%), DO (65.2%), CT (60.7%), F (49.1%), ATM (27.7%) AMP (23.2%), CAZ (23.2%), CPM (22.3%), CRO (19.6%), CTX (13.4%) and the lowest sensitive drug is CIP which is (7.1%). The drugs which show high resistance are CIP (92.9%), CTX (86.6%), CRO (80.4%), CPM (77.7%), CAZ (76.8%), AMP (76.8%), ATM (72.3%), CT (39.3%) DO (34.8%), SCF (21.4%), CN (10.7%), TGC (8.9%), F (8.9%), MEM (6.3%), TZP (2.7%), AK (1.8%), and the lowest resistance drug is FOS (0.9%) as shown in Table.No.7 and Figure.No.2.

Table 9: Antibiogram of E. coli in clinical isolates

Antibiotics	Sensitive	percentage	Resistance	Percentage	P-value
AMP	26	23.2%	86	76.8%	974
SCF	88	78.6%	24	21.4%	391
TZP	109	97.3%	3	2.7%	476
CTX	15	13.4%	97	86.6%	592
CAZ	26	23.2%	86	76.8%	168
ATM	31	27.7%	81	72.3%	555
MEM	110	93.8%	7	6.3%	845
AK	110	98.2%	2	1.8%	184
DO	73	65.2%	39	34.8%	660
CIP	8	7.1%	104	92.9%	599
FOS	111	99.1%	1	0.9%	350
F	55	49.1%	10	8.9%	281
CPM	25	22.3%	87	77.7%	123
CN	100	89.3%	12	10.7%	726
TGC	102	91.1%	10	8.9%	026
CRO	22	19.6%	90	80.4%	708
CT	68	60.7%	44	39.3%	825

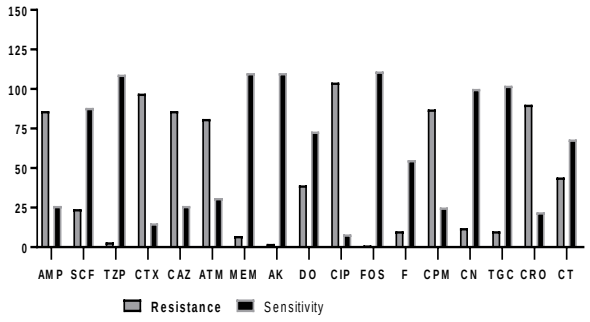

Figure 2: Anti program of E. coli in clinical isolates

DISCUSSION

Antimicrobial drug perform vital role In reducing the illness and death ratio in animal and human as well. However, the choosy force exerted by antimicrobial drug use also has been the main driving force behind the spread and emergence of drug-resistance traits between pathogenic and commensal bacteria (14,15). Observation data show that resistance in E. coli is constantly maximum for antimicrobial agents that have been in use the longest time in human and veterinary medicine (16,17). The previous 2 periods shave been observed major increases in the emergence and spread of multidrug-resistant bacteria and increasing resistance to newer compounds, such as fluoroquinolones and certain cephalosporins (18,19). The

results of the present study exposed that 27 % of isolates yielded the growth ofE. coli in a total of 415 clinical samples. A study conducted in 2013 also reported the same prevalence of 32% in clinical isolates while 27% prevalence was reported in 2009 which is like our study (20,21). While the low prevalence of 18.2% was reported between July 2011 and June 2012 (22). Another study also reported a low prevalence of 8% among the E. coli isolates recovered from clinical samples. The current study reported a high prevalence (60%) in female patients than males (40%) among the clinical isolates of E. coli. The same results were reported in a study in which 63% of isolates were obtained from female patients (23,24). These findings were on the same line reported is that the females have open genitalia, predisposing it to fecal contamination, as compared to males, whose relatively closed genital prevents the establishment of pathogens. E. coli easily spread to vaginal passage through fecal contamination, where it invades and colonizes in the urinary tract leading to infection (25) A present study reported different age groups of clinical samples collected from infected patients visiting Khyber teaching hospital Peshawar. The highest prevalence was observed in the group age of 0 – 10 years (24.1%), followed by the age groups 21-40 years (22.3%),41-60 years (22.3%), and above 61years (21.4%) while the lowest prevalence was recorded in the age group of 11-20 years (3.0%). The previous study reported the different age groups ranged from newborn to 88 years of patients having E. coli infection which are on the same line with our results. The majority of patients were observed in another study in which E. coli were found in age group >60 years (20%), followed by 31-40 years (18%) and 41-50 years (16%) which is like our findings. The current study observed that 58.0% of isolates were obtained from urine, followed by pus (14.3%), sputum (10.7%), swabs (8.9%), and others (8.0%). It was reported in another study that the majority of the E. coli isolates were recovered from urine (47%) followed by pus (26%), blood (11%), stool (8%), sputum (5%), and body fluids (3%). (26).The results of the antibiogram revealed that the antibiotics; FOS(99.1%), AK(98.2%), TZP(97.3%), MEM(93.8%), TGC (91.1%), CN (89.3%), SCF (78.6%), DO (65.2%), CT (60.7%) and F (49.1%) were highly sensitive against E. coli isolates while the high resistance are observed in antibiotics; CIP (92.9%), CTX (86.6%), CRO (80.4%), CPM (77.7%), CAZ (76.8%), AMP (76.8%) and ATM (72.3%) against E. coli isolates. In another study, it was reported that the antibiogram results show antibiotics against meropenem (41%), piperacillin-tazobactam (23%), Amikacin (23%), amoxicillin-clavulanic acid (21%), followed by aztreonam (18%), Gentamicin (12%), Ceftazidime (11%) Cefepime (10%), Ampicillin (6%).

CONCLUSION

The present study concludes that 27% of isolates yielded the growth of E. coli among clinical isolates. The results of antibiogram revealed that some antibiotics; FOS (99.1%), AK (98.2%), TZP (97.3%), MEM (93.8%), TGC (91.1%) and CN (89.3%) were effective against E. coli isolates while CIP (92.9%), CTX (86.6%), CRO (80.4%) and CPM (77.7%) were resistant. Proper culture and sensitivity testing should be performed to stop the extend of antibiotic resistance. Drug-resistance observation programs should be start in hospitals to maintain a ensure on antimicrobial resistance. Hospital Policies should be implemented to design antibiograms for each clinical unit to overcome the resistance mechanism.

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