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Comparative *in vitro* activity of selected antibacterial agents against *Escherichia coli* isolated from hospitalized patients suffering UTI

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Abstract: *Escherichia coli* is the most studied among those bacteria causing urinary tract infections. This study was aimed to find out antibacterial activity and minimum inhibitory concentration of selected antibacterial agents against *E. coli* isolates of hospitalized UTI patients. The specimens were inoculated on Eosin Methylene Blue medium. *E. coli* isolates were identified via colonial morphology, biochemical testing and API-20 kit. The susceptibility pattern of antibacterial agents was determined applying disc diffusion method (Kirby-Bauer) and dilution tube method. Among all, 38.82% (n=158/407) specimens were positive for *E. coli,* while the rest showed either no growth or exhibited colonies other than *E. coli*. while observing the susceptibility pattern, Imipenem was found the most effective (73.42%) antibacterial agent, followed by nitrofurantoin (52.53%), cefpirome (44.94%) and tazobactam/ piperacillin (44.94%), whereas the *E. coli* isolates were highly resistant to sulfamethoxazole/trimethoprim (71.52%), followed by Amoxicillin-clavulanic acid (67.72%), nalidixic acid (66.46%) and Tobramycin (62.03%), when tested by disc diffusion method. The isolates were susceptible to cefpirome (39.87%) and tobramycin (39.87%) and resistant to sulfamethoxazole/trimethoprim (75.32%), followed by levofloxacin (61.39%), when tested by tube dilution method. The study concluded high degree of resistance against Sulfamethoxazole/trimethoprim, in contrast, cephalosporin and Imipenem exhibited good potency which can be recommended for UTI.

Keywords: Antibacterial agents, tube dilution method, *E. coli,* MIC, susceptibility, UTI.

INTRODUCTION

Urinary tract infections (UTIs) are one of the frequent infections with around 10% of people facing UTI once in their life (Gharbi *et al*., 2019). It affects people of all ages from newborn to elderly (Prakasam *et al*., 2012). Every year, approximately 150 million people are being detected with UTIs, around the world (Khoshnood *et al*., 2017). Since UTI is a common hospital acquired infection, it is linked with high rates of illness and death (Sharma *et al*., 2013). *E. coli,* being the most common etiologic agent in humans (Zalewska-Piątek and Piątek, 2019), accounts for more than 80% of all UTIs (Edlin *et al*., 2013).

In hospitals, drug resistance serves as a continuous risk, with a greater chance of illness and death when infected by antibiotic resistant strains (Maillard, 2018). Antibiotic resistance has been emerged as a universal problem, threatening our potential to treat infections (Cars *et al*., 2008). Infections with drug resistant strains may result in serious ailment, higher death rates, prolonged stays in hospitals with greater chance of complications (Livermore, 2012) and higher medical expenses (Oxford and Kozlov, 2013). The overuse and misuse of antibiotics in Pakistan has played a key role in increasing and emerging the problems of antibiotic resistance (Tanvir *et al*., 2012). As UTI accounts for a huge proportion of antimicrobial agents' utilization, it is very essential to have knowledge causing infections, their susceptibility pattern towards different antibiotics as well as epidemiological characteristics. This information is very essential for therapeutic improvement and to avoid the emergence of bacterial resistance, responsible for a wide range of treatment failure (Adedeji and Abdulkadir, 2009).

Globally, rates of resistance against antibiotics needed for the therapy of UTI differ markedly area to area. The evaluation of causative agents and their antibiotic sensitivity profile can help obtaining the best empirical treatment (Farajnia *et al*., 2009). Generally, doctors prescribe drugs before isolating the causative agent or performing the susceptibility test. Careless use of drugs like improper dosages, incomplete drug courses or the use of left-over medication as a result of illiteracy and lack of awareness may become a reason for increasing resistant varieties (Ali *et al*., 2020). While increasing UTI cases and drug resistance, antibiotics should be used only when prescribed by a doctor (Asl *et al*., 2017).

While keeping the above discussion in mind, the current study was aimed to evaluate the degree of emerging resistance in *E. coli* as well as the susceptibility pattern and MIC of selected antibacterial agents against *E. coli* isolates of UTI patients by applying both disc diffusion method (Kirby-Bauer) and tube dilution method respectively to help clinicians in the study area towards right selection of antibiotics for the treatment of UTIs.

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MATERIALS AND METHODS

Specimen collection and identification

The research work examined and approved by the Institutional Bioethical Committee of Hazara University, Mansehra and was found in accordance with the ethical principles and polices followed by this university. The current clinical research was conducted in Microbiology section of Ayub Medical complex and microbiology department of Hazara University, Mansehra from July-December 2019. In which, 407 urine specimens were sampled both from male and female indoor patients in key hospitals of Hazara Division. The collected urine specimens were inoculated on EMB culture medium suitable for uropathogens. The culture plates were allowed for growth for overnight incubation at 37°C and were examined for the colonies of *E. coli*. All the *E. coli* isolates were identified through colonial morphology, different biochemical tests and API 20 Kit (api20E, Biomeriieux SA, Ref 20 100) as conducted by (Ali *et al.,* 2021).

Susceptibility pattern of E. coli isolates

The susceptibility pattern of each isolated *E. coli* was determined using Mueller Hinton agar (MHA) (CM0337-OXOID) by Kirby-Bauer disc diffusion method as prescribed (Wayne, 2011). As a susceptible control, an American Type Culture Collection bacterial strain *E. coli* (ATCC 25922) was tested simultaneously with each batch for the cross checking of any contamination. Prior to inoculation, tryptic soy broth was prepared and each isolated *E. coli* strain was inoculated and incubated in capped test tubes containing 5ml broth each for 18 hours at 35°C. Growth of each culture tube was adjusted up to 0.5 McFarland index as prescribed by Clinical and Laboratory Standards Institute (CLSI). Ten selected antimicrobial agents (table 2) were tested on MHA medium against the confirmed isolated strains of *E. coli* and after overnight incubation at 37°C, the obtained result of each sample was examined, measured and was compared with the standard Kirby-Bauer disc susceptibility chart for *E. coli* to classify the result as sensitive, intermediate and resistant (Wayne, 2011).

Minimum inhibitory concentration (MIC)

Six antibacterial agents (AMC, CTX, CPR, LEV, SXT and TOB) of the selected ten were tested via tube dilution method (TDM) (Owuama, 2017) to estimate MIC against each *E. coli* isolate. Each antibacterial agent was diluted into different concentrations in test tubes containing sterile nutrient broth. 10µl (loopful) of the fresh *E. coli* culture (0.5 McFarland standard) was inoculated into each test tube containing nutrient broth with various concentration and were incubated overnight at 37°C. Thereafter, the tubes were examined for *E. coli* growth (Turbidity). Subsequently, the broth (10µl) also was inoculated on nutrient agar plate from each test tube that exhibited no turbidity, and were allowed for overnight incubation at 37°C. The Petri dishes were examined for the colonies of *E. coli*. Those that showed colonies on agar medium was considered as MIC of that antibacterial agent.

Data analysis

MS Excel 2016 and SPSS software version 20.0 were used for the analysis of relationship among Zone of inhibition and MIC. Linear regression analysis was performed taking zone of inhibition as fixed variable (x) and MIC as random variable (y). Heterogeneity of proportion was tested in different classes using Chi-Square test.

RESULTS

Among 407 collected specimens, 61.18% (n=249/407) exhibited either no colonies on culture medium or it grew colonies other than *E. coli,* while the colonies of 38.82% (n=158/407) specimens were confirmed as *E. coli*.

Frequency distribution of the total collected specimens amongst the key hospitals varied from n=47-98 specimens, although the proportion of positive cases amongst the key hospitals varied from 31.63% to 48.91%. Frequency distribution and positive cases of each hospital are given in fig. 1. No significant difference (*p=0.1*) was detected among the specimens confirmed as positive for *E. coli*.

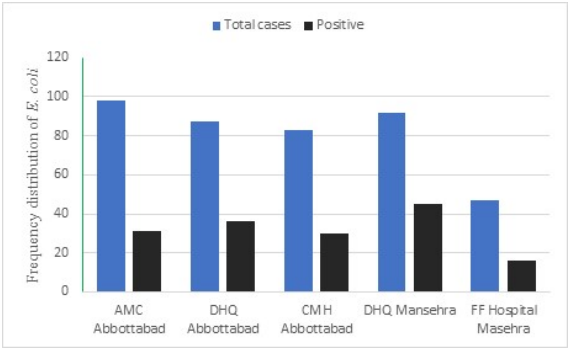

Fig. 1: Frequency distribution of all collected and positive specimens for *E. coli* collected from the key hospitals of Hazara Division

Among 407 urine specimens, 246 specimens were collected from female, while 161 specimens were collected from male population in which 97 and 61 specimens exhibited colonies for *E. coli* on culture media, respectively. It was also revealed that, amongst the clinically diagnosed female patients, *E. coli* infection was more common in the group of old age (56.41%), followed by late reproductive age (47.83%) than in early post reproductive age (20.75%) and young adults (19.57%).

Among the clinically diagnosed male patients, *E. coli* infections were less common in young adults than in later age groups (table 1). Specimens could not be collected for the age group 1-15, as no UTI patients of this age group were available at the time.

Table 1: Age and gender wise distribution of *E. coli* isolated from collected specimens

Age group (Year)	Total frequency		Male		Female	
	Total	Positive N (%)	Total	Positive N (%)	Total	Positive N (%)
1-15	0	0 (0)	0	0 (0)	0	0 (0)
16-30	77	15 (19.48)	31	6 (19.35)	46	9 (19.57)
31-45	104	47 (45.19)	35	14 (40.00)	69	33 (47.83)
46-60	99	29 (29.29)	46	18 (39.13)	53	11 (20.75)
≥ 61	127	67 (52.76)	49	23 (46.94)	78	44 (56.41)
Total	407	158 (38.82)	161	61 (37.89)	246	97 (39.43)

Table 2: Susceptibility pattern of *E. coli* isolates against selected antibacterial agents using disc diffusion method

Antibiotic (code)	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Amoxicillin-clavulanic acid (AMC)	30 (18.99)	21 (13.29)	107 (67.72)
Cefotaxime (CTX)	63 (39.87)	22 (13.92)	73 (46.20)
Cefpirome (CPR)	71 (44.94)	11 (6.96)	76 (48.10)
Imipenem (IMP)	116 (73.42)	12 (7.59)	30 (18.99)
Levofloxacin (LEV)	60 (37.79)	9 (5.70)	89 (56.33)
Nalidixic acid (NA)	30 (18.99)	23 (14.56)	105 (66.46)
Nitrofurantoin (F)	83 (52.53)	22 (13.92)	53 (33.54)
Sulfamethoxazole/ Trimethoprim (SXT)	41 (25.95)	4 (2.53)	113 (71.52)
Tazobactam/piperacillin (TZP)	71 (44.94)	27 (17.09)	60 (37.97)
Tobramycin (TOB)	40 (25.32)	20 (12.66)	98 (62.03)

Table 3: Minimum inhibitory concentration susceptibility pattern of *E. coli* isolates to six selected antibiotics tested by broth tubes dilution method.

Antibiotic (code)	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Amoxicillin-clavulanic acid (AMC)	36 (22.78)	30 (18.99)	92 (58.23)
Cefotaxime (CTX)	60 (37.97)	40 (25.32)	58 (36.71)
Cefpirome (CPR)	63 (39.87)	33 (20.89)	62 (39.24)
Levofloxacin (LEV)	45 (28.48)	16 (10.13)	97 (61.39)
Sulfamethoxazole/Trimethoprim (SXT)	14 (8.86)	25(15.82)	119 (75.32)
Tobramycin (TOB)	63 (39.87)	25 (15.82)	70 (44.30)

While comparing with the standard chart, the obtained susceptibility results of the current study indicated that IMP (73.42%) was found to be the most potent among all the selected antibacterial agents, followed by F (52.53%), while the isolated *E. coli* strains were found to be the most resistant to SXT (71.52%), AMC (67.72%), NA (66.46%) and LEV (56.33%). See summary of the susceptibility tests (table 2).

Comparing the obtained results of MIC with standard values, CPR (39.87%) and TOB (39.87%) were found the most potent antibacterial agents against tested *E. coli* isolates, followed by CTX (37.97%), while SXT was found the most resistant. Results of the current study also revealed that 37.97% of the *E. coli* isolates were noted to be susceptible to CTX when tested by MIC. A drug of choice Trimethoprim-sulfamethoxazole (SXT), uses as a first-line antibiotic for the treatment of UTI, exhibited the highest resistance rate (75.32%) when tested for MIC (table 3).

Almost all except five *E. coli* isolates were found in the current research having multi drug resistant pattern, when disc diffusion method was applied. None of the *E. coli* strain was completely sensitive or resistant to the selected antibacterial agents. Out of 158 *E. coli* isolates, 38 (24.05%) were noted to be resistant against half of the antibacterial agents. In case of TDM, all of the *E. coli* isolates, except 25, were noted to have multi drug resistant pattern. Only one isolate was found to be sensitive to all the six antibacterial agents, while six isolates were resistant against all the six antibacterial agents. When tested for MIC, 45 (28.48%) strains were found to be resistant against half of the selected antibacterial agents.

Since, the susceptibility was conducted in triplicates, so, the average values of zone of inhibition (ZI) against each *E. coli* isolate found by disc diffusion method were cross plotted with those found by TDM for MIC and linear regression as well as correlation amongst the obtained values of 6 selected antibacterial agents were determined in the form of scatter graph. As expected, the two variables of each antibacterial agent exhibited a strong inverse relationship. Strong inverse correlations were observed for all 6 selected antibacterial agents as reflected by the highest values of coefficients of determination (range 0.747-0.928). The fitted regression lines in the scatter graph explain 74.7 to 92.8% variations amongst the data lines (fig. 2). The

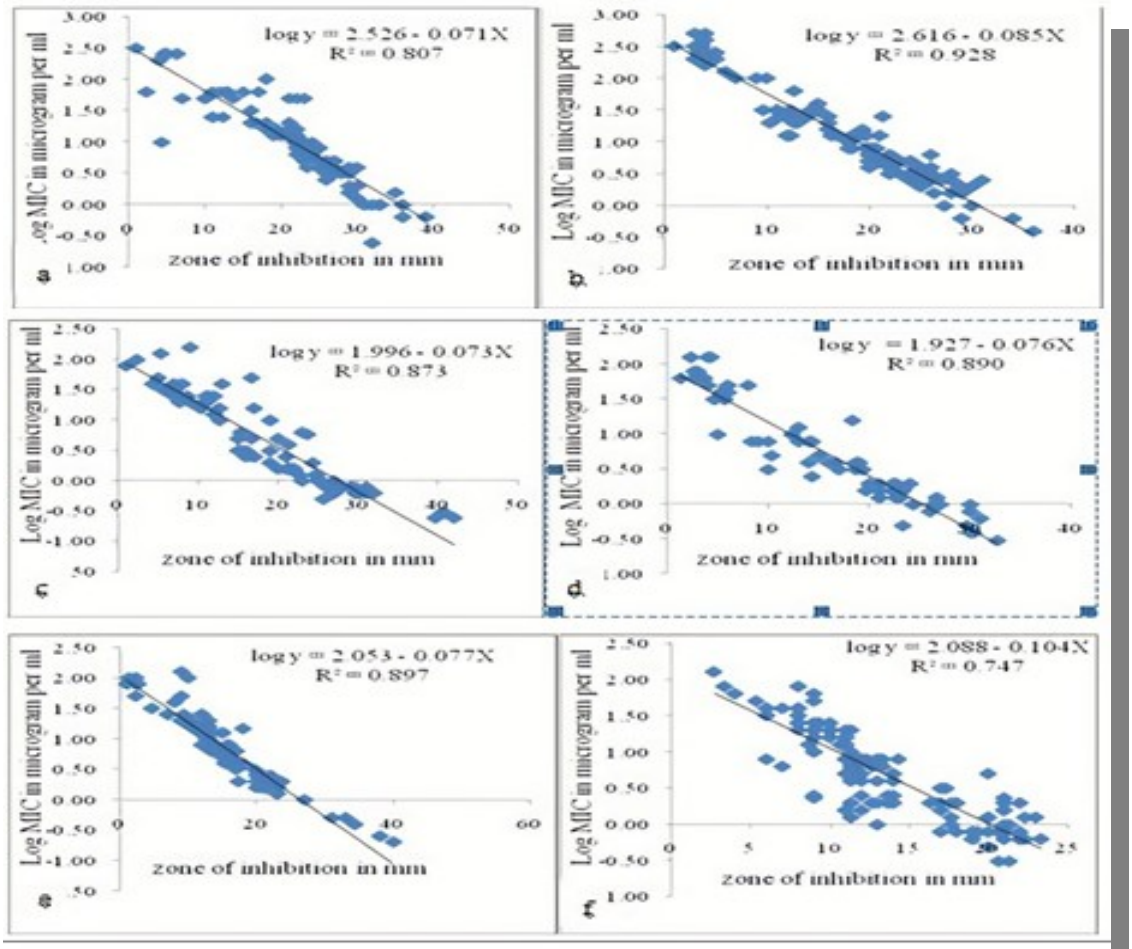

Fig. 2: Scatter graph showing relationship between disc zone diameters and logarithmic values of MICs. (a) CTX: r = -0.898; (b) CPR: r = -0.963; (c) LEV: r = -0.934; (d) SXT: r = -0.943; (e) AMC: r = -0.947; (f) TOB: r = -0.864. Slope observed in all cases was highly significant with the value p<*0.001*.

susceptibility data of selected antibacterial agents obtained by both methods exhibited a strong inverse relationship for each antibacterial agent confirms the accuracy of methodology practiced in this study.

DISCUTION

E. coli strains were isolated and found to be the most causative agents for the UTIs both in male and female population (Tansarli *et al*., 2013), therefore, it was considered to be studied as a causative agent for UTI in this study. Among 407 collected specimens, 38.82% (n=158/407) specimens exhibited colonies for *E. coli* whereas the rest exhibited either no colonies on culture medium or it grew colonies other than *E. coli*. In contrast, the previous study reported 66% of the collected samples grew colonies for *E. coli* and were found as the UTI pathogen (Bashir *et al*., 2008).

Fig. 1 revealed the frequency distribution of the total collected specimens amongst the key hospitals varied from n=47-98 specimens, although the proportion of positive cases amongst these varied from 31.63%-48.91%. No significant difference (*p=0.1*) was detected among the specimens confirmed as positive for *E. coli*. Results of the current study revealed that, almost two-fifth, 38.82% (n=158/407), of the indoor patients suffering from UTI were infected by *E. coli*. Whereas, the previous study reported more than three-fifth (66%) of the UTI patients were infected by *E. coli*. (Bashir *et al*., 2008). So, both studies agreed on the fact that the hospitalized patients suffering from UTIs were mostly infected by *E. coli*. It means that *E. coli* is the leading causative agent for UTI amongst all bacterial species.

The female population in the current study contributed more for positive, 61.39% (n=97/158) as compare to male population, 38.61%. More proportion of the female patients reveals that women are more exposed to UTIs as the previous study agrees with the current study (Hossain *et al*., 2020). It was also revealed that, amongst the clinically diagnosed female patients, *E. coli* infection was more common in the group of old age (56.41%), followed by late reproductive age (47.43%) than in early post reproductive age (20.75%) and young adults (19.57%). A study supported the current study in this regard and they reported highest infection rate in the age above 30 years (Hossain *et al*., 2020).

The obtained susceptibility results of the current study indicated that IMP (73.42%) was found to be the most potent among all the selected antibacterial agents, followed by F (52.53%), while the isolated *E. coli* strains were found to be the most resistant to SXT (71.52%), AMC (67.72%), NA (66.46%) and LEV (56.33%). See summary of the susceptibility tests (table 2). Results of the previous study (Kiffer *et al*., 2006) revealed that IMP (99.96%) was found one of the most highly potent antibiotic against *E. coli,* while another study reported the lowest resistance (7.2%) of *E. coli* to IMP (Gupta *et al*., 2006). So, the situation alarming the researchers for only few antibacterial agents including IMP are exhibiting potency against the UTI causing *E. coli,* while due to the emergence of resistance a wide range of previously potent antibacterial agents (including SXT, AMC, NA and LEV) are now exhibiting no or less potency against it.

Comparing the obtained results of MIC of Six antibacterial agents (Table 3) by TDM with standard values, CPR (39.87%) and TOB (39.87%) were found the most potent against *E. coli* isolates, followed by CTX (37.97%), while SXT was found the most resistant. While in a previous study, CPR exhibited the highest activity (99.3%) against *E. coli* (Sirot *et al*., 2002). In contrast, the decrease sensitivity rate in our study may be due to the misuse or self-medication, as in the communities of developing countries like Pakistan, self-medication and misuse of medication are at peak. Results of the current study revealed that 37.97% of the *E. coli* isolates were noted to be susceptible to CTX when tested by MIC (table 3). While another study noted just 10.2% sensitivity of CTX against *E. coli* (Sabir *et al*., 2014). The decreased rate may be due to the elevated development of resistance in *E. coli* against CTX or self-medication.

A drug of choice SXT, used as a first-line antibiotic for the treatment of UTI, exhibited the highest resistance rate (75.32%) when tested for MIC (table 3). The increased rate of resistance to SXT in our study is quite similar with the increase resistance (80.6%) against SXT in a previous study (Manikandan *et al*., 2011). The resistance in *E. coli* to SXT may be due to inappropriate prescription, such as a single drug of choice for the UTI treatment. The variances in susceptibility pattern of *E. coli* isolates in the current study as well as in those reported previously may be due to the time lag amongst these studies. Ignorance, poverty, and poor sanitary conditions may be one of the key reasons for developing antibacterial resistance. Easy availability and accessibility of forget and counterfeit medications may be one of the key reasons to emerge resistance to a wide range of potent antibiotics (Ali *et al*., 2020).

Almost all except five *E. coli* isolates were found in the current research having multi drug resistant pattern, when disc diffusion method was applied. None of the *E. coli* strain was completely sensitive or resistant to the selected antibacterial agents. Out of 158 *E. coli* isolates, 38 (24.05%) were noted to be resistant against half of the antibacterial agents. In case of TDM, all of the *E. coli* isolates, except 25, were noted to have MDR pattern. Only one isolate was found to be sensitive to all the six antibacterial agents, while six isolates were resistant against all the six antibacterial agents. When tested for MIC, 45 (28.48%) strains were found to be resistant against half of the selected antibacterial agents. The previous studies are in accordance with the current research. They reported that, most of the *E. coli* isolates showed MDR at high level (Solomon *et al.*, 2017).

The two variables of each of the 6 selected antibacterial agents exhibited a strong inverse relationship when cross plotted. Strong inverse correlations were observed for all 6 selected antibacterial agents as reflected by the highest values of coefficients of determination (range 0.747-0.928) (fig. 2). The susceptibility data of selected antibacterial agents obtained by both methods (dilution tube method and disc diffusion method) exhibited a strong inverse relationship for each antibacterial agent confirms the accuracy of methodology practiced in this study. A similar comparison was done in a previous study (Joly-Guillou *et al*., 2010).

CONCLUSION

While observing the obtained results of both methods, disc diffusion method and MIC, cephalosporin was found favorably effective against almost all *E. coli* isolates, while SXT exhibited no or less efficacy. The highest MDR pattern of *E. coli* strains isolated from urine specimens was challenging for researchers. Therefore, regular and proper monitoring mechanism need to be established for antibiotics prescriptions and therapy of UTI patients and other bacterial infections, which will ultimately be resulted to overcome the emergence of resistance in *E. coli* to broad-spectrum antibiotics like SXT and spread of these resistant strains.

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