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Research Article

Detection of extended spectrum beta lactamase from multidrug resistance *Escherichia coli* from various clinical sample in District Peshawar, Pakistan

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Citation

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Abstract

Gram negative bacteria's such as *Escherichia coli* is one of the most common community-acquired as well as the nosocomial pathogen responsible for a large number of infections. Extended-spectrum beta-lactamase producing strains of *Escherichia coli* has become a great therapeutic challenge to the clinicians in managing such infections. This study was carried out in order to find out multidrug-resistant *Escherichia coli* from various clinical samples. A total of 70 *E. coli* isolates were obtained from various clinical samples. These already identified samples were obtained from Lady Reading Hospital. Antibiotic susceptibility test was done by disc diffusion method. The present study shows that (55.71%) samples were ESBL positive. The percentage of isolates sensitive to current antibiotics were highest for Polymyxin b (98.57%) and highest for Ceftriaxone (12.85%). While the percentage of isolates resistant to current antibiotics were highest for that Co-trimoxazole (84.28%), and lowest for Polymyxin b (1.42%). ESBL producing strains of *E. coli* causes therapeutic failure and also contribute to multidrug resistance. Therefore monitoring of antimicrobial resistance in developing countries is necessary to optimize empiric treatment and the prudent use of antimicrobials.

Keywords: *Escherichia coli;* Extended-spectrum β-lactamase; Multidrug resistance

Introduction

Escherichia coli is one of the most common community-acquired pathogens responsible for a large number of nosocomial infections [1]. *E. coli* is a gram-negative, anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia*, commonly occur as normal flora of small intestine of warm-blooded

organisms [2]. The majority of *E. coli* strains are harmless. These harmless strains are part of the normal flora of the gut [3-5], which are benefited by producing vitamin K in their hosts [6] and prevent the colonization of the intestine with other pathogenic bacteria [7].

The *E. coli* related disease for the first time is diagnosed in 1982 from undercooked cow meat and unpasteurized milk [8]. The transmission of *E.coli* occurs through contaminated water (water which comes in contact with the feces of animals), while using it for drinking, for washing vegetables and fruits, and swimming in contaminated pools and lakes. It is important to note that the age and immune system of a person affected by a disease play a major role in its progress. Recent studies show's that infants aged younger than 5 years are more susceptible to this bacterium. Eating undercooked meat and burgers, eating on surfaces at service restaurants, using the immune-suppressing medication can attract *E. coli* related disease [9]. Most of the *E. coli* strains do not cause any disease [10], but virulent strains are responsible for gastroenteritis, urinary tract infections, and neonatal meningitis. It can also cause severe abdominal cramps, diarrhea that normally turns bloody within 24 hours, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to the hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia [11]. The Urinary tract infection is mostly caused by *E. coli.* [12].

The frequent use of antibiotics in rural farms increases the antimicrobial resistance in developing countries, which promotes the multiple drug resistance (MDR) in *E. coli* in both human and veterinary medicines [13, 14]. In clinical settings, mostly *E.coli* strains are treated β-lactam antibiotics, the extensive use of which has led to the emergence of ESBL's producing strains that can hydrolyze β-lactam antibiotics [15]. The ESBLs are chromosomal or plasmid-mediated β-lactamases (Enzymes that cleave the β-lactam ring), have mutated from pre-existing broad- spectrum β-lactamases (TEM-1, TEM-2, SHV-1), as a consequence of frequent use of 3rd generation antibiotics like Cephalosporins and Aztreonam. ESBL-producing genes are normally found on plasmids and its size is 80kb or larger which carry resistance determinants for aminoglycosides, fluoroquinolones, tetracyclines, Chloramphenicol and Cotrimoxazole, making the micro-organisms resist a wide variety of antibiotics [16]. Several different methods have been used for the detection of ESBL producing strains, but the most widely used technique is Double-disk synergy or disk approximation [17].

Although bacterial resistance to broad-spectrum β- lactam antibiotics are major concerns and the primary focus for clinicians and researcher, until recently not many studies have conducted in Peshawar, Pakistan to detect ESBL's producing *E coli* strains. The present study was conducted with an aim to determine the ESBL enzyme in multidrug-resistant *E. coli* isolated from various clinical samples.

Materials and methods

Study design

Prospective study was designed to determine the ESBL enzyme in multidrug-resistant *E. coli* isolated from various clinical samples.

Sampling

The study was centered on 70 samples of *E. coli*, which were collected from Lady Reading Hospital (LRH) in Peshawar, during the period of (February 2018 to August 2018). The Samples were obtained using sterile techniques to circumvent contamination

Antibiotic sensitivity testing

Antibiotic susceptibility test was done by applying the Kirby-Bauer disk diffusion method, according to the Clinical and

Laboratory Standard Institute (CLSI). Commercially available antibiotic discs were used. For example Amikacin (AK), Cefepime (FEP), Ciprofloxacin (CIP), Ceftriaxone (CRO), Amoxicillin (AMC), Tazobactam (TZP), Imipenem (IPM), Co-trimoxazole (SXT) and Polymyxin b (PB).

Phenotypic detection of ESBL

ESBL production was confirmed phenotypically by Double-disc synergy test (DDST) according to the Clinical and Laboratory Standards Institute (CLSI) criteria for ESBL screening. According to the CLSI protocol, DDST was done by using amoxicillin (30 µg)/clavulanic acid (10 µg) and cefepime (30 µg). The discs were placed 25 mm apart from each other on Muller-Hinton agar (MHA) plate inoculated with 0.5 McFarland suspension of the tested isolates. The plate was incubated overnight at 37ºC. AMC may or may not be sensitive, and FEP will be resistant but will be sensitive upto some extent towards AMC disc, which is due synergistic effect of clavulanic acid. The window formation indicated the presence of ESBL

Statistical analysis

All the data were presented in graphs and figures and was expressed in percentages.

Results

A total of 70 identified samples of *E. coli* were collected from the patients with the age 1day-90years, out of which 32 (45.71%) males and 38 (54.28%) females. Different specimens e.g urine, pus, wound swab were received from the indoor or admitted patients in Lady reading hospital Peshawar KPK (Fig. 1) depicts the sample wise distribution of clinical isolates of *E. coli*.

Antibiotic susceptibility testing in our isolates, we have found increased percentage (98.57%) of isolates showed sensitivity to polymyxin b followed by imipenem, which showed sensitivity of (90%). 84.28% of *E .coli* isolates showed resistance to co.trimoxazole. However, we have observed an elevated level of resistance to other routinely used antibiotics. The Growth of Resistant *E. coli* strain has been shown (Fig. 2), while cumulative susceptibility pattern of *E.coli* isolates were shown (Table 1).

The resistant antibiotic strains were tested for their ability to produce ESBL, the percentage of which has been shown (Fig. 3), while ESBL positive strain is shown (Fig. 4).

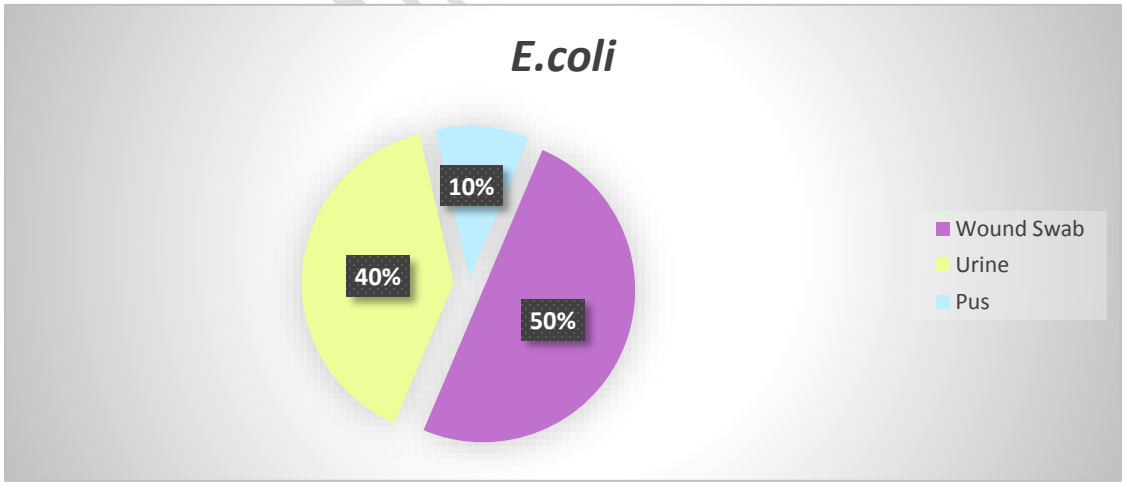

Figure 1. Shows the Percentage of *E. coli* in various clinical samples

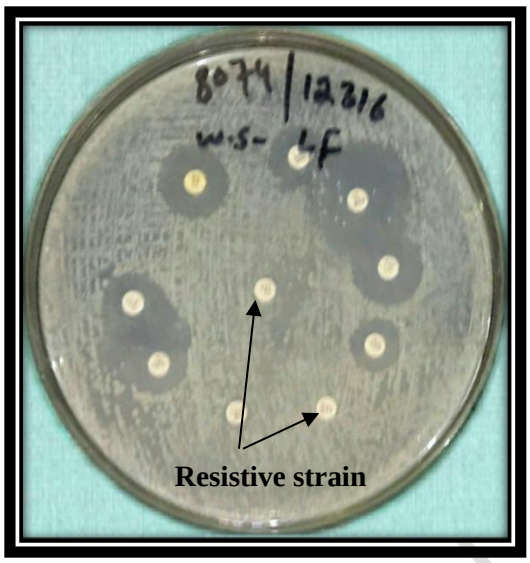

Figure 2. Growth of Resistant *E. coli* strain on MHA media

Table 1. Cumulative susceptibility patterns of *E. coli* to current antibiotics

Antibiotic disk	Total no	Sensitive		Resistance		Intermediate	
		No	%	No	%	No	%
Amoxicillin	70	43	61.42%	24	34.28%	3	4.28%
Cefepime	70	43	61.42%	25	35.71%	2	2.85%
Ceftriaxone	70	9	12.85%	49	70%	12	17.14%
Ciprofloxacin	70	11	15.71%	53	75.71%	6	8.57%
Imipenem	70	63	90%	4	5.71%	3	4.28%
Amikacin	70	52	74.28%	5	7.14%	13	18.57%
Tazobactam	70	56	80%	5	7.14%	9	12.85%
Co-trimoxazole	70	10	14.28%	59	84.28%	1	1.42%
Polymyxin b	70	69	98.57%	1	1.42%	0	0%

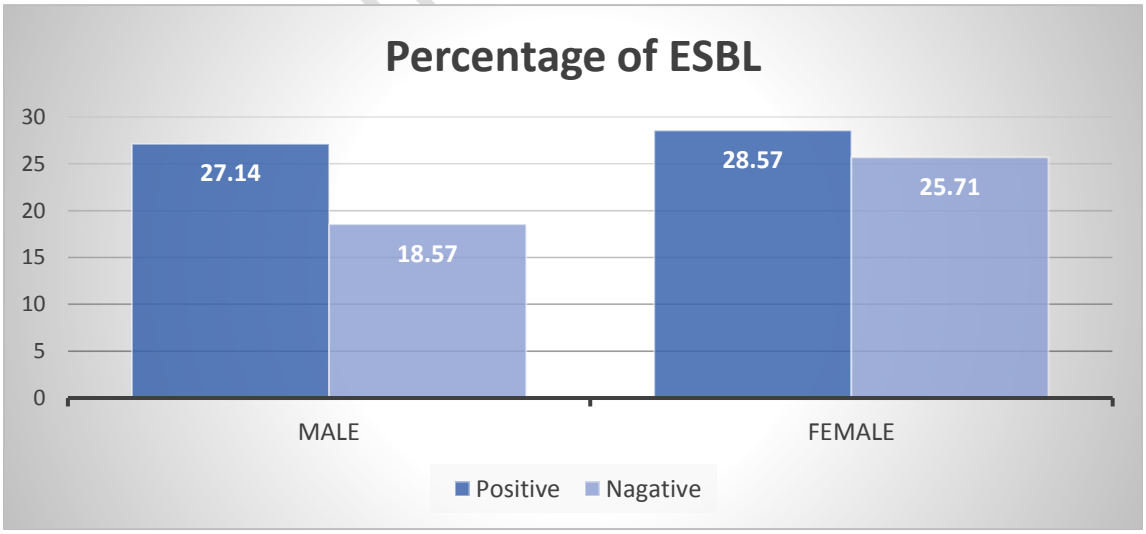

Figure 3. Shows Percentage of ESBL positive and negative in male and female

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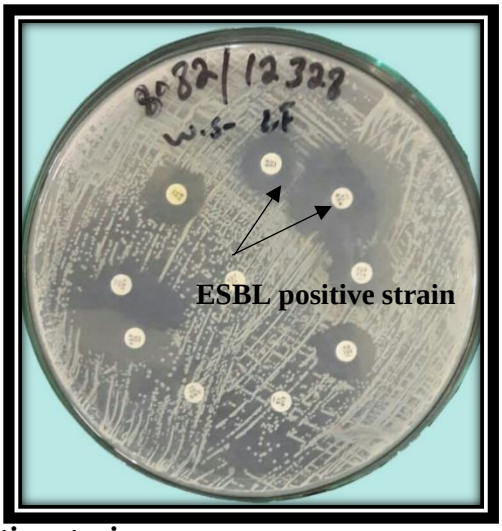

Figure 4. Shows ESBL positive strains

Discussion

Pakistan is a developing country with insufficient health infrastructure and poor feedback system. The solution to a problem is to understand the importance of it. In this study *E. coli* strain was isolated from various clinical samples, bearing out the suggestion that like other microorganisms. *E.coli* is also responsible for various clinical Infections As anticipated, the strain was resistant to Co-trimoxazole, Ciprofloxacin, Ceftriaxone, Cefepime, Amoxicillin, Tazobactam, Amikacin, Imipenem and Polymyxin b respectively, representing the appearance of multi-drug resistant forms. The present work demonstrated the resistance rate in the following direction SXT, CIP, CRO, FEP, AMC, AK or TZP, MEM and PB. Antibiogram showed a high level of resistance in the location, observed in Co-trimoxazole and Ciprofloxacin while Polymyxin b shows greater susceptibility to all the antibiotics (98.57%). In conformity with earlier observation, our result is supported by the study of Gales *et al.* **[18]** in the USA. They observe that 99.9% of *E. coli* isolates were sensitive to Polymyxin b (PB). After Polymyxin b the second most sensitive antibiotic is Imipenem (IPM) which is 90% sensitive. Our results were lower than in comparison to other study carried out in India 93.3% by Swaroop *et al.* **[19]**. It was 97% according to the study of Goudarzi *et al.* **[20]** in Iran, and 99.7% in the study conducted by Shah *et al.* **[21]** in Pakistan, moreover Ullah *et al.* **[22]** in Peshawar, Pakistan reported it is 97.4% respectively. A reason for this lower percentage of sensitivity from other studies may be due to the extensive use of antibiotics in this region. Therefore they show lower sensitivity as compared to others.

On the percentage of antibiotic resistivity, the highest resistivity is that of Co-trimoxazole (84.28%). Our result is supported by the study of Goudarzi *et al.* **[20]** in Iran, which is 80% and Ullah *et al.* **[22]** in Peshawar, Pakistan it was 81.0%.

In this study, the most important thing is ESBL which is produced by *E. coli* spp. This enzyme enables bacteria to cause serious infections by acquiring resistance against different kinds of antibiotics. The present study showed that out of 70 tested samples 39 (55.71%) were ESBL positive. Our result is supported by the study carried out in Iran (55.5%) by Goudarzi *et al.* **[20]** and in Pakistan (56.9%) by Ullah *et al.* **[22]**.

Our results were lower than in comparison to other study carried out in Turkey 84% Bali *et al.* **[23]**, whereas in the study conducted by

Ibrahim *et al.* [24] in Sudan reported it as 92.2%, It was 67.9% in the study conducted by Fernandes *et al.* [25] in Portugal. Moreover, Salem *et al.* [26] in Egypt reported it is 87% respectively.

While our result is higher than those reported in Colombia 11.7% Martinez *et al.* [27], whereas in a study conducted by Harada *et al*. [28] in Japan reported it as 20.4%. It was 36.7% in the study conducted by Yu *et al.* [29] in China, and 13.2% in the study conducted by Kiratisin *et al.* [30] in Thailand. Moreover, Goudarzi *et al.* [20] in Saudi Arabia reported it is 30.6% respectively. The inconsistency in reported results can be attributed to the frequent use of antibiotics in that particular region which will make an organism more resistant to extended-spectrum beta-lactam antibiotics.

Conclusion

The present study shows the prevalence of ESBL in *E. coli* was found to be 55.71% in Lady reading hospital Peshawar. PB (Polymyxin b) (98.57%) followed by IMP (Imipenem) (90%) are the best choice for "*E. coli*" with the highest sensitivity. Among the resistivity of antibiotics, the highest resistivity is that of SXT (Co-trimoxazole) (84.28%), which is the lowest acting antibiotic on these bacteria. It is concluded that the Multidrug resistance (MDR) is one of the major problems in various infections from all over the world. Several Hospitals belong to developing countries do not perform routine culture test procedure, because of limited resources, however, the present study supports the culture sensitivity test for every kind of infection, and also take some steps for reducing the resistivity, by minimizing the use of antibiotics, use of synergistic combinations, improving the hygienic measures and conduct further research to find the antibiotic pattern of resistance in *E. coli.*

Authors' contributions

Conceived and designed the experiments: M Zahid, Performed the experiments: M Afzaal, MF Afridi & A Haseeb, Analyzed the data: M Shah, Contributed materials/ analysis/ tools: M Haroon & R Ahmad, Wrote the paper: FU Hassan & M Sajid.

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