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Cefepime Resistance among *Escherichia coli* Isolates from Clinical Specimens in Mayo Hospital, Lahore

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Abstract:

In this study, a total of sixty clinical samples of blood, pus, urine, HVS, and miscellaneous were collected from patients and examined for microbiological evaluation and cefepime (fourth-generation cephalosporin) susceptibility pattern at Mayo Hospital, Lahore. Gram staining and various morphological, as well as biochemical tests, were performed to identify *Escherichia coli*. The antimicrobial susceptibility of bacterial isolates against cefepime was determined by the disk diffusion method. The results revealed that among sixty purified *E.coli* isolates, 53% (n=32) were resistant while 47% (n=28) showed sensitivity towards Cefepime. The bacterial isolates from lower age group patients showed higher resistance. Higher resistance of *E. coli* against cefepime among females (58%) than males (42%) was found. These findings suggest that some mechanism or genetic mutations are there which inducing resistance in *E.coli* towards antibiotics. There is an urgent need to control such alarming resistance. Education programs and improving the hygienic measures are necessary to prevent contamination with *E. coli* and minimize the use of cephalosporin antibiotics.

Keywords: Urinary tract infections, *Escherichia coli*, cefepime susceptibility.

INTRODUCTION

Antimicrobial resistance is a growing public health concern where the microorganism can survive exposure to antibiotic treatment phenomenon (Iqbal and Ashraf, 2018; Raghunath, 2008; Shawish *et al.*, 2020; Yunus *et al.*, 2016). Resistance against antimicrobial agents is increasing among Gram-negative bacteria. *Escherichia coli* is developing resistance against antimicrobial agents (particularly third and fourth generation cephalosporins) in hospital settings by the production of ESBLs (Extended-spectrum β -lactamases) (Goossens and Grabein, 2005). The spread of ESBLs has become a major public health concern due to limited therapeutic options. The herbal medicines (Morais-Braga *et al.*, 2012) can be a therapeutic alternative due to the presence of certain unique phytochemicals which possess activity against bacteria, fungi, and pests (Hussain *et al.*, 2016; Iqbal *et al.*, 2019; Iqbal *et al.*, 2015; Iqbal and Ashraf, 2019; Kalim *et al.*, 2016; Mouffouk *et al.*, 2019; Sattar *et al.*, 2016; Shahzad *et al.*, 2017).

E. coli is usually a commensal bacterium of humans and animals. Pathogenic variants cause intestinal and extraintestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia (Sodha *et al.*, 2011). Surveillance data showed that resistance in *E. coli* is consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine (Karp *et al.*, 2017).

Antibiotics are used in humans for the treatment and control of bacterial infections (Groth *et al.*, 2012). Cephalosporins are a group of semi-synthetic antibiotics, used to treat a wide variety of bacterial infections, such as respiratory tract infections (pneumonia, tonsillitis, and bronchitis), skin infections, and urinary tract infections (UTIs) (Khoshbin *et al.*, 2015). Fourth-generation cephalosporins being zwitterionic compounds, can rapidly penetrate the outer membrane of gram-negative bacteria. Besides, research has

demonstrated that beta-lactamase enzymes have a lower affinity for fourth-generation cephalosporins (Garau *et al.*, 1997).

Cephalosporins are prescribed for a wide variety of infections every day. Cephalosporins resistant bacteria are considered a serious public health threat (Lalak *et al.*, 2016). Cefepime has been referred to as a fourth-generation cephalosporin because of its extended spectrum of activity and stability to beta-lactamase hydrolysis (Sanders, 1993). Cefepime-resistant *E. coli* is one of the most important pathogens that result in a wide range of digestive symptoms, including fever, headaches, and diarrhea in children and adults in developing and developed countries (Lang *et al.*, 1994). The infectious diseases caused by cefepime-resistant *E. coli* are spreading worldwide and about 50% to 60% of nosocomial infections caused by *E. coli* are cefepime-resistant (Khalili *et al.*, 2012). Although widely accepted as broad-spectrum antibiotics, cephalosporins are not active against all the bacteria commonly isolated in a hospital microbiology laboratory (Livermore, 1987).

In Pakistan, like other developing countries, there is generally an increase in antibiotic resistance especially to all commonly used antibiotics because the availability and use of antibiotics are poorly controlled. There is no systematic national surveillance of antibiotic resistance and insufficient data is available to quantify the problem (Muhammad *et al.*, 2020). There is an urgent need to strengthen the microbiological and epidemiological capacities of health care workers internationally to prevent the transmission of nosocomial infections. This study aimed to determine cefepime resistance among *Escherichia coli* isolates from clinical specimens in Mayo Hospital, Lahore.

MATERIALS AND METHODS

Sample collection

The observational and prospective study was approved by the institutional research committee and the anonymity of patients was protected. During the study period, a total of sixty samples of blood, pus, urine, HVS, and miscellaneous were randomly collected in sterile bottles from patients at Mayo Hospital, Lahore for the period of 1st March to 30th May 2019. The date, time, and number of patients were labeled on the container and transported to the laboratory within 2 hours of collection (Chakraborty *et al.*, 2011; Saleem *et al.*, 2018a; Saleem *et al.*, 2018b).

Primary culture and preservation of bacterial strains

Blood agar, MacConkey agar, Nutrient agar, and CLED agar media were prepared following the manufacturer's instructions; pH was adjusted, autoclaved, poured in sterilized Petri plates, and was incubated at 37 °C for 24 hours for sterility check. Only sterile agar plates were selected for primary culturing after processing the samples. Nutrient agar and blood agar were used to culture the isolates of blood, and pus specimens, whereas MacConkey agar was used for the cultivation of urine isolates. Then these culture plates were incubated at 37 °C for 24 hours (Saleem *et al.*, 2018b).

Purification of Bacterial Isolates

Bacterial colonies having different morphology were selected for purification by the multiple streak method. Then bacterial colonies with different morphological characteristics were picked by a loop from primary culture plates and cultured on Blood agar, MacConkey agar, and Nutrient agar plates. The pure cultured plates were labeled and incubated at 37 °C for 24 hours (Hussain *et al.*, 2016).

Identification of bacterial isolates

All of the purified bacterial isolates (n=60) were identified based on colony morphology, microscopy, and biochemical tests following the standard protocols of Bergey's Manual of

Determinative Bacteriology (Bergey and Holt, 1994; Iqbal *et al.*, 2016; Saleem *et al.*, 2018b).

Antibiotic Sensitivity Testing

Antimicrobial susceptibility of isolates was tested for all bacterial isolated by the disk diffusion method using Muller Hinton agar following the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016). The antibiotic discs of fourth-generation cephalosporin (Cefepime) were used. Nutrient agar media was poured into sterile Petri plates and let to solidify. The plates were kept in an incubator for 24 hours in an upside-down position at 37°C. Bacterial culture from a freshly grown plate was inoculated on the nutrient agar plate. Cefepime discs were placed on the surface of the inoculated agar plate. Plates were incubated at 37°C for 24 hours. Examined the inoculated agar plates having Cefepime disks after 24 hours and measured the diameter of the zone of inhibition. Identify the sensitivity or resistance of the bacteria against Cefepime by using the available CLSI guidelines.

RESULTS

Prevalence of clinical samples

Escherichia coli was isolated from different clinical samples (n=60) such as blood (n=7), urine (n=4), pus (n=12), HVS (n=9), and miscellaneous (n=28) from male and females from hospitalized and outpatients at Mayo hospital Lahore (Table 1). Among the collected samples, (n=25) were males and (n=35) were females. It shows that the infection ratio was higher in female patients than in male patients.

Table 1. Prevalence of *E. coli* within genders.

Sr. No.	Type of sample	No. of Patients		
		Male	Female	Total
1	Miscellaneous	16	12	28
2	Pus	2	10	12
3	HVS	0	9	9
4	Blood	5	2	7
5	Urine	2	2	4
Total		25	35	60

Identification of bacterial isolates

All of the purified *E. coli* isolates (n=60) were identified based on culture characteristics,

microscopic morphology, gram stain (Table 2), and biochemical profiles (Table 3).

Table 2. Microscopic and Colonial characteristics of *E.coli* from clinical samples.

Bacterial species	Colony characteristics			Morphological characteristics			
	Color on agar	Color on MacConkey agar	Color on blood agar	Gram staining	Motility test	Oxygen requirement test	
<i>E.coli</i>	Opaque large yellow or off-white with shiny texture and mucoid elevated colonies.	Pink to rose-red, well isolated colonies may be surrounded by a zone of precipitated bile salt.	Form zone of beta hemolysis slightly convex, greenish grey	- rods	Motile	Aerobe or Facultative anaerobe	

Table 3. Biochemical identification of *E.coli* isolates from clinical samples.

Biochemical test	<i>E.coli</i>	
Gram staining	–	
Motility test	+	
Catalase test	+	
Oxidase test	–	
Indole production test	+	
Methyl red test	+	
Vogues Proskauer test	–	
Lactose fermentation test	+	
Mannitol salt agar	+	
Citrate utilization test	–	
Eosin methylene blue	+	
	Slant	A
	Butt	A
Triple sugar iron test	Gas	+
	H ₂ S	–

Cefepime Resistant *E. coli* among different age groups in clinical samples

Among the blood samples tested, two resistant isolates of *E. coli* were found in the age groups 0-20 years and 41-60 years each. Out of 4 urine

samples collected, two resistant isolates were reported in the age group between 0-40 years and most were females. Our results showed that the total number of HVS samples was nine and four resistant isolates were reported in the age group 21-40 years. A total of twelve pus samples were collected. It was found that the number of resistant samples was greater in lower ages. Among miscellaneous samples, 17 resistant strains were isolated (Table 4). The resistant strains of *E. coli* were 67% in HSV samples followed by miscellaneous samples (61%), blood samples (57%), urine samples (50%), and pus samples (33%) (Table 5).

Antibiotic sensitivity (S)/resistant (R) profile of *E. coli* isolates against fourth-generation cephalosporin (Cefepime)

Out of a total of 60 clinical samples only (n=32), 53% were resistant and the remaining (n=28) 47% were sensitive (Table 6). It shows that *E. coli* resistance is increasing so rapidly. The use of medicines not prescribed by the doctor should be avoided. Medicines should be recommended after the susceptibility test so that the resistance rate could be minimized.

Table 4. Prevalence of Cefepime Resistant *E. coli* among different age groups in clinical samples.

Sr. No.	Age group	Sample Type									
		Blood samples		Urine samples		HVS samples		Pus samples		Miscellaneous samples	
		No. of patients	Resistant isolates	No. of patients	Resistant isolates	No. of patients	Resistant isolates	No. of patients	Resistant isolates	No. of patients	Resistant isolates
1	0-20	2	2	3	2	3	0	3	1	2	0
2	21-40	1	0			4	4	3	2	6	5
3	41-60	2	2	1	0	2	1	2	1	10	7
4	61-80	2	0					2	0	5	3
5	81-100							2	0	5	2
Total		7	4	4	2	9	5	12	4	28	17

Table 5. Results of fourth-generation cephalosporin (Cefepime) resistance tested against *Escherichia coli* isolates.

Sr. No.	Type of samples	Cefepime Resistance in <i>E. coli</i>					
		Resistant strains		Sensitive strains		Total strains	
		n	%age	n	%age	n	%age
1	Miscellaneous	17	61	11	39	28	100
2	Pus	4	33	8	67	12	100
3	Blood	4	57	3	43	7	100
4	Urine	2	50	2	50	4	100
5	HVS	6	67	3	33	9	100
	Total	33		27		60	

Table 6. Antibiotic sensitivity profile of *Escherichia coli* against Cefepime.

Sensitivity / resistance profile	No. of bacterial isolates	Percentages
Sensitive	28	47%
Resistant	32	53%
Total	60	100%

DISCUSSION

In the present study, an attempt was made to determine the resistance in *E. coli* against fourth-generation cephalosporin (CEFEPIME) and the causes of this much rise in resistance. From this study, it has been seen that *E. coli* are the main cause of resistance. In various clinical samples, the cefepime resistant *E. coli* were identified and it was found that resistance is rising so rapidly. The prevalence of metal-o-beta lactamases among *E. coli* is the major cause of resistance towards beta-lactam antibiotics (Rawat and Nair, 2010).

Antibiotic resistance in bacteria isolated from human infections is increasing day by day making it a major public health and nosocomial problem (O'Connell *et al.*, 2013; Zetola *et al.*, 2005). It is very important to determine the antibiotic resistance patterns in bacterial isolates of human origin (Ashraf, 2014; Iqbal and Ashraf, 2018; Marra *et al.*, 2011). Various studies have reported the occurrence of bacteria in clinical samples (Ashraf, 2014; Iqbal and Ashraf, 2018; Sewify *et al.*, 2016).

Multidrug-resistant gram-negative bacteria show resistance towards many antibiotics. Infections

caused by MDR strains are associated with increased morbidity, mortality, and prolonged hospitalization. Thus, not only do these bacteria like *E. coli* pose a threat to global public health, but also create a significant burden to healthcare systems (Cerceo *et al.*, 2016).

In the present study (n=60) clinical samples were identified as containing *E. coli* which showed sensitivity and resistance against fourth-generation cephalosporin (CEFEPIME). In this research, 42% of samples were taken from male patients and 58% samples were female patients. The infection rate was high among females because women's gut is more hospitable to *E. coli* due to weak or underdeveloped immune systems. Most women do not take care of their diet, and also women are more prone to have UTI than men because in females, the urethra is much shorter and closer to the anus than in males, and they lack the bacteriostatic properties of prostatic secretions (Kampf, 2018; McDonnell and Russell, 1999). A previous study reported the rate of infection was higher in females (Ojo and Anibijuwon, 2010). However, both males and females of any age are prone to UTIs (Ashraf, 2014; KV and Shafiya, 2011).

We found that the highest infections were found in young age between 0-20 years and among 41-60 years the rate of resistance was also high. This is because of poor hygiene conditions among young age, greater exposure to bacteria and viruses, and poor immune systems. Old patients had higher resistance than younger in the present study and agreed with previous findings (KV and Shafiya, 2011). Urinary tract infection is a major problem for all age groups (Jha and Bapat, 2005). Another study documented that patients having 41-50 years of age were commonly infected (Kolawole *et al.*, 2009). Nosocomial infections are serious threats to a community. These infections have close associations with morbidity and mortality due to an increase in resistance towards advanced medicines like cefepime. It was found in this research that resistance is increasing at an alarming rate. Almost 53% of samples were

resistant against cefepime and only 47% showed sensitivity towards it. This is supported by a previous study that documented that the prevalence of resistance to this bacterium in Iran is high (Bechashk *et al.*, 2019).

The purpose of this research was only to know the resistance and sensitivity profile of *E. coli*. Some samples showed sensitivity and some showed resistance against fourth generation cephalosporin (cefepime). Further researches are necessary to evaluate and combat nosocomial infections caused by resistant *E. coli*.

CONCLUSION

In this study relatively, higher resistance was found in females than in males against fourth-generation cephalosporin. This is threatening to the world as the resistance rate is rapidly increasing not only against basic medicines but also against advanced medicines as seen in this study. *E. coli*, the most commonly found pathogen and is highly resistant to multi-drugs as well as advanced generation cephalosporin. Shortly it was concluded and reported that *E. coli* acquires resistance naturally or through a genetic mutation day by day. The prevalence of cefepime-resistant *E. coli* is increasing rapidly and is a threat to all. There is a crucial need to work and control epidemics.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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