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***Corresponding author:**
Mehwish Saleem;
Email: rafia_1@yahoo.com

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Characterization of Ceftazidime Resistance in Clinical Isolates of Bacteria in Lahore, Pakistan

Mehwish Saleem[1,2*], Ayesha Batool[1,2], Muhammad Naeem Iqbal[3,4], Asfa Ashraf[4,5]

[1]Department of Zoology, Govt.Post Graduate Islamia College (W) Cooper Road, Lahore, Pakistan.
[2]Microbiology Section, Sir ganga Ram Hospital, Lahore, Pakistan.
[3]The School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China.
[4]Pakistan Science Mission (PSM), Noor Kot 51770, Pakistan.
[5]The School of Life Sciences, Fujian Normal University, Fuzhou 350117, China.

Abstract
The aim of the current study was to determine Ceftazidime resistance in clinical isolates of bacteria in Lahore, Pakistan. This study was carried out at Lahore in Pathology Laboratory, Sir Ganga Ram Hospital from January to June 2018. In order to study the Ceftazidime resistant pattern, a total of 190 clinical samples were collected from different patients. From these collected samples exceeding number 88 were of pus samples followed by 56 blood, 25 sputum and 21 urine samples. All clinical samples were subjected to conventional cultural and biochemical methods for microbial enumeration. Antibiotic sensitivity was analyzed using agar disk diffusion method. The results revealed a higher prevalence of clinical samples among females (53.68%), and in age group 0-20 years (67%). Prevalence of bacteria isolated was *Escherichia coli* (81.57%), followed by *Staphylococcus aureus* (7.89%), *Pseudomonas sp.* (4.22%), *Klebsiella sp.* (3.16%), and *Proteus sp.* (3.16%). Rate of resistance to Ceftazidime was higher in females than in the males. Teenagers were highly resistant to Ceftazidime. Among the bacterial isolates, higher resistance to Ceftazidime was shown by *E.coli* (56.31%), followed by *S.aureus* (6.84%), *Pseudomonas sp.* (4.21%), *Klebsiella sp.* (1.57%), and least resistance by *Proteus sp.* (1.05%). Resistance to Ceftazidime illustrated that more consumption of a specific antibiotic leads to sustainability of resistance against those antibiotics.
Keywords: Ceftazidime, antibiotic sensitivity, bacteria, Sir Ganga Ram Hospital.

INTRODUCTION

Antimicrobial resistance (AMR) is the ability of a microbe to resist the effects of drugs formerly used to treat them. The term "antibiotic resistance", relates only to bacteria becoming resistant to antibiotics. Resistant microbes are more difficult to treat, requiring alternative drugs or higher doses, both of which may be more exclusive or more toxic. Microbes that are resistant to multiple antimicrobials are called multidrug resistant (MDR); or occasionally superbugs (Iqbal *et al.,* 2015a). Infection with antibiotic-resistant bacteria may cause severe illness, increased mortality rates, and an increased risk of problems and admission to hospitals (Kollef, 2008).

Antibiotics serve many purposes beyond treating “routine” bacterial infections. Antibiotics are often used after a medical treatment, as well as an important addition to the treatment of patients with cancer. Thus antibiotics are essential to saving individuals from infections (Piddock, 2011). Since the introduction of antibiotics in the 1930s, millions of lives have been saved and positive outcomes achieved (Hughes, 2017).

According to the European Centre for Disease Prevention and Control, 25,000 people in Europe die each year as a direct consequence of resistant infection. A recent assessment verified that the additional cost of resistance could be of £20,000 per patient episode in hospital (Smith 2013). Increased antimicrobial resistance is the cause of harsh infections, complications, longer hospital stays and increased mortality. Antibiotic overprescribing is a fastidious problem in primary care, where viruses cause most infections. Many scientists and healthcare providers, as well as policymakers, believe that the resistance levels of microbes to antibiotics has now put patients in danger (Carlet *et al*., 2012). Gram-negative rods such as *Escherichia coli*, have become resistant to almost all current antibiotics. This resistance has recently led to a widespread outbreak in Europe (Buchhols *et al*., 2018). Recent reports have shown that antibiotic resistance is a global threat (Kumarusamay *et al*., 2010).

Ceftazidime, publicized under the brand names Fortaz among others, is an antibiotic useful for the treatment of a number of bacterial infections. Exclusively, it is used for joint infections, meningitis, pneumonia, sepsis, urinary tract infections, malignant otitis externa, *Pseudomonas aeruginosa* infection, and vibrio infection. Common side effects include nausea, allergic reactions, and pain at the place of injection. It is not suggested in people who have had previous anaphylaxis to Penicillin. Its use is comparatively safe during pregnancy and breastfeeding (Hamilton, 2015).

Cephalosporins stay important agents in the treatment of many types of bacterial infections because of their broad-spectrum activity, well-characterized pharmacokinetic and pharmacodynamic properties, and confirmed safety and efficacy (Andes and Craig, 2013*).* Ceftazidime is a third-generation cephalosporin that was introduced into clinical use in the 1980s because of its verified broad spectrum activity against Gram-positive cocci and Gram-negative bacilli, including *Pseudomonas aeruginosa* (Turner, 2009). Resistance to Ceftazidime in *E.coli* isolates from intra-abdominal infections and urinary tract infections currently exceeds 10% in many North American hospitals (Hoban *et al.,* 2011).

In Pakistan like other developing countries, there is generally increase in antibiotic resistance especially to all commonly use antibiotics because the availability and use of antibiotic is poorly controlled. The physicians prescribe broad spectrum antibiotics without antibiotic susceptibility test. There is no systematic national surveillance of antibiotic resistance and insufficient data is available to quantify the problem (Abdul *et al.,* 2008). The present study was carried out to report the current Ceftazidime resistance in clinical isolates of bacteria in Lahore, Pakistan.

MATERIALS AND METHODS

Sample collection

A total of one hundred and ninety clinical samples of blood, pus, sputum and urine, from patients were randomly collected in sterile bottles at Sir Ganga Ram Hospital, Lahore. 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). A questionnaire was used about Bacterial isolates identified, sex and age of patients. The study was approved by the institutional research committee and anonymity of patients was protected. The samples were processed for microbiological examination at Pathology Laboratory, Sir Ganga Ram Hospital.

Primary culture

Blood agar, MacConkey agar and Nutrient agar and CLED agar media were prepared by using the product instructions. The pH was adjusted and the product was autoclaved. Sterile agar plates were selected for primary culturing. Samples were centrifuged at 6000 rpm for 5 minutes after sediments settled into the bottom of tubes and the supernatant was then discarded. Clinical samples were centrifuged to obtain the primarily sediments and cultured on BA, MA, Nutrient agar and CLED agar by spread out technique. Then, cultured plates were placed in incubator at 37°C for 24 hrs (Chakraborty *et al.,* 2011).

Purification and Identification of Bacterial Isolates

Isolation was done by multiple streaking of Bacterial colonies having diverse morphological characteristics (Iqbal et al., 2012; Iqbal *et al.,* 2015b; Iqbal *et al.,* 2016; Shahzad *et al.,* 2017). Bacterial colonies were picked by loop from primary culture plates and cultured on Blood agar,

MacConkey agar and Nutrient agar plates, labeled and incubated at 37°C for 24 hours. To identify unknown pure bacterial cultures, we studied colony morphology, performed microscopy and genus and species level, biochemical tests using the standard protocols of Bergey's Manual of Determinative Bacteriology (Bergey, 1984; Yunus *et al.,* 2016; Yunus *et al.,* 2017a; Yunus *et al.,* 2017b).

Antibiotic Sensitivity Testing

The antibiotic discs (Ceftazidime) were applied using disk diffusion method according to CLSI procedure (Bauer and Kirby, 1966; NCCLS. 2006) using Mueller Hinton agar. Using sterile inoculating loop, we picked the bacterial colony and dispensed it into saline solution. Plates were placed in incubator at 37°C and examined after 24 hours. Measurement and interpretations were following international guidelines.

Statistical analysis

The date obtained from the questionnaires and biochemical analysis was processed in SSPS windows version 18. Appropriate test statistics one way ANOVA were used.

RESULTS

A total of one hundred and ninety clinical samples of blood, pus, sputum and urine, from patients were included in this study. Out of which 102 (53.68%) were female and 88 (46.32%) were male. Infection rate was higher in females than males. Among age groups, 0-20 years, there were 67 (35%) clinical samples; among the age group of 21-40 years, there were 57 (30%); among 41-60 years there were 49 (25.7%); among 61-80 years there were 15 (7.8%) and the remaining 2 (1%) were within 81-100 years (Table 1).

Tabe 1. Prevalence of clinical samples among age groups

Age group	Pus	Sputum	Blood	Urine	Total
0-20	12	4	48	3	67
21-40	44	7	2	4	57
41-60	26	9	6	8	49
61-80	5	4	0	6	15
81-100	1	1	0	0	2
Total	88	25	56	21	190

Identification of bacterial isolates

All of the purified bacterial isolates (n=190) were identified on the basis of culture characteristics, microscopic morphology, gram stain (Table 2) and biochemical profiles (Table 3).

Prevalence of bacterial isolates

Among the biochemically identified bacterial isolates (n=190), the highest number was of *Escherichia coli* 155 (81.57%), followed by *Staphylococcus aureus* 15 (7.89%), *Pseudomonas sp.* 8 (4.22%), *Klebsiella sp.* 6 (3.16%) and *Proteus sp.* 6 (3.16%) (Table 4).

Antibiotic sensitivity testing

Antibiotic Sensitivity was observed by Disc diffusion protocol (Kirby Bauer). Rate of resistance to Ceftazidime was higher in females than in the males. Teenagers were highly resistant to Ceftazidime (Table 5). Among the bacterial isolates, the highest resistance was observed with Ceftazidime in *E.coli* 56.31%, *Staphylococcus aureus* 6.84%, *Pseudomonas aeruginosa* 4.21%, *Klebsiella* 1.57%, and least resistance by *Proteus sp.* 1.05% (Table 6).

DISCUSSION

Antibiotic resistance is a serious worldwide hazard to public health due to the appearance of multidrug resistant bacteria and is considered as a great problem in the treatment of bacterial infections both in hospital in addition to community settings (Pawan, 2017). Multidrug-resistant bacterial infections, particularly those caused by Gram-negative pathogens, have appeared as one of the world's greatest health threat.

Most of the samples were collected from pus 47.52% (88/190) in females because females were more prone to respiratory infections. Our results are agreed with (Khurshid *et al*., 2002) who also reported high prevalence of resistance in pus samples from females and urine specimen from males.

Table 2. Microscopic and Colonial characteristics of pathogens

Bacterial species	Colony characteristics			Morphological characteristics		
	Color on agar	Color on MacConkey agar	Color on blood agar	Gram staining	Motility test	Oxygen requirement test
E.coli	Opaque large yellow colonies and non mucoid colony elevation	Pink to rose-red. Colonies may be surrounded by a zone of precipitated bile.	Slightly convex, grey	- rods	Motile	Aerobe or Facultative anaerobe
S.aureus	Uniform, opaque and Deep yellow colonies	No growth to slight growth (pale pink)	Yellow to cream or white colonies	+ cocci	Non-motile	Facultative anaerobe
Klebsiella spp.	Large Yellowish/white and mucoid colony elevation	Pink, mucoid.	White grey and usually mucoid	- rods	Non-motile	Facultative anaerobic
Pseudomonas spp.	Pale blue green with irregular edges	Colorless to pink.	Slightly opaque colony	- rods	Motile	Aerobe
Proteus spp.	Blue grey with irregular edges and slightly elevated	White / colorless	Swarming growth pattern	- rods	Motile	Facultative anaerobe

Table 3. Biochemical identification of pathogens

Biochemical test		*E.coli*	*S. aureus*	*Proteus mirabilis*	*Pseudomonas* spp.	*Klebsiella* spp.
Oxidase test		–	–	–	+	–
Catalase test		+	+	+	+	+
Indole production test		+	–	+/–	–	–
Methyl red test		+	+	–	–	+
Vogues proskaur test		–	+	–	–	+
Lactose fermentation test		+	+	–		+
Mannitol salt agar		+	+	–	+	+
Citrate utilization test		–	+	+/–	+	+
Eosin methyline blue		+	–	–		+
Urease production test			–	+	+	+
Tripple sugar iron test	Gas	+	–	+	–	+
	H_2S	–	–	+/–	–	–

Table 4. Prevalence of bacterial isolates identified by conventional biochemical characterization in patients suffering from pathogenic infections

Name of bacteria isolated	Total number of samples	Number of samples positive for pathogens	Percentages
E. coli	190	155	81.57%
Staphylococcus aureus	190	15	7.89%
Pseudomonas spp.	190	08	4.22%
Klebsiella spp.	190	06	3.16%
Proteus spp.	190	06	3.16%
Total		190	100%

Table 5. Ceftazidime susceptibility profile among gender

Gender	Resistance	Sensitive	Intermediate
Male	61	17	13
Female	71	15	13

Table 6. Antibiotic sensitivity profile of bacterial isolates against ceftazidime

Serial No.	Bacterial isolates	Resistant	Sensitive	Intermediate
1	*E.coli*	107	25	23
2	*Staphylococcus aureus*	13	2	0
3	*Pseudomonas* spp.	8	0	0
4	*Klebsiella* spp.	3	2	1
5	*Proteus* spp.	2	2	2

Escherichia coli, *Staphylococcus aureus*, *Pseudomonas sp.*, *Klebsiella sp.* and *Proteus sp.* were isolated from clinical samples. There are several reports about the incidence of bacteria in clinical samples. This study is similar to the research of Ibrahim et al. (2012) who explained the increased multiple-drug resistant *E. coli* from hospitals in Khartoum state, Sudan. *E.coli* was the most prevalent pathogen in UTIs, also supported by previous studies (Saleem *et al.,* 2011; Vasquez and Hand, 2004).

The results showed that females are more resistant to the Ceftazidime because of higher number of recommendation of Cephalosporin in primary care than men do. These results are in accord with the results of (Schroder *et al.,* 2016) who studied data from forthcoming national or regional examination of community pharmacy, insurance or national health care systems. Women were more likely than men to receive an antibiotic treatment in their lifetime. Cephalosporin's recommended to women was 44% and 32% higher than those recommended for men respectively. Thus, as their prescription is higher, they become used of this Cephalosporin and then become resistant. Multidrug-resistances are an extremely serious public health issues associated with major epidemic outbreaks (Prescott *et al.,* 2002).

The striking increase in the rates of resistance to third-generation cephalosporins primarily results from the spread of plasmid-borne extended-spectrum beta-lactamase (ESBL), especially those be in the right place to the CTX-M family (Ruppé, 2015). Control of resistant gram-negative bacterial infections entails a comprehensive approach, including plans for risk factor recognition, detection and identification of resistant organisms, and achievement of infection-control and anticipation strategies.

CONCLUSION

Many of the bacterial strains were shown resistant to Ceftazidime which are still frequently prescribed in South Asia as they are the least expensive drugs and readily available. Females are more infected with pathogenic bacteria than the males. Now a day's *E.coli* are the more resistant bacteria to Ceftazidime which are more frequently prescribed and it indicated that utilization of a particular antibiotic leads to getting hold of resistance by that pathogenic bacteria. Continuing studies of community resistance pattern are essential and so is continuing up-to-date education of the medical community in this field.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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