Identification and Characterization of Urinary Tract Infectious Bacteria and Antibiotic sensitivity

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Abstract

Etiological patterns of uropathogens is different in different regions due to continuous evolution, of bacteria, antibiotics sensitivity patterns, misuse and overuse of antibiotics. Therefore, it is important to know the antibiotic susceptibility patterns for prescription of suitable antibiotic. This study was conducted to determine the prevalence of uropathogens and their antimicrobial sensitivity pattern from Kohat region of Pakistan. In this study 100 samples were collected from both males and females of all ages in which 70 samples contained microbes. In 30 samples no microbial growth was recorded. The Percentage of positive culture from both male and female were 57% and 43% respectively Both Gram (+) and Gram (-) bacteria were found in UTI but *E.coli* (34.21%) was predominant followed by *K. pneumoniae* (10.52%), *P. aeruginosa* (9.21%), *K. oxytoca* (6.57%), *C. albicans* (5.26%), *E. faecium* (5.26%), *E. faecalis* (3.94%), *S. aureus* (3.94%), *E. cloacea* (2.63%), *C. freundii* (2.63%), *P. mirabalis* (2.63%) and *A. baumannii* (1.31%). Many of the isolates showed resistance to commonly used antibiotics. The sensitivity

percentage of different commonly used antibiotics against both Gram (+) and Gram(-) bacteria were Ampicillin 13%, Ceftriaxone 25%, Amikacin 77%, Gentamicin 41%, Augmentin 44.77%, Fosfomycin 64%, Cotrimoxazole 36%, Nitrofurantoin 68%, Ciprofloxacin 37%, Imipenem 78%, Meropenem 67%, Cefepime 25% and Tetracycline 40%. The most effective antibiotics against both Gram (+) and Gram (-) bacteria were Fosfomycin, Imipenem, Meropenem Amikacin and Nitrofurantoin. In light of the findings of this study, it is strongly recommended to discover new antimicrobial compounds and evaluate the resistant pattern at genomic and proteomics level to discover the genes which are responsible for antibiotics resistant pattern.

Keywords: Urinary tract infection, E. coli, Uropathogens, antimicrobial sensitivity, K. pneumoniae

1. Introduction

Urinary Tract Infection (UTI) is mainly caused by pathogenic invasion of the urinary tract resulting in the inflammatory response of the urothelium. The primary cause of infection is proliferation of pathogenic bacteria in the urinary tract. Various pathophysiological factors determines the clinical manifestations of UTI such as the etiologic organism(s), associated part of the urinary tract, the infection severity and response of the patient's immune system [1]. Fever, chills, dysuria, and urinary urgency, malodorous or cloudy urine are main symptoms and signs of UTI. Infections are almost always mounting in origin. The primary cause is proliferation of bacteria in periurethra and the distal urethra [2]. Uterus, kidney, bladder and urine within the urethra of mammals are sterile under normal conditions. The low pH, urea in urine, enzymes and other end products of metabolism maintain a sterile environment. Only few organisms can survive the hypertonic medulla of the kidney. The flushing with urine and mucus clears the lower urinary tract 4-5 times a day eliminating any potential infectious organism [3]. Moreover, in men the anatomical length of urethtra (20cm) also act as a barrier against microorganisms. But in females, the short urethra (5cm) is easily crossed by microorganisms. That's why UTI in females are 14 times more common as compared to males. The vaginal and cervical epithelium produces mucus, acidic environment due to Doderlein's bacillithat degrades glycogen to lactic acid. Thus the vaginal (pH 3.5) is more acidic making it intolerant to most of the microorganisms [4].

UTI is one of the most common nosocomial infection which is caused by a variety of gram (+)

and gram (-) bacteria. Gram (-) bacteria such as *Klebsiella sp*, *Escherichia sp*, *Citrobacter sp*, *Enterobacter sp*, *Proteus sp*, *Serratia sp*, and *Pseudomonas sp* and Gram (+) bacteria such as *Staphylococcus sp*, *Streptococcus sp* and *Enterococcus sp* are frequently associated with UTIs. Among these bacteria, *E. coli* causes 80-90% of UTIs. *Klebsiella pneumonia*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Enteroccus faecalis* are most frequently isolated in ambulatory patients and nosocomial infections. [5].

The detection of significant bacteriuria, which refers to the presence of more than 100000 pathogenic bacteria per milliliter of urine, in the presence of illness, is a gold standard for diagnosis of UTI. Other scientific literature suggests 10^3 cfu/ml, depending on the type of causative agent [6]. Diagnosis of UTI is not possible on only clinical grounds. The profiling of bacteria in urine in bladder is necessary for confirmation of UTI [7]. However, the most commercial screening methods are neither easily available nor inexpensive to allow for their use in routine practice. The Screening tests are advantageous as they are rapid and hence useful in a situation where a large number of negative cultures are being processed [8]. Urinary infections cause less complications as compared to nosocomial infections, but they sometimes can cause bacteremia and then death [2].

Antibiotic resistance is dangerously increasing at high levels in all parts of the world. Our ability to treat common infectious disease is threatening due to new resistance mechanism. Antibiotics can be used in the treatment of UTIs. However, the choice of antibiotics depends upon sensitivity and the type of bacteria to several antibiotics such as Trimethoprim-Sulfamethoxazole (TMP-SMX). However, prolonged administration of antibiotics because side effects in the patients and due to mutation or through plasmid, the bacteria may develop resistance [9]. Pathogens causing UTI have developed resistance to most of the antibiotics available. This resistant developed due to misuse and prolonged use of wide spectrum antibiotics. As a result the intestinal flora changes leading to emergence of bacterial resistance [10].

It is very important to gain insights into the current knowledge of the causative organisms of UTI and their antibiotic is susceptibility. This study aimed to isolate and identify microorganisms in urine culture of suspected patients of UTI in the Kohat region of Pakistan and test the sensitivity to various antibiotics.

2. Methodology

2.1. Study site and sample collection

The sampling site was KDA Hospital Kohat, Liaquat Memorial Hospital Kohat, Combined Military Hospital Kohat and Alkhidmat Naseem Khan Memorial Hospital Kohat. Study was conducted in the Department of Microbiology, Kohat University of Science and Technology, Kohat from February 2020 to September 2020. The collection of a first morning urine samples from 100 patients suspected of UTI was done in sterile containers. The collection of a first morning urine samples was done because the overnight growth of microorganisms increases the microbial counts in the urinary bladder. Samples were collected carefully to avoid contamination. The labeled urine samples were instantly transferred to the research laboratory, Microbiology department, Kohat University of Science and Technology for analysis. Patient's demographics such as age, sex, and parameters for microbiological findings were collected on a self-developed data collection Performa. Parameters for microbiological findings include culture morphology results, and in vitro antibiotics susceptibility results of isolates. The study was undertaken with the approval and is conducted according to the declaration of Helsinki. Verbal consent was taken from each participant.

2.2 Isolation of pathogens

The 100 urine samples were cultured on nutrient agar by using pour plate method (1.0 ml) after serial dilution. The plates were then incubated aerobically for 24 hours at the temperature of 37ºC for bacterial growth respectively. On the basis of morphological cultural and biochemical properties, individual colonies were selected [11].

2.3 Identification of isolates

The isolates were identified by using a slightly modified version of method previously used by Gul et al, 2004 [12]. The cultures were examined with naked eye to observe the colonial morphology which includes size, surface, color, shape, edge, color and opacity. To notice the shape, arrangement, size and staining reaction, Gram's stain were prepared from the colonies. Oxidase, catalase and indole tests were performed and brought by Clinical and Laboratory Standards Institute, USA.

2.4 Preparation for Sensitivity Test

The sub-culturing of bacterial isolates on nutrient broth was followed by incubation

aerobically for 24hrs at the temperature of 37°C. Broth cultures (100µl) of each bacterial isolates was diluted separately in tests tubes with 250µl of normal saline solution or sterile phosphate buffer saline (PBS). McFarland standard (a chemical solution of 99.4ml of 1% conc. H_2SO_4 and 0.6ml of 1 % $BaCl_2.H_2O$) was used to compare the transparency with spectrophotometer at 540nm

2.5 Antibiotics sensitivity testing

To test the antibiotic sensitivity of isolated bacteria, the disc diffusion method was used. The antibiotics used were obtained from Karachi Market, Peshawar. The antibacterial sensitivity of isolates were evaluated against 12 different antibiotics. For all selected antibiotics, the susceptibility break points for isolates was observed the (Table: 1(a) and 1(b)). For each test organism separate plates with MHA media was used. With help of sterile cotton, isolates were streaked on petri plates and pressed for uniform contact [13]. After incubated at the temperature of 37°C for 24 hours, the plates were kept for 3 minutes [12]. Around each disc, the inhibition zone (mm) was measured with the help of meters from back of the plates and correlate with standardize chart provided by Clinical and Laboratory Standards Institute, USA. For determination of resistance against antimicrobial agents (moderate resistant (MR) or resistant (R) or susceptible (S))

Table 1.(a) Antibiotics discs used with their susceptibility break point for *Enterobacteriaceae*

No	Antibiotics	Disc Code	Discs	Zone Diameter (mm)		
				R	MR	S
1.	**Amoxicillin**	AML	25 µg	≤ 13	14–17	≥ 18
2.	**Cephalothin**	KE	30 µg	≤14	15–17	≥ 18
3.	**Amphicillin**	AMP	10 µg	≤13	14–16	≥17
4.	**Cefepime**	FEP	30 µg	≤18	19–24	≥25
5.	**Ceftriaxone**	CRO	30 µg	≤19	20–22	≥23
6.	**Imipenem**	IPM	10 µg	≤19	20–22	≥23
7.	**Tetracycline**	TE	30 µg	≤11	12–14	≥15
8.	**Gentamicin**	CN	10 µg	≤12	13–14	≥15
9.	**Amikacin**	AK	30 µg	≤14	15–16	≥17

10.	**Cefoperazone**	CFP	75 µg	≤ 15	16–20	≥21
11.	**Penicillin**	P	10 µg	≤ 13	14–17	≥ 18
12.	**Ciprofloxacin**	CIP	5 µg	≤15	16–20	≥21

All chemicals were provided by Clinical and Laboratory Standards Institute, USA.

Table 1.(b) Antibiotics discs used with their susceptibility break point for *S.aureus and P. aeruginosa*

No	Antibiotics	Disc Code	Discs	Zone Diameter (mm)		
				R	**MR**	**S**
1.	**Amoxicillin**	AML	25 µg	-	-	-
	Staphylococcus			-	-	-
	P. aeruginosa					
2.	**Cephalothin**	KE	30 µg	-	-	-
	Staphylococcus			-	-	-
	P. aeruginosa					
3.	**Amphicillin**	AMP	10 µg	≤ 28	-	≥ 29
	Staphylococcus			-	-	-
	P. aeruginosa					
4.	**Cefepime**	FEP	30 µg	-	-	-
	Staphylococcus			≤ 14	15–17	≥ 18
	P. aeruginosa					
5.	**Ceftriaxone**	CRO	30 µg	-	-	-
	Staphylococcus			-	-	-
	P. aeruginosa					
6.	**Imipenem**	IPM	10 µg	-	-	-
	Staphylococcus			≤ 15	16–18	≥ 19
	P. aeruginosa					
7.	**Tetracycline**	TE	30 µg	≤ 14	15–18	≥ 19
	Staphylococcus			-	-	-
	P. aeruginosa					
8.	**Gentamicin**	CN	10 µg	≤ 12	13–14	≥ 15
	Staphylococcus			≤ 12	13–14	≥ 15
	P. aeruginosa					
9.	**Amikacin**	AK	30 µg	≤ 14	15–16	≥ 17
	Staphylococcus			≤ 14	15–16	≥ 17
	P. aeruginosa					
10.	**Cefoperazone**	CFP	75 µg	-	-	-
	Staphylococcus			-	-	-
	P. aeruginosa					

11.	**Penicillin**	P	10 µg	≤ 28	-	≥ 29
	Staphylococcus			≤ 14	15–20	≥ 21
	P. aeruginosa					
12.	**Ciprofloxacin**	CIP	5 µg	≤ 15	16–20	≥ 21
	Staphylococcus			≤ 15	16–20	≥ 21
	P. aeruginosa					

3. RESULTS

According to the inclusion criteria, data from 100 urine specimens from patients suspected of UTI were collected conveniently during a period of three months from March 2020 to May 2020. Among the 100 cultures analyzed, 56% (56/100) yielded bacterial growth, 14% (14/100) yielded mix growth and 30% (30/100) yielded no growth of bacteria.

3.1. Identification of isolates

The morphological characteristics of isolates including size, color and morphology was observed from the incubated nutrient agar plates (Table: 2). The isolated bacteria were *p. aeruginosa*, *S. aureus*, *K. pneumonia*, *E. coli*, *E. aerogenes* and *P. mirabilis.*

Table 2. Morphological characteristics of the Test Isolates

No	**Isolates**	**Colony/Culture Characteristics**				
		Elevation	**Color**	**Margins**	**Texture**	**Opacity**
1.	Isolate #1	Raised	Blue-green (pigments)	Undulate	Glistening	Transparent
2.	Isolate #2	Flat	Grayish white	Regular	Smooth	Opaque
3.	Isolate #3	Raised	Creamy	Entire	Smooth shiny	Opaque
4.	Isolate #4	Flat (rounded knob)	Whitish pale	Undulate	Muciod	Transparent

5.	Isolate #5	Raised (convex)	Creamy (pigments)	Entire	Smooth	Transparent
6.	Isolate #6	Raised (convex)	Grayish	Entire	Smooth shiny	Transparent

3 .2. Male to Female Ratio

During this study from 100 clean catch mid-stream urine specimens there were 42 males (42%) and 58 females (58%). Out of these 100 cultures examined, 56% (56/100) yielded bacterial growth of which 42.85% (24/56) were males and 57.14% (32/56) were female. In both males and females the predominant microorganism was *E.coli* followed by *K. pneumoniae* and *P.aeruginosa*. The Gender wise distribution of various isolates is illustrated in (Figure 1).

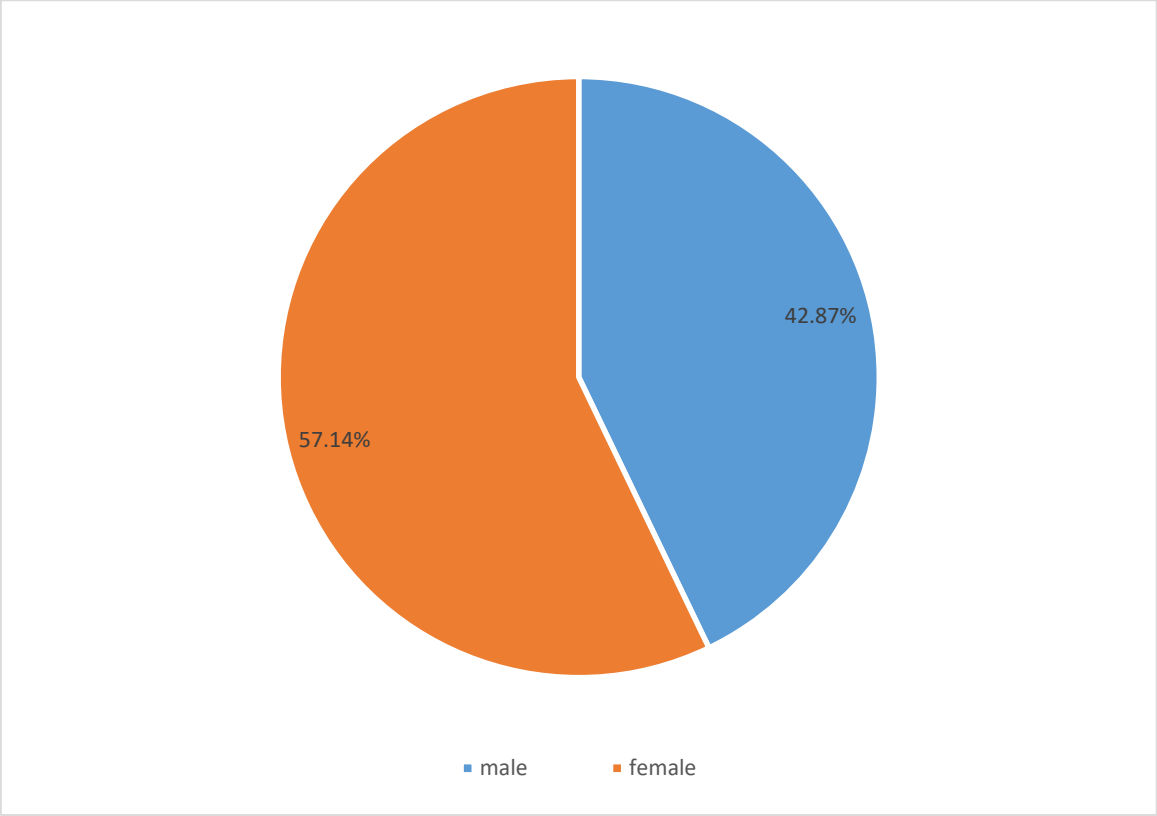


Figure 1. Percentage of positive cultures with respect to gender.

3.3. Biochemical identification

The result of confirmation of pathogens after biochemical tests is given in Table 3. This included rod shaped and cocci bacteria.

Table 3. Biochemical Identification of Test Isolates in positive samples.

No	Isolates	Cell Morphology		Biochemical tests						
		Shape	**Gram**	**Cat**	**Oxi**	**Ind**	**Cit**	**Ure**	**DNase**	**Motile**
1.	Isolate #1	Rods	- ve	+	+	-	+	-	-	+
2.	Isolate #2	Rods	- ve	+	-	+	-	-	-	+
3.	Isolate #3	Rods	- ve	+	-	-	+	+	-	-
4.	Isolate #4	Rods	- ve	+	-	-	+	+	-	-
5.	Isolate #5	Cocci	+ ve	+	-	-	+	+	+	-
6.	Isolate #6	Rods	- ve	+	-	-	+	+	Variable	+

***Catalase=Cat, Oxidase=Oxi, Indole=Ind, Citrate=Cit, Urease=Ure**

With blue pigmentation, colonial morphology of isolate 1 was fluctuated. Colonies were transparent with regular margins and glistering texture (Table 2). The isolates of this colony were Gram (-) rods and biochemical tests (Table 3). The biochemical tests confirmed that the isolate is of *pseudomonas* spp. (Table 3)

Colonies of isolate 2 were slightly grey in color with flat margins. Colonial margins was regular with opaque and smooth texture (Table 2). From this colony, bacteria were Gram (-) rods (Table 3). The biochemical tests confirmed that the isolate is a strain of *E. coli*.

The colonies of isolate 3 was creamy in colour. Colonial margins was intact with shiny and smooth opaque texture (Table 2). From this colony, bacteria were Gram (-) rod (Table 3) and biochemical tests (Table 3) of this isolate shown that it is *Enterobacter* spp.

The colonies of isolate 4 was pale white and it was flat with rounded knobs. Colonial margins fluctuated having mucoid texture and it was transparent (Table 2). The isolates of this colony were Gram (-) rods. The biochemical tests confirmed that the isolate is of *Klebsiella* spp. (Table 3).

The colonies of isolate 5 was creamy in colour. Colonies had intact margins, appeared transparent. The texture was smooth (Table 2). Isolates of this colony were Gram positive (+) cocci (Table 3). Biochemical tests confirmed that the isolate is of *Staphylococcus* spp. (Table 3).

The colonies of isolate 6 were a little raised as convex surface with slightly grey pigmentation. Colonial margin was intact with shiny and smooth texture and it was transparent (Table 2). The isolates of this colony were Gram negative rods (Table 3). The biochemical tests confirmed that the isolate is of *Proteus* spp. (Table 3)

4.5. Distribution of Gram (+), Gram (-) bacteria and Fungi among uropathogens

In positive samples, 20/56 (35.71%) were *E.coli*, 14/56 (25%) were *candida spp*. 14/56 (25%) were *klebsiella spp*. and the remaining 2/56 (3.57%) were *enterococcus spp*. and 2/56 (3.57) were *Serratia marcescens*.

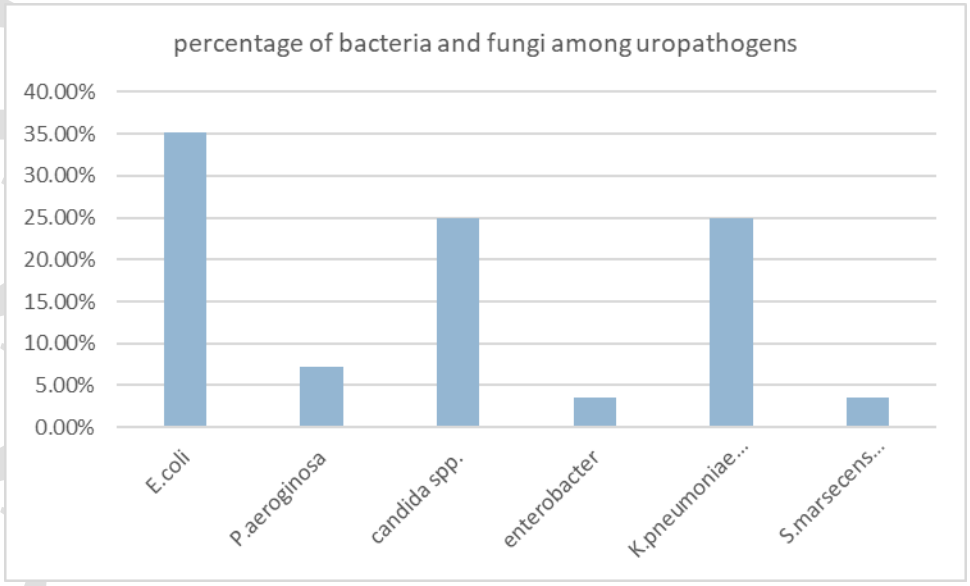


Figure 2.Percentage of different bacteria and fungi among uropathogens

3.7. Antibiotics sensitivity pattern of test isolates

By using disc diffusion method, the sensitivity test was performed. For each isolate with the use of all antibiotics, the zones of mean inhibition were recorded. The break point of the selected antimicrobial and antibiotics susceptibility of test isolates are given in Table 2(a) and 2.(b). For the isolated bacteria, the antimicrobial patterns were determined and it was shown that *P. arogenosa* intermediate resistant to 3 antibiotics and completely resistant to 5 antibiotics that are

commonly administered against *P. aregnosa* (Figure 3.3). Antimicrobial susceptibility test of *Proteus maribillis* shown that it was resistant to 7 antibiotics (Figure 4(b). *K. pneumonae and E. arogenes* were resistant to 9 antibiotics (Figure 3.5). *E. coli* was intermediate resistant to 2 antibiotics and resistant to 6 antibiotics while *S. aureus* was intermediate resistant to 1 antibiotic and resistant to 5 antibiotics (Figure 3.4). All the isolates showed resistance to *Cephalothin and Penicillin* (Table 3.3 and Figure 3.8).

Table: 4(a) Inhibition zone (mm) of different test isolates

No	**Antibiotics**	**Test Isolates**					
	P. arogenosa		***E. coli***	***E. arogenes***	***K. pneumonae***	***S. aureus***	***Proteus maribillis***
1.	Penicillin	R	11	R	R	R	17
2.	Cephalothin	R	11	R	R	13	R
3.	Amikacin	15	23	26	24	23	17
4.	Amphacillin	R	6	R	R	R	17
5.	Amoxicillin	R	11	R	R	R	24
6.	Imipenem	18	36	30	28	34	34
7.	Cefoperazone	20	20	9	8	29	15
8.	Ciprofloxacin	18	23	R	R	22	22
9.	Gentamicin	18	23	17	17	19	11
10.	Tetracycline	11	14	R	R	R	8
11.	Ceftriaxone	17	19	R	R	28	16
12.	Cefepime	28	R	8	7	27	R

R=Resistant

Table: 4(b) Antibiotic Sensitivity Pattern shown by the Test Isolates

No	Antibiotics	Test Isolates					
	P. arogenosa		***E. coli***	***E. arogenes***	***K. pneumonae***	***S. aureus***	***Proteus maribillis***
1.	Penicillin	R	R	R	R	R	R
2.	Cephalothin	R	R	R	R	R	R
3.	Amikacin	IR	S	S	S	S	S
4.	Amphacillin	R	R	R	R	R	S
5.	Amoxicillin	R	R	R	R	R	S
6.	Imipenem	IR	S	S	S	S	S
7.	Cefoperazone	S	IR	R	R	S	R
8.	Ciprofloxacin	IR	S	R	R	S	S
9.	Gentamicin	S	S	S	S	IR	R
10.	Tetracycline	R	IR	R	R	R	R
11.	Ceftriaxone	S	R	R	R	S	R
12.	Cefepime	S	R	R	R	S	R

Sensitive=S, Resistant=R, Intermediate Resistant=IR

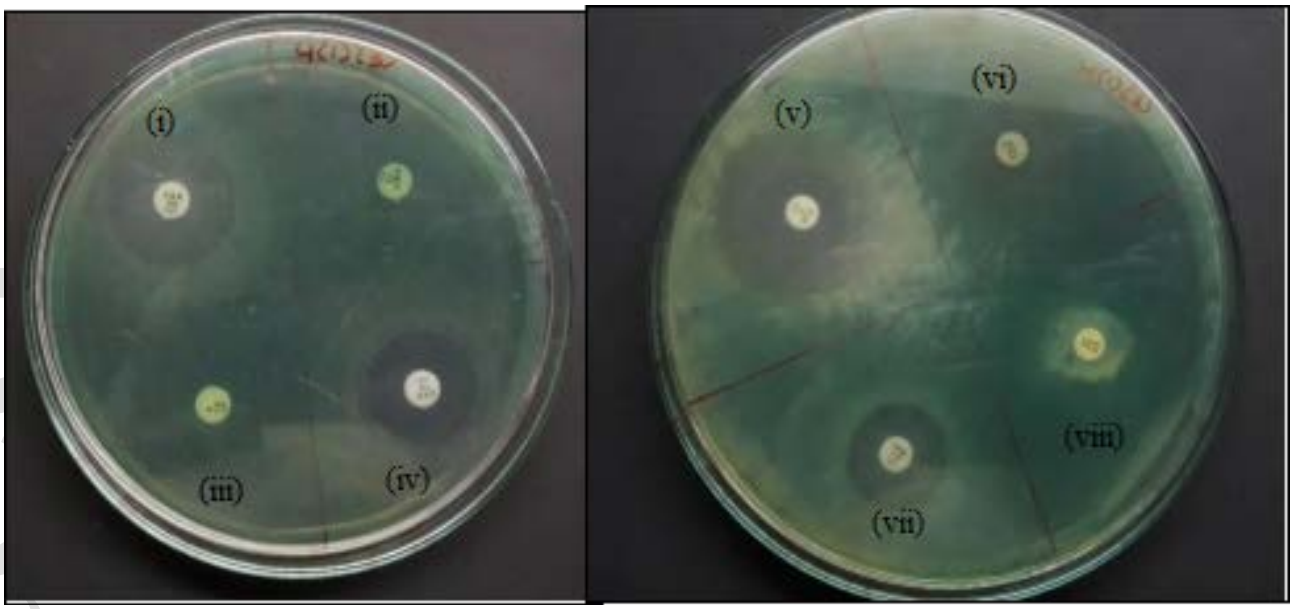


Figure 3 (a). Antibiotic Susceptibility test of *P. aeruginosa* against i. Cefepime, ii. Ampicillin, iii. Penicillin, iv. Cefoperazone, v. Ciprofloxacin, vi. Tetracycline, vii. Imipenem and viii. Cephalothin

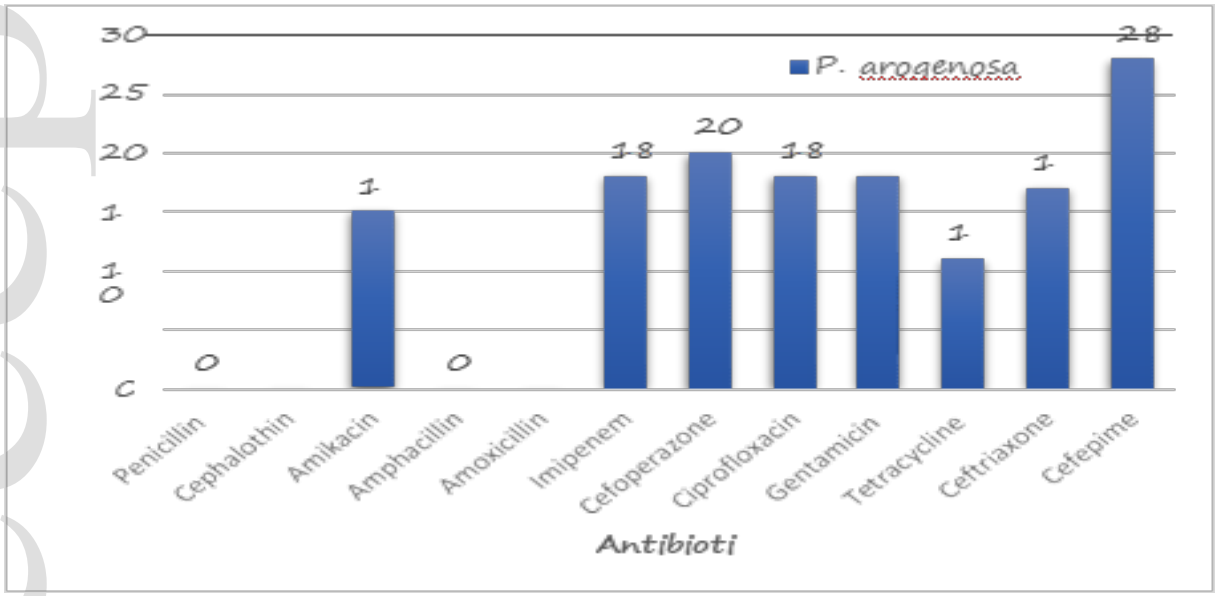


Figure 3 (b). The bar graph shows zone of inhibition produced by different antibiotics against *P. aregnosa*

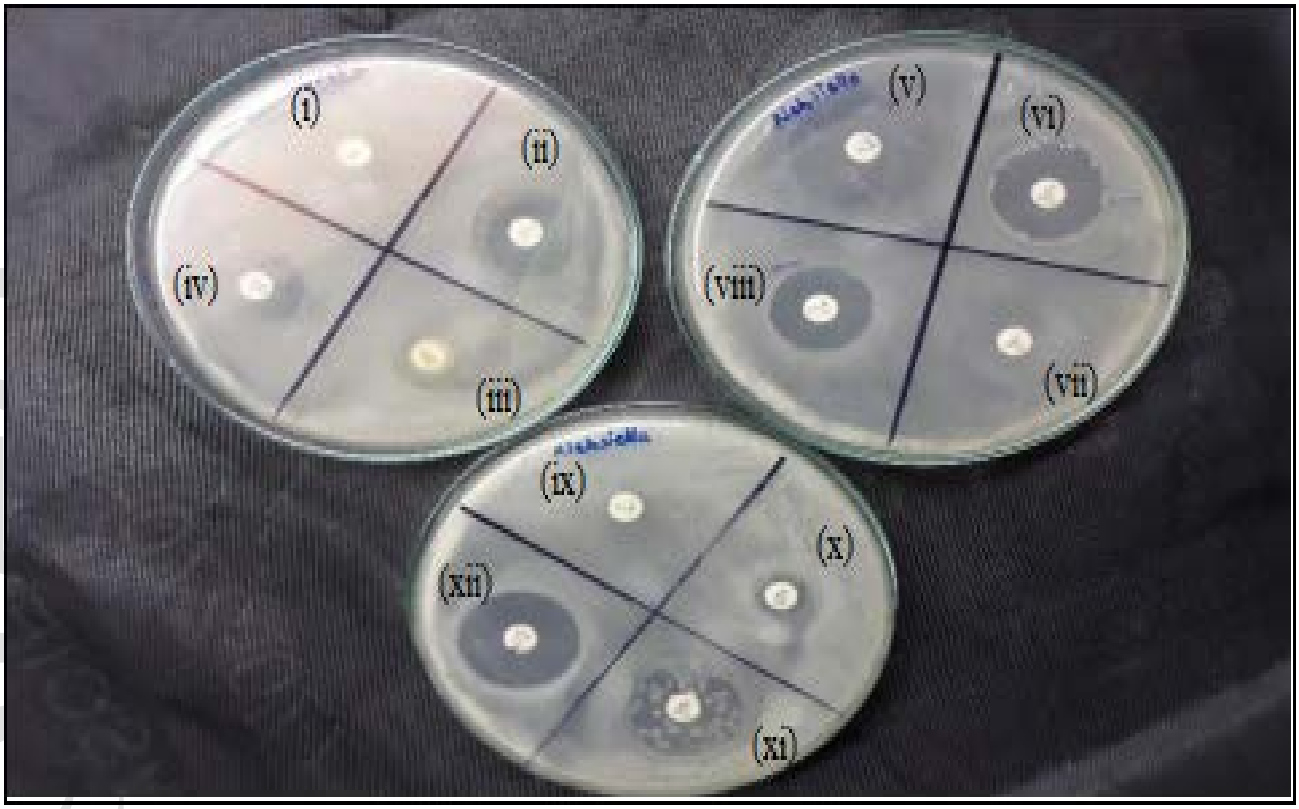


Figure 4 (a). Antibiotic Resistance Pattern of *Proteus maribillis* against i. Ceftriaxone, ii. Imipenem, iii. Amoxicillin, iv. Ciprofloxacin, v. Penicillin, vi. Gentamicin, vii. Tetracycline, viii. Ampicillin, ix. Cephalothin, x. Cefoperazone, xi. Cefepime and xii. Amikacin

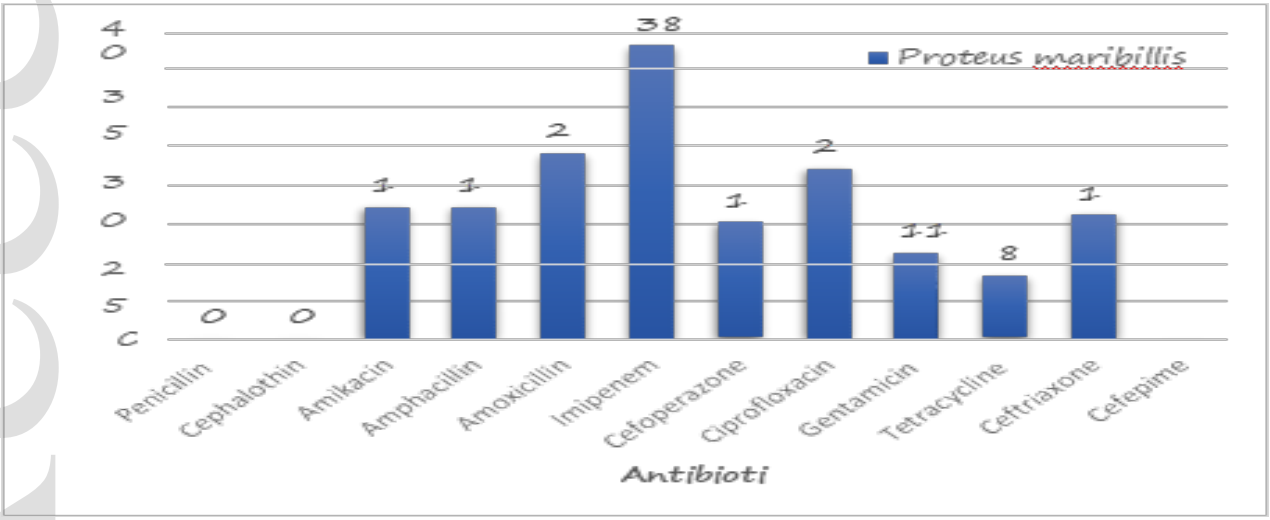
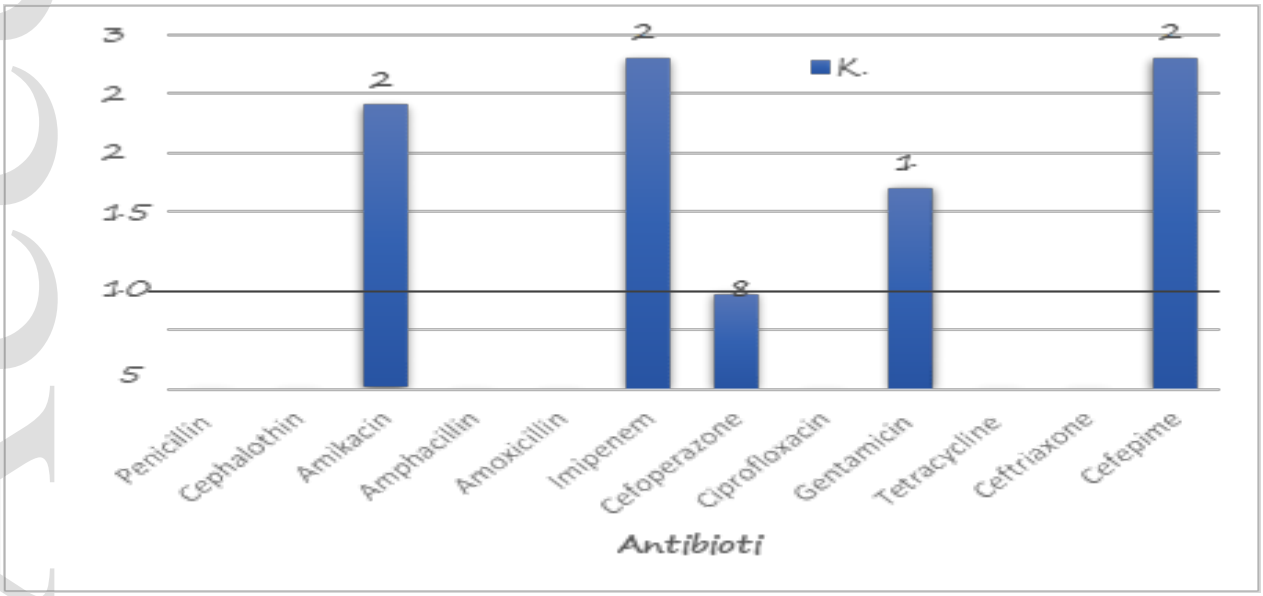


Figure 4 (b). The bar graph shows zone of inhibition produced by different antibiotics against *P. maribillis***Figure 5 (a).** Antibiotic Resistance Pattern of *K. pneumonae* against i. Penicillin, ii. Amikacin, iii. Tetracycline, iv. Gentamicin, v. Imipenem, vi. Ceftriaxone, vii. Ampicillin, viii. Ciprofloxacin, ix. Amoxicillin, x. Cephalothin, xi. Cefepime and xii. Cefoperazone

Figure 5 (b). The bar graph shows zone of inhibition produced by different antibiotics against *K. pneumonae*



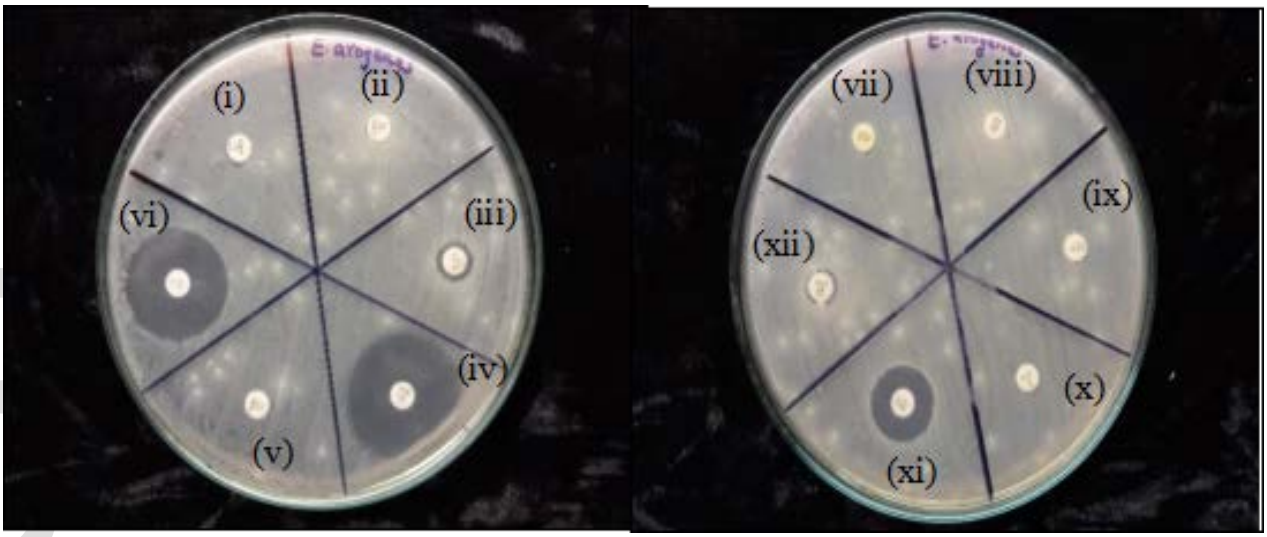


Figure 6 (a): Antibiotic resistant pattern of *Enterobacter aerogenes* against i. Ampicillin, ii. Ceftriaxone, iii. Cefoperazone, iv. Imipenem, v. Amoxicillin, vi. Amikacin, vii. Tetracycline, viii. Cephalothin, ix. Penicillin, x. Ciprofloxacin, xi. Gentamicin and xii. Cefepime

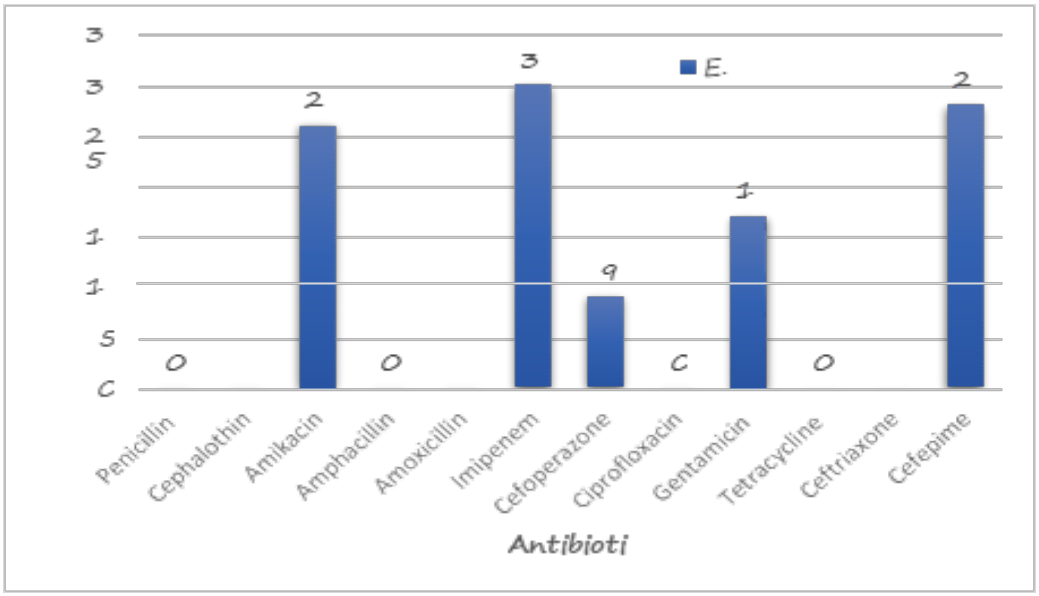


Figure 6 (b): The bar graph shows zone of inhibition produced by different antibiotics against *Enterobacter aerogenes*

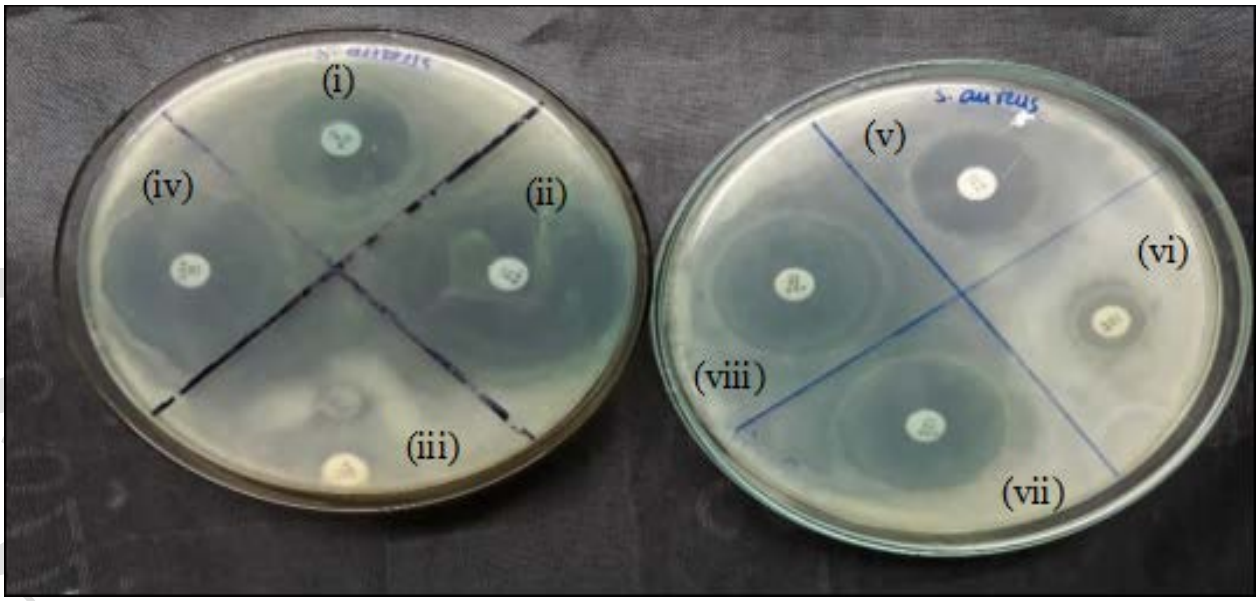


Figure 7 (a). Antibiotic Resistance Pattern of *S. aureus* against i. Ciprofloxacin, ii. Imipenem, iii. Amoxicillin, iv. Ceftriaxone, v. Amikacin, vi. Cephalothin, vii. Cefoperazone and viii. Cefepime inhibition of *S. aureus* introduced by different antibiotics

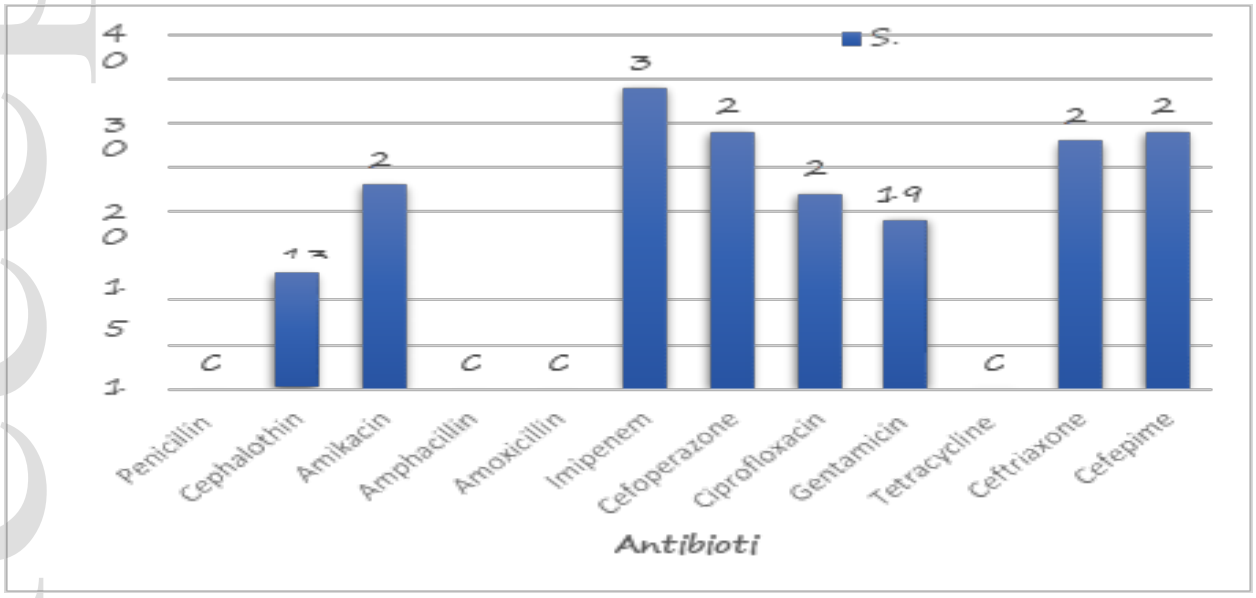


Figure 7 (b). The bar graph shows zone of inhibition produced by different antibiotics against *S.aureus*

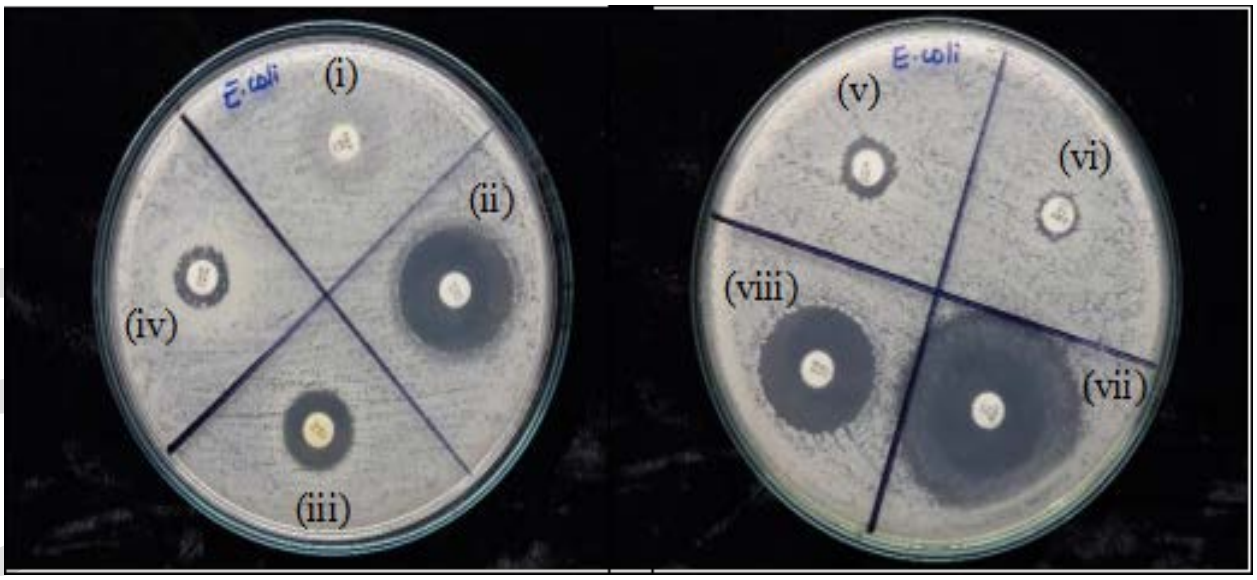


Figure 8 (a). Antibiotic Resistance Pattern of *E. coli* against i. Cefepime, ii. Amikacin, iii. Tetracycline, iv. Cephalothin, v. Penicillin, vi. Ampicillin, vii. Imipenem and viii. Gentamicin

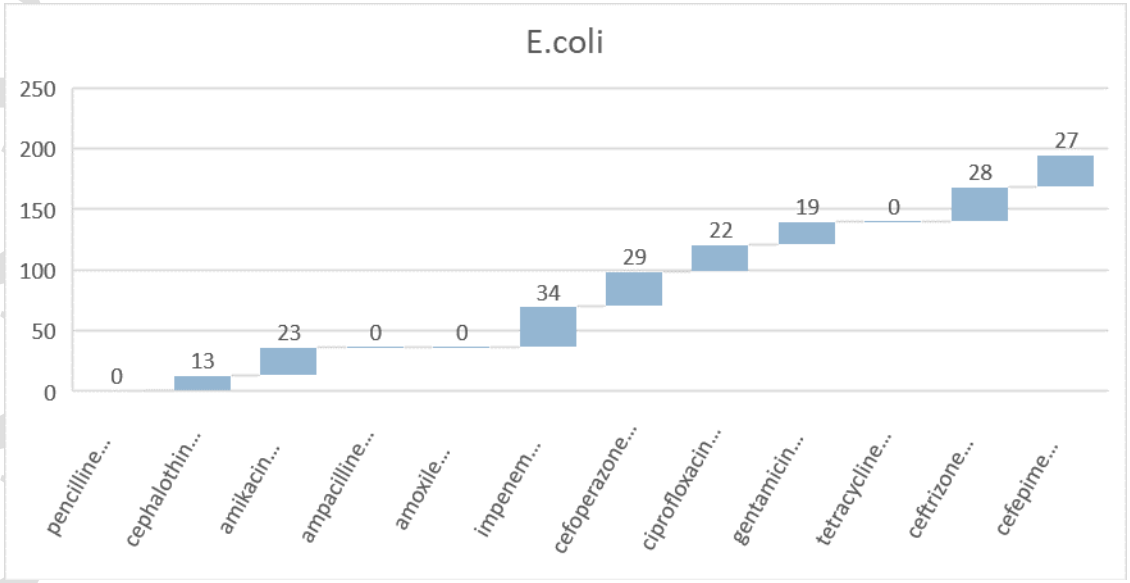


Figure 8 (b). The bar graph shows zone of inhibition produced by different antibiotics against *E. coli*

The response of isolates of bacteria against various antibiotics was varied. Their sensitivity was strongest towards Amikacin and Imipenem whereas lowest sensitivity was recorded towards Ampicillin and Amoxicillin. No sensitivity was recorded for Cephalothin, Penicillin and Tetracycline. S. aurues (Table 4(b)) shown sensitivity towards Imipenem, Amikacin, Cefepime, Ceftriaxone and Cefoperazone as shown in sensitivity breakpoint (Table 4(a)). *P. mirabilis* shown resistance towards Amoxicillin and Amoicillin whereas *E. arogenes and K. pneumonia* shown sensitivity towards Imipenem, gentamicin and Amikacin. P. aeruginosa shown sensitivity towards Imipenem, Amikacin, Ciprofloxacin, Ampicillin and Amoxicillin. The sensitivity of E. coli is towards Imipenem, Amikacin, Ciprofloxacin and gentamicin (Table 4(b)).

DISCUSSION

Urinary tract infection (UTI) is one of the most common bacterial infections. The disease infects men, women and children of all age groups. They are implicated in both communities acquired and hospital acquired infections [13]. Effective management depends upon its prompt identification and selection of adequate antibiotic. The present study was organized to assess the prevalence of uropathogens caused UTI and achieve anti-biogram of clinical isolates from our local area against commonly prescribed antibiotics. Most common pathogen detected was *E. coli* (35%) followed by *Klebsiella* spp (25%) and candida spp (25%) which is similar to the results of another study carried out in Hungary [14]. Another retrospective study reported that out os 1176 urine samples, *E.coli* was the most common pathogen with 47.3% prevalence, Klebsiella spp was 10.3% and Candida spp was 8.8% [15].

In our study 79.1% of the infection are caused by Gram (-) rods Ano. ther study has reported similar result where the gram negative rods appeared asmost common pathogen associated with UTI [16]. A study from India reports that 71.6% and 28.3% of inpatients and outpatients of UTI had gram negative bacteria [15].

In this study, *E.coli* was the most common cause of UTI in males and females and it was followed by *K.pneumoniae,* respectively. This was in agreement with other studies from Pakistan [17, 18]. However, in a study conducted on diabetic patients *Proteus spp* was the second most common cause of UTI [15]. Morever, UTI was more prevalent in females (57.1%) which is in accordance with previous reports from Pakistan and other parts of the world [17, 19].

Resistance was high against ampicillin, Cotrimoxazole as reported by Aghamahdi F however gentamicin, ciprofloxacin, ceftriaxone and tetracycline were also found resistant to most isolates in contrast to this study and another study from Nepal by Gupta UP, et al. [20]. Whereas amikacin, fosfomycin, nitrofurantoin, imipenem, meropenem, vancomycin, teicoplanin and combinations like sulbactam-cefoperazone and Tazobactam- piperacillin were found sensitive to most of isolates as reported by other studies [21].

This study was to investigate the antimicrobial susceptibility test for all isolates obtained from urine samples. Results revealed that the bacterial isolates obtained from urine samples have high resistance against various antibiotics. Bacterial resistance to antibiotics is a major threat for all over the world. But for developing countries like Pakistan, this is an even greater public health problem. This is because Pakistan has one of the big problems of bacterial diseases in the world and thus, antibiotics have a significant role in decreasing mortality and morbidity in the country [22].

In present study, urine samples from the patients indicated the presence of the highest number of uro-pathogens it means that populations were anguish from severe urinary tract infection (UTI). The existence of UTI among the patients could be credited to the poor sanitary conditions of the environment due to overcrowding and unhygienic conditions of the hospitals. Related conclusions were drawn by different researchers [23]. It indicates that in Pakistan, people are using antibiotics in very high frequency for unnecessary purposes and in most cases, these were prescribed from the medical institution [24, 25]. Such practices have contributed to a warning development of rising antibiotic resistance in the country.

The study also shown the presence of *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli* and *Proteus mirabilis;* responsible for UTI. Most of these organisms were also well documented by many researchers [26]. Most of the infections could attributed toward *E. coli*, responsible for over 50% outdoor patients which is followed by *Enterobacter* spp, *Klebsiella pneumonia*, *Proteus* spp and *Pseudomonas aeruginosa*, respectively while *Staphylococcus aureus* is then most frequent isolates among Gram (+) cocci [27, 28].

All of the isolates shown highest resistivity towards antibiotics on the basis of sensitivity pattern, that is generally used against these pathogens. Furthermore, the isolated uro-pathogens were also sensitive towards several antibiotics. Approximately all isolates shown strongest sensitivity to

Imipenem and Amikacin. Cephalothin, Tatracycline and Pencillin resistance shown no sensitivity to bacteria whereas lowest sensitivity were shown by Amoxicillin and Ampicillin. The organism may developed a different mode of action due to the increased resistant pattern, which could attributed to the recurrent use of these antibiotics [28]. In this study, Imipenem was the most useful antibiotic which is compared to most frequently used antibiotics and found relatively expensive. This makes the organisms susceptible to it because this has probably limited its unselective use and procurement [29]. All isolates were susceptible to Imipenem and approximately similar results were reported when studied [30]. When isolates were tested against Imipenem, some other researchers also reported similar findings [31, 32]. So, there is a need to point up the rational use of antimicrobials strictly adhere to the concept of "reserve drugs" to minimize the misuse of available drugs.

Conclusion

Base on the above findings, the Imipenem proved as the most sensitive antibiotic against urinary tract bacteria. It is recommended to keep this as a reserve drug. Since the antibiotic susceptibility patterns vary greatly, it is important to know the resistance pattern in order to identify the effective drug, especially in t conditions where experimental therapy is essential. .. *E.coli* is the most common cause of urinary tract infections in our setup. Fosfomycin, carbapenems, combination drugs and nitrofurantoin are most effective drugs and be used to treat urinary tract infections. Resistance to most commonly used antibiotics like nitrofurantoin and gentamicin is also on rise There is a need to discover new antimicrobial compounds to combat the resistant bacteria involved urinary tract infections and further research should be carried out. It's also strongly recommend to evaluate the resistant pattern at genomic and proteomics level to discover the genes which are responsible for antibiotics resistant pattern.

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