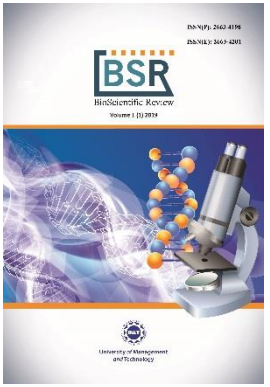

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Identification and Characterization of Urinary Tract Infectious Bacteria and its Antibiotic Sensitivity

Madeeha Mehboob[1], Mohammad Hakim[1], Obaid Ullah[1], Sumaira Salahuddin Lodhi[2], Irum Khalil[3], Muhammad Anees[1], Malik Nawaz Shuja[1*]
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Abstract

Etiological patterns of uropathogens are different in different geographical regions due to the continuous evolution of bacteria, antibiotic sensitivity patterns and their misuse and overuse. Therefore, it is important to know the antibiotic susceptibility patterns for the prescription of suitable antibiotics. This study was conducted to determine the prevalence of uropathogens and their antimicrobial sensitivity patterns in the Kohat region of Pakistan. In this study, 100 samples were collected from both male and female subjects of all ages. Out of these 100 samples, 70 samples contained microbes. In 30 samples, no microbial growth was recorded. The percentages of positive culture from male and female subjects were 57% and 43%, respectively. Both Gram (+) and Gram (-) bacteria were found in UTI. Among them *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 commonly used antibiotics against both Gram (+) and Gram (-) bacteria are as follows: 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 find new antimicrobial compounds. Moreover, it is imperative to evaluate the resistant patterns at genomic and proteomic levels to discover the genes responsible for antibiotic resistant patterns.

1. Introduction

Urinary Tract Infection (UTI) is mainly caused by pathogenic invasion of the urinary tract resulting in the inflammatory response of urothelium. The primary cause of infection is the proliferation of pathogenic bacteria in the urinary tract. Various pathophysiological factors determine the clinical manifestations of UTI, such as etiologic organism(s), associated part of the urinary tract, infection severity and the response of the patient's immune system [1]. Fever, chills, dysuria, urinary urgency, and malodorous or cloudy urine are main symptoms and signs of UTI.

Infections are almost always mounting in origin. Their primary cause is the proliferation of bacteria in periurethra and 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, thus eliminating any potential infectious organisms [3]. Moreover, in men, the anatomical length of urethtra (20cm) also acts as a barrier against microorganisms. However, in women, the short urethra (5cm) is easily crossed by them. This is why UTI in women is 14 times more common as compared to men. The vaginal and cervical epithelium produces mucus that contain glycogen. This glycogen is metabolized to lactic acid by Doderlein's bacilli. Thus, the vagina (pH 3.5) becomes acidic making it intolerant to most microorganisms [4].

UTI is among the most common nosocomial infections caused by a variety of Gram (+) and Gram (-) bacteria. Gram (-) bacteria such as *Klebsiella spp*, *Escherichia spp*, *Citrobacter spp*, *Enterobacter spp*, *Proteus spp*, *Serratia spp*, and *Pseudomonas spp* and Gram (+) bacteria such as *Staphylococcus spp*, *Streptococcus spp* and *Enterococcus spp* are frequently associated with UTIs. Among these bacteria, *Escherichia coli* causes 80-90% of all UTIs. *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Enteroccus faecalis* are most frequently isolated in ambulatory patients and in the case of nosocomial infections [5].

The detection of significant bacteriuria, which refers to the presence of more than 100000 pathogenic bacteria per milliliter of urine is the gold standard for the diagnosis of UTI. Other scientific literature suggests an amount of 10^3 cfu/ml, depending on the type of the causative agent [6]. The diagnosis of UTI is not possible solely on clinical grounds. The profiling of bacteria in urine (in bladder) is necessary for the confirmation of UTI [7]. However, most commercial screening methods are neither easily available nor inexpensive enough to allow for their use in routine practice. Screening tests are advantageous as they yield rapid results and hence remain 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. However, occasionally, they can cause bacteremia leading to death [2].

Antibiotic resistance is dangerously increasing to high levels in all parts of the world. Our ability to treat common

infectious diseases is being threatened due to the new resistant bacterial strains. Antibiotics can be used for the treatment of UTIs. However, the choice of antibiotics depends upon the sensitivity of bacteria to several antibiotics, such as trimethoprim-sulfamethoxazole (TMP-SMX). Indeed, prolonged administration of antibiotics causes side effects in 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 resistance developed due to the misuse and prolonged use of wide spectrum antibiotics. As a result, the intestinal flora changed leading to the emergence of bacterial resistance [10].

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

2. Methodology

2.1. Study Site and Sample Collection

Sampling sites were KDA Hospital Kohat, Liaquat Memorial Hospital Kohat, Combined Military Hospital Kohat and Alkhidmat Naseem Khan Memorial Hospital Kohat. The study was conducted in the Department of Microbiology, Kohat University of Science and Technology, Kohat from February 2020 to September 2020. A first morning urine sample was collected from 100 patients suspected of UTI in sterile containers. The collection of a first morning urine sample was done because the overnight growth of microorganisms increases the microbial count in 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 pro forma. The parameters for microbiological findings included culture morphology results and *in vitro* antibiotic susceptibility results of isolates. The study was undertaken with prior approval and conducted according to the declaration of Helsinki. Verbal consent was taken from each participant.

2.2. Isolation of Pathogens

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

2.3. Identification of Isolates

Isolates were identified using a slightly modified version of the method previously used by Gul et al. [12]. The cultures were examined with naked eye to observe the colonial morphology which included their size, surface, color, shape, edge, and opacity. To notice their shape, arrangement, size and staining reaction, Gram's stain was prepared from the colonies. Oxidase, catalase and indole tests were performed 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 aerobic incubation for 24 hours at 37°C. Broth culture (100µl) of each bacterial isolate was diluted separately in test 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. Antibiotic Sensitivity Testing

To test the antibiotic sensitivity of isolated bacteria, disc diffusion method was used. The antibiotics used were obtained from Karachi Market, Peshawar. The antibacterial sensitivity of isolates was evaluated against 12 different antibiotics. For all selected antibiotics, the susceptibility break points for isolates were observed (see Table 1 and 2). For each test organism, separate plates with MHA media were used. With the help of sterile cotton, isolates were streaked on petri plates and pressed for uniform contact [13]. After being incubated at 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 the back of the plates and correlated with the standardized chart provided by Clinical and Laboratory Standards Institute, USA. For the determination of the level of resistance against antimicrobial agents, the isolates were labeled as moderate resistant (MR) or resistant (R) or susceptible (S)).

Table 1. Antibiotic Discs used with their Susceptibility Break Point for *Enterobacteriaceae*

No	Antibiotics	Disc Code	Discs Contents_____ (µg)	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 2. Antibiotics Discs used with Their Susceptibility Break Point for *S. Aureus and P. Aeruginosa*

No	Antibiotics	Disc Code	Discs Contents_____ (µg)	Zone Diameter (mm)		
				R	MR	S
1.	Amoxicillin *S. aureus* *P. aeruginosa*	AML	25 µg	- -	- -	- -
2.	Cephalothin *S. aureus* *P. aeruginosa*	KE	30 µg	- -	- -	- -
3.	Amphicillin *S. aureus* *P. aeruginosa*	AMP	10 µg	≤ 28 -	- -	≥ 29 -
4.	Cefepime *S. aureus* *P. aeruginosa*	FEP	30 µg	- ≤ 14	- 15–17	- ≥ 18
5.	Ceftriaxone *S. aureus* *P. aeruginosa*	CRO	30 µg	- -	- -	- -
6.	Imipenem *S. aureus* *P. aeruginosa*	IPM	10 µg	- ≤ 15	- 16–18	- ≥ 19
7.	Tetracycline *S. aureus* *P. aeruginosa*	TE	30 µg	≤ 14 -	15–18 -	≥ 19 -
8.	Gentamicin *S. aureus* *P. aeruginosa*	CN	10 µg	≤ 12 ≤ 12	13–14 13–14	≥ 15 ≥ 15
9.	Amikacin *S. aureus* *P. aeruginosa*	AK	30 µg	≤ 14 ≤ 14	15–16 15–16	≥ 17 ≥ 17
10.	Cefoperazone *S. aureus* *P. aeruginosa*	CFP	75 µg	- -	- -	- -
11.	Penicillin *S. aureus* *P. aeruginosa*	P	10 µg	≤ 28 ≤ 14	- 15–20	≥ 29 ≥ 21
12.	Ciprofloxacin *S. aureus* *P. aeruginosa*	CIP	5 µg	≤ 15 ≤ 15	16–20 16–20	≥ 21 ≥ 21

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 their size, color and morphology were observed from the incubated nutrient agar plates (Table 3). The isolated bacteria were *Pseduomonas* aeruginosa, *S. aureus*, *K. pneumonia*, *E. coli*, *Enterobacter aerogenes* and *P. mirabilis.*

Table 3. Morphological Characteristics of 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

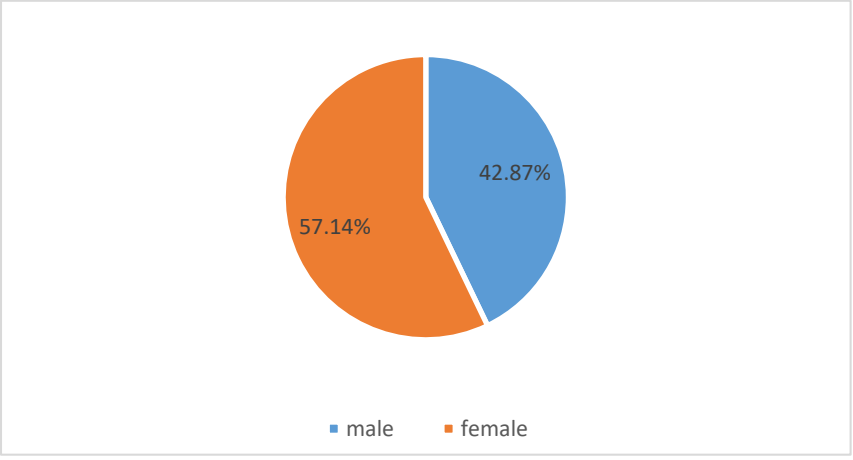

Figure 1. Percentage of positive cultures with respect to gender

3.2. Male to Female Ratio

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

3.3. Biochemical Identification

The results of the confirmation of pathogens after biochemical tests are given in Table 4. These pathogens include rod shaped and cocci bacteria.

With blue pigmentation, Isolate 1showed varied colonial morphology. Colonies were transparent with regular margins and glistering texture (Table 3). The isolates of this colony comprised Gram (-) rods and were confirmed via biochemical tests (Table 4). Biochemical tests confirmed that the isolates were *pseudomonas spp* (Table 4).

The colonies of Isolate 2 were slightly grey in color with flat margins. Colonial margins were regular with an opaque and smooth texture (Table 3). In this colony, bacteria comprised Gram (-) rods (Table 4). Biochemical tests confirmed that the isolates were a strain of *E. coli*.

The colonies of Isolate 3 were creamy in colour. Colonial margins were intact with a shiny, smooth and opaque texture (Table 3). In this colony, bacteria comprised Gram (-) rods (Table 4). Biochemical tests (Table 4) of these isolates showed that they were *Enterobacter spp*.

The colonies of Isolate 4 were pale white and flat with rounded knobs. Colonial margins had mucoid texture and it was transparent (Table 3). The isolates of this colony comprised Gram (-) rods. Biochemical tests confirmed that the isolates were *Klebsiella spp* (Table 4).

The colonies of Isolate 5 were creamy in colour. They had intact margins and were transparent. The texture was smooth (Table 2). The isolates of this colony comprised Gram positive (+) cocci (Table 4). Biochemical tests confirmed that the isolates were *Staphylococcus spp*. (Table 4).

Table 4. 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

The colonies of Isolate 6 were a little raised as convex surface with slightly grey pigmentation. Colonial margin was intact with a shiny and smooth texture and it was transparent (Table 3). The isolates of this colony comprised Gram (-) rods (Table 4). Biochemical tests confirmed that the isolates were *Proteus spp* (Table 4).

3.4. Distribution of Gram (+) and Gram (-) bacteria and fungi among uropathogens

Among positive samples, 20/56 (35.71%) were *E. coli*, 14/56 (25%) were *candida spp*, 14/56 (25%) were *Klebsiella spp*, 2/56 (3.57%) were *Enterococcus spp*, and the remaining 2/56 (3.57) were *Serratia marcescens* (Figure 2).

3.5. Antibiotics Sensitivity Pattern of Test Isolates

Using disc diffusion method, sensitivity test was performed. The zones of mean inhibition were recorded for all isolates using antibiotics. The break points of the selected antimicrobial and antibiotic susceptibility of test isolates are given in Table 5 and Table 6. For the isolated bacteria, antimicrobial patterns were determined and it was shown that *P.aeruginosa* was intermediately resistant to 3 antibiotics and completely resistant to 5 antibiotics commonly administered against it (Figure 3 and 4). Antimicrobial susceptibility test of *P. maribillis* showed that it was resistant to 7 antibiotics (Figure 5 and 6). *E. arogenes* (Figure 7 and 8) and *K. pneumoniae* (Figure 9*)* and were resistant to 9 antibiotics. *E. coli* was intermediately resistant to 2 antibiotics and completely resistant to 6 antibiotics (Figure 10 and 11), while *S. aureus* was intermediately resistant to 1 antibiotic and completely resistant to 5 antibiotics (Figure 12 and 13). All the isolates showed resistance to cephalothin and penicillin (Table 6).

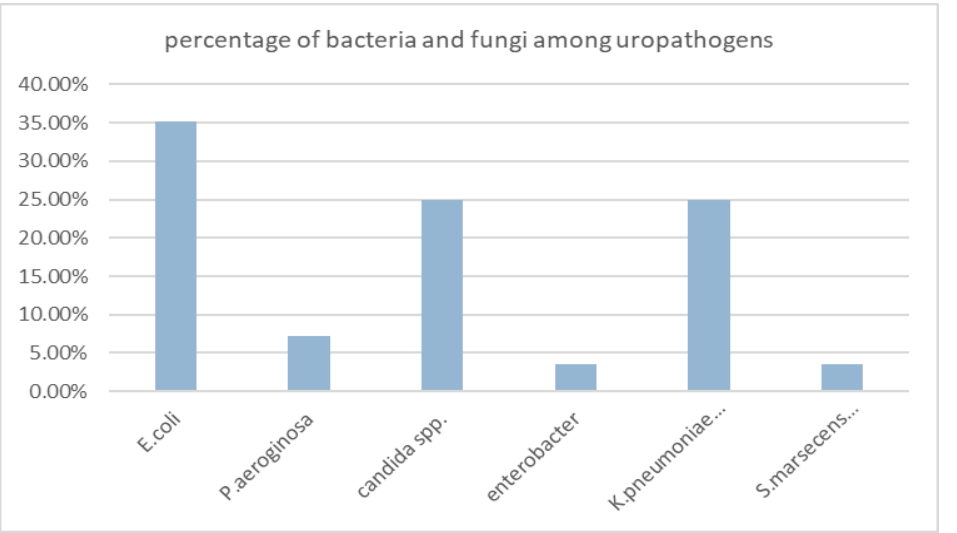

Figure 2. Percentage of different bacteria and fungi among uropathogens

Table 5. Inhibition Zone (mm) of Different Test Isolates

No	Antibiotics	Test Isolates					
		P. aeruginosa	***E. coli***	***E. arogenes***	***K. pneumoniae***	***S. aureus***	***P. 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

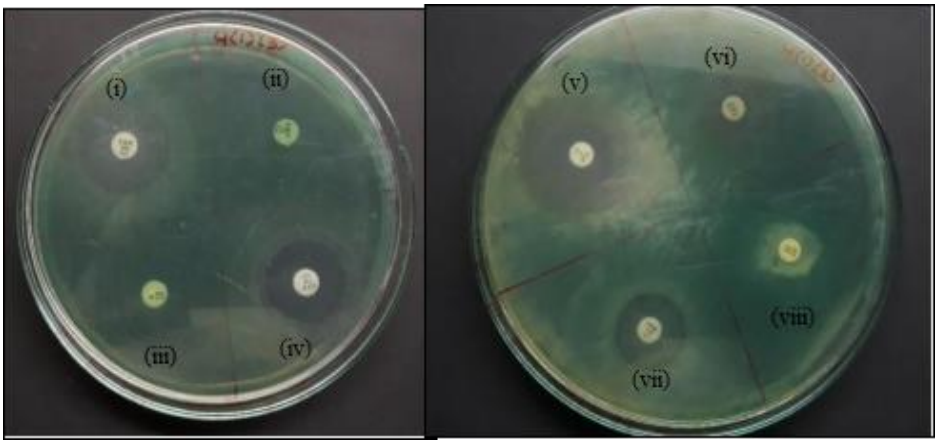

Figure 3. Antibiotic susceptibility test of *P. aeruginosa* against i. Cefepime, ii. Ampicillin, iii. Penicillin, iv. Cefoperazone, v. Ciprofloxacin, vi. Tetracycline, vii. Imipenem, and viii. Cephalothin

The response of the 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 towards cephalothin, penicillin and tetracycline. *S. aurues* (Table 6) showed sensitivity towards imipenem, amikacin, cefepime, ceftriaxone and cefoperazone, as shown by their sensitivity breakpoints (Table 5). *P. mirabilis* showed

resistance towards amoxicillin and amoicillin, whereas *E. arogenes and K. pneumonia* showed sensitivity towards imipenem, gentamicin and amikacin. *P. aeruginosa* showed sensitivity towards imipenem, amikacin, ciprofloxacin, ampicillin and Amoxicillin. *E. coli* showed sensitivity towards imipenem, amikacin, ciprofloxacin and gentamicin (Table 6).

Table 6. Antibiotic Sensitivity Pattern Shown by Test Isolates

	Antibiotics		**Test Isolates**				
No	***P. aeruginosa***		***E. coli***	***E. arogenes***	***K. pneumoniae***	***S. aureus***	***P. 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

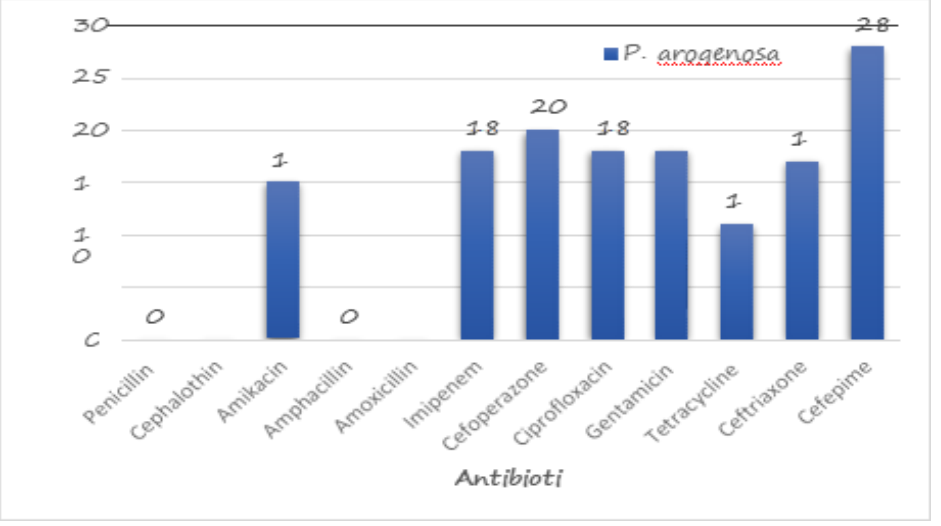

Figure 4. Bar graph shows the zones of inhibition produced by different antibiotics against *P. aeruginosa*

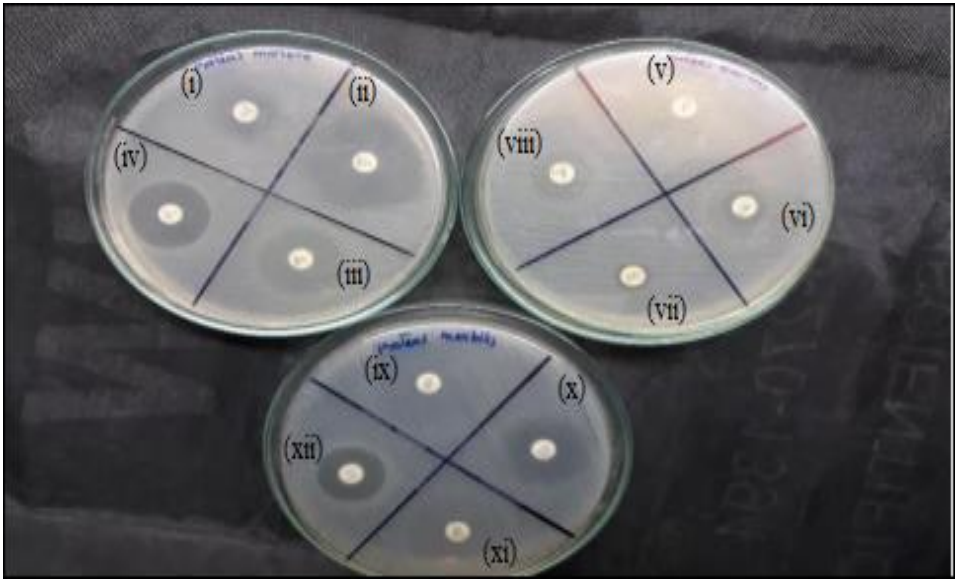

Figure 5 . Antibiotic resistance pattern of *P. maribillis* against i. Ceftriaxone, ii. Imipenem, iii. Amoxicillin, iv. Ciprofloxacin, v. Penicillin, vi. Gentamicin, vii. Tetracycline, viii. Ampicillin, ix. Cephalothin, x. Cefoperazone, xi. Cefepime, and xii. Amikacin

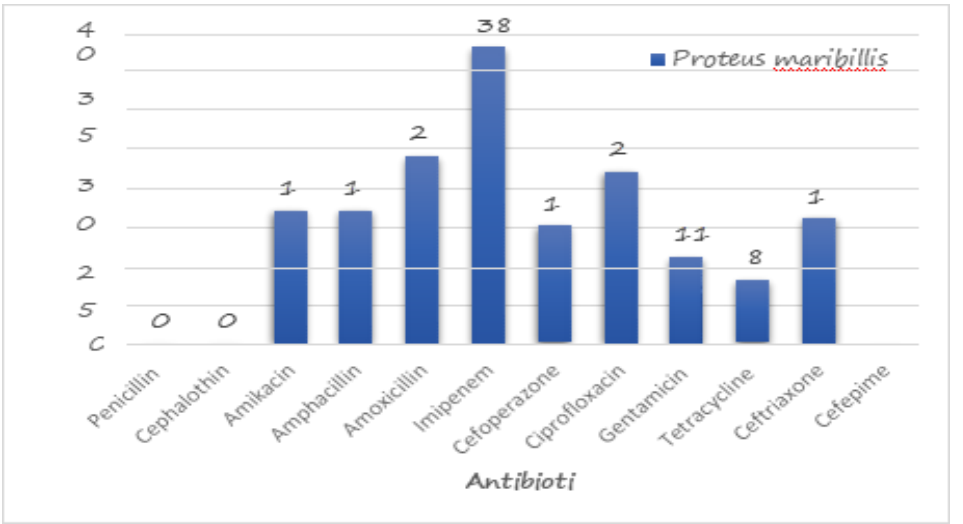

Figure 6. Bar graph shows the zones of inhibition produced by different antibiotics against *P. maribillis*

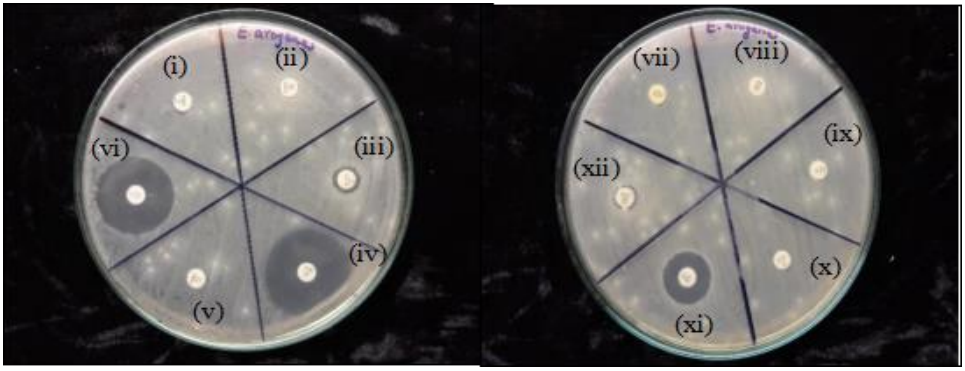

Figure 7. Antibiotic resistance pattern of *E. aerogenes* against i. Penicillin, ii. Amikacin, iii. Tetracycline, iv. Gentamicin, v. Imipenem, vi. Ceftriaxone, vii. Ampicillin, viii. Ciprofloxacin, ix. Amoxicillin, x. Cephalothin, xi. Cefepime and xii. Cefoperazone

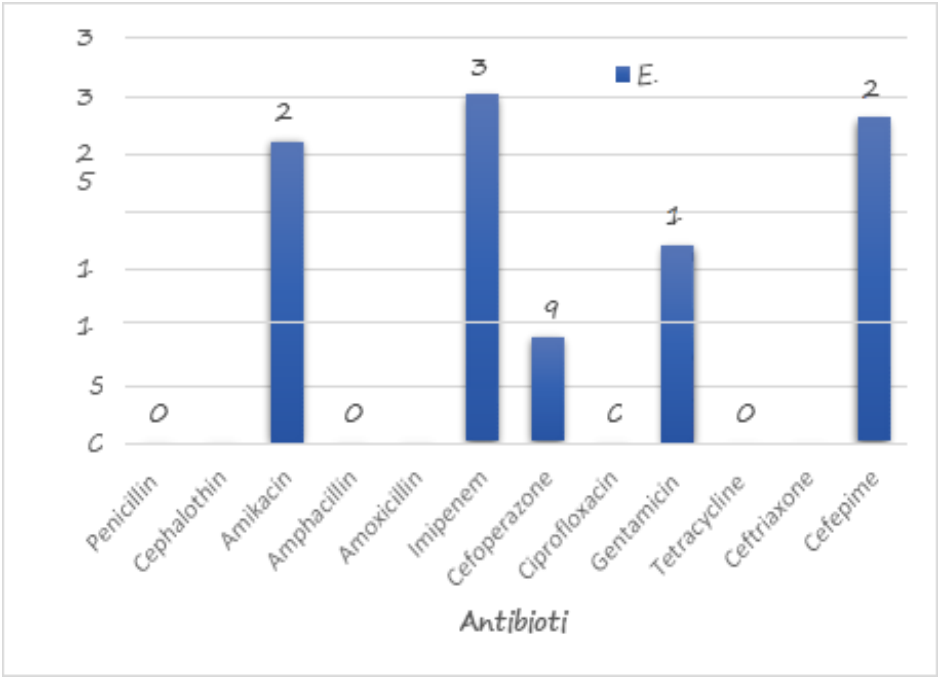

Figure 8. The bar graph shows zone of inhibition produced by different antibiotics against *E. aerogenes*

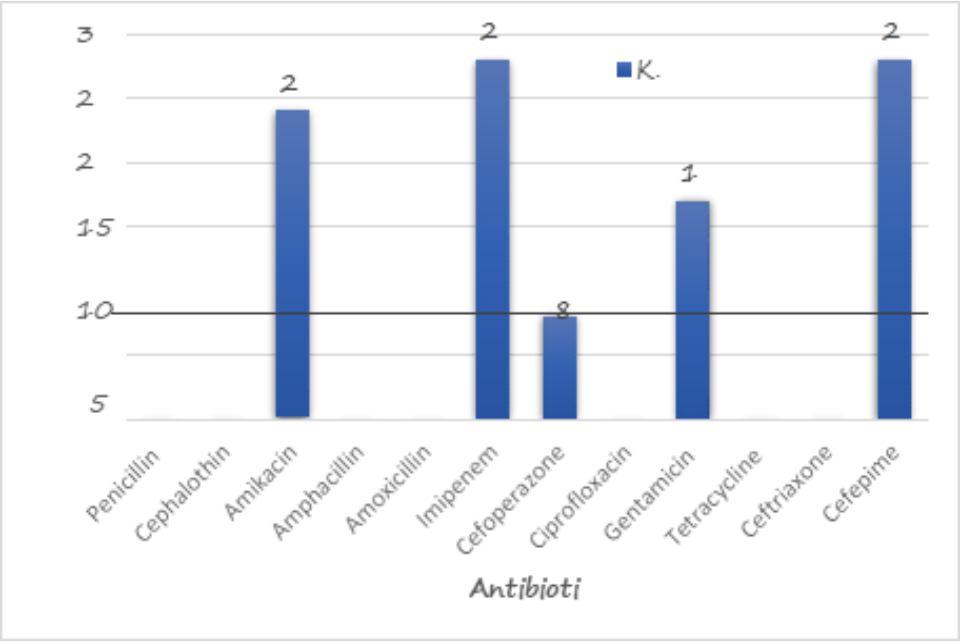

Figure 9. Bar graph shows the zones of inhibition produced by different antibiotics against *K. pneumonia*

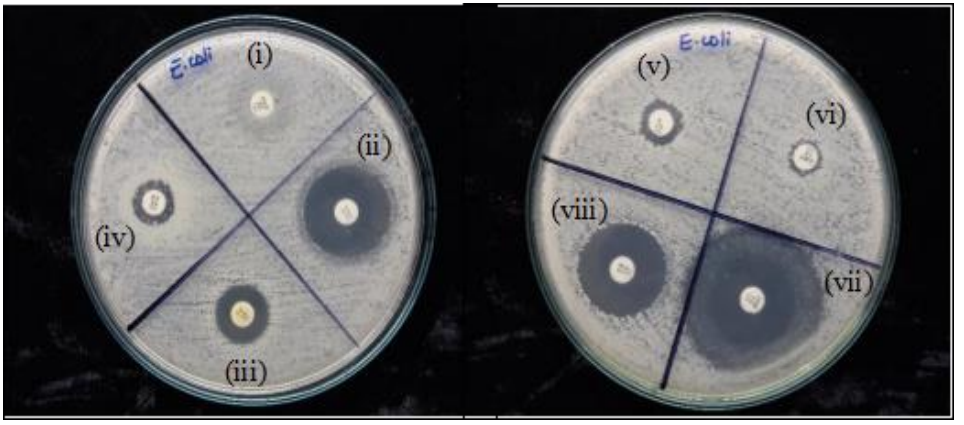

Figure 10. Antibiotic resistance pattern of *E. coli* against i. Cefepime, ii. Amikacin, iii. Tetracycline, iv. Cephalothin, v. Penicillin, vi. Ampicillin, vii. Imipenem, and viii. Gentamicin

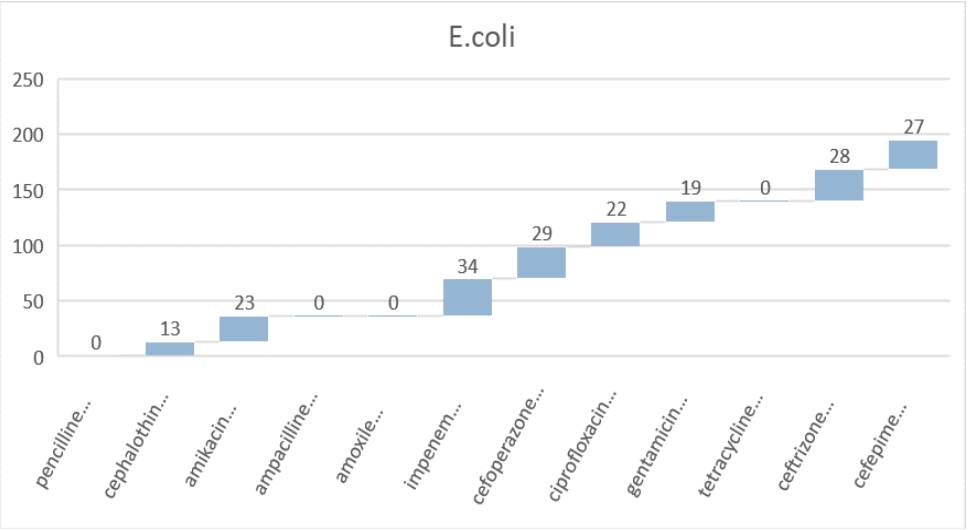

Figure 11. Bar graph shows the zones of inhibition produced by different antibiotics against *E. coli*

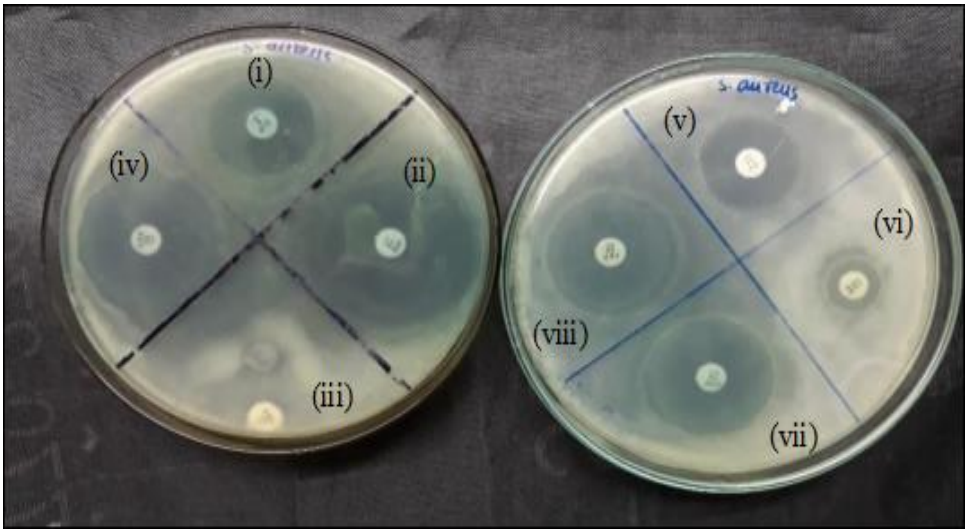

Figure 12. Antibiotic resistance pattern of *S. aureus* against i. Ciprofloxacin, ii. Imipenem, iii. Amoxicillin, iv. Ceftriaxone, v. Amikacin, vi. Cephalothin, vii. Cefoperazone, and viii. Cefepime inhibition of *S. aureus* introduced by different antibiotics

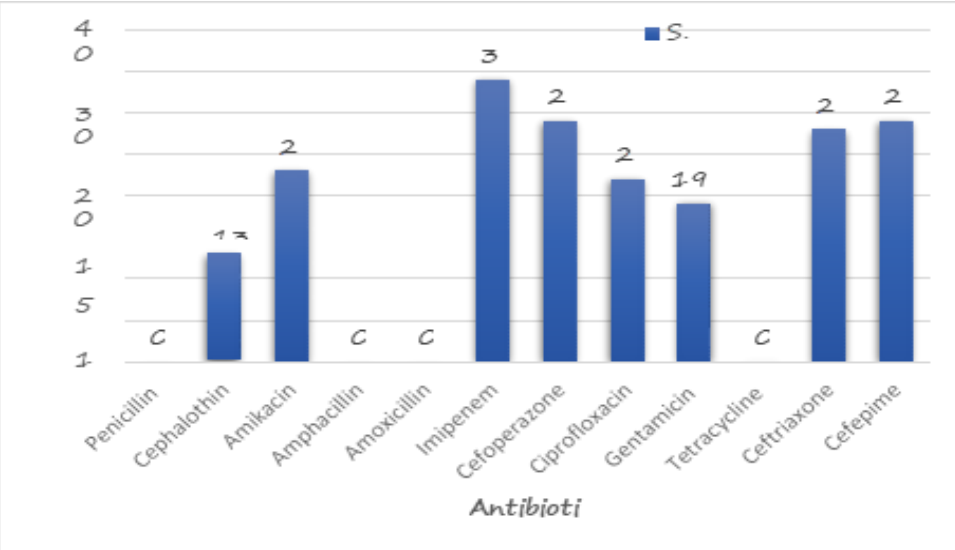

Figure 13. Bar graph shows the zones of inhibition produced by different antibiotics against *S. aureus*

4. Discussion

Urinary Tract Infection (UTI) is among the most common bacterial infections. It infects men, women and children of all age groups. They are associated with both community acquired and hospital acquired infections [13]. Effective management of this disease depends upon its prompt identification and selection of adequate antibiotics. The current study was organized to assess the prevalence of uropathogens causing UTI and to achieve anti-biogram of clinical isolates from the local area against commonly prescribed antibiotics. The most common pathogen detected was *E. coli* (35%), followed by *Klebsiella spp* (25%), and *candida spp* (25%). These findings are similar to the results of another study carried out in Hungary [14]. Another retrospective study reported that out of 1176 urine samples, *E. coli* was the most common pathogen with 47.3% prevalence, while the prevalence of *Klebsiella spp* and *Candida spp* was 10.3% and 8.8%, respectively [15].

In our study, 79.1% of the infection was caused by Gram (-) rods. Another study reported similar results, where Gram negative rods appeared as the most common pathogen associated with UTI [16]. A study from India reported that 71.6% and 28.3% of the inpatients and outpatients of UTI, respectively, had Gram negative bacteria [15].

In the current study, *E. coli* was found to be the most common cause of UTI in both male and female subjects, followed by *K. pneumoniae.* This confirms the findings of other studies from Pakistan [17, 18]. However, in a study conducted on diabetic patients, *Proteus spp* was reported to be the second most common cause of UTI [15]. Moreover, UTI was found to be more prevalent in female subjects (57.1%),

which is in accordance with the previous reports from Pakistan and other parts of the world [17, 19].

Resistance was high against ampicillin and cotrimoxazole, as reported by Aghamahdi F. A study based on the data collected from Mexico City reported the prevalence of high resistance towards ampicillin, co-trimoxazole and ciprofloxacin among uropathogenic *E. coli* isolates [20]. Whereas, amikacin, fosfomycin, nitrofurantoin, imipenem, meropenem, vancomycin, teicoplanin and combinations such as sulbactam-cefoperazone and tazobactam-piperacillin were found sensitive to most isolates as reported by other studies [21].

This study was conducted to investigate the antimicrobial susceptibility test for all isolates obtained from urine samples. The results revealed that bacterial isolates obtained from urine samples have high resistance against various antibiotics. Bacterial resistance to antibiotics is a major threat all over the world. However, for developing countries such as Pakistan, this threat is even worse and antibiotic resistance has emerged as a new challenge [22].

In the current study, urine samples from the patients indicated the presence of the highest number of uropathogens, which showed that populations were suffering from severe UTI. The existence of UTI among the patients can be credited to poor sanitary conditions due to overcrowding and unhygienic conditions prevailing at the hospitals. Related conclusions were drawn by different researchers [23]. This research indicates that in Pakistan, people use antibiotics in a very high frequency for unnecessary purposes and in most cases, they are prescribed by the medical practitioner [24, 25]. Such practices contribute to a alarming development of rising antibiotic resistance in the country.

The current study also shows the presence of *K. pneumonia*, *S. aureus*, *P. aeruginosa*, *E. aerogenes*, *E. coli* and *P. mirabilis* in the samples, which are responsible for UTI. Most of these organisms are well documented by many researchers [26]. Most of the infections can be attributed to *E. coli*, which was found responsible for over 50% of outdoor patients. It was followed by *Enterobacter* spp, *K. pneumonia*, *Proteus spp* and *P. aeruginosa*, respectively. While *S. aureus* was the most frequent type of isolates among Gram (+) cocci [27, 28].

All isolates showed high resistance towards antibiotics generally used against these pathogens on the basis of sensitivity patterns. Furthermore, the isolated uropathogens also proved to be sensitive towards several antibiotics. Overall, all isolates showed the strongest sensitivity towards imipenem and amikacin. cephalothin, tatracycline and pencillin whereas the lowest sensitivity was recorded for amoxicillin and ampicillin. The organisms may develop a different mode of action due to the increased resistance pattern, which could be attributed to the recurrent use of these antibiotics [28]. In this study, imipenem was found to be the most useful antibiotic as compared to other most frequently used antibiotics. It is relatively expensive. This makes the organisms susceptible to it because its cost has probably limited its unrestricted use and procurement [29]. All isolates were found to be susceptible to imipenem. Similar results were reported by [30]. When

isolates were tested against imipenem, other researchers also reported similar findings [31, 32]. So, there is a need to point up the rational use of antimicrobials, while strictly adhering to the concept of "reserve drugs" in order to minimize the misuse of available drugs.

5. Conclusion

Based on the above findings, imipenem was identified as the most sensitive antibiotic against UTI. It is recommended here to maintain its status as a reserve drug. Since antibiotic susceptibility patterns vary greatly, it is important to know the resistance pattern in order to identify the effective drug, especially in the conditions where experimental therapy is essential. *E. coli* is the most common cause of UTI in our country. Fosfomycin, carbapenems, combination drugs and nitrofurantoin are the most effective drugs and they should be used to treat UTI. Resistance to most commonly used antibiotics such as nitrofurantoin and gentamicin is also on the rise. There is a need to discover new antimicrobial compounds to combat the resistant bacteria involved in UTI and further research should be carried out for this purpose. It is also strongly recommended to evaluate the resistant patterns at genomic and proteomic levels in order to discover the genes responsible for resistant patterns.

Conflict of Interest

The authors declare no conflict of interest.

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