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Antibiotic susceptibility testing of bacterial Isolates Associated with Urinary Tract Infection in the northern region of Thi-qar Province.

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Abstract

Urinary tract infections (UTIs) are among the most prevalent infectious diseases, affecting millions worldwide and incurring significant healthcare costs. The study aimed to assess antibiotic resistance to commonly used antibiotics among bacterial pathogens linked to UTIs. Antibiotic susceptibility testing was performed using the disk diffusion method and in accordance with CLSI guidelines (2024).After identification and isolation, only 43 samples were positive for bacterial urinary tract infections (28.7%). The predominant uropathogen was Escherichia coli 15 (34.9%), followed by Klebsiella pneumoniae 11 (25.6%), Pseudomonas aeruginosa 8 (18.6%), Proteus mirabilis 5(11.6) and Staphylococcus aureus 4 (9.3%). E. coli isolates showed variable resistance rates to Cefotaxime (60%), Ciprofloxacin (53.3%), Tetracycline (66.7%), Trimethoprim (73.3%) and Trimethoprim/sulfamethoxazole (66.7%). K. pneumonaie isolates were high resistant to Ciprofloxacin (63.63%), Tetracycline (100%), Tobramycin (63.63%), Trimethoprim (81.8%) and Trimethoprim/sulfamethoxazole (63.63%). Most of P. aerugenosa and P.mirabilis were resistance to Trimethoprim (100% for each) and Trimethoprim/sulfamethoxazole (100% and 80%, respectively). All P.aerugenosa isolates were resistant to Nitrofurantoin (100%). Isolates of S.aureus were significantly resistant to Trimethoprim/sulfamethoxazole (75%), Tobramycin (100%), Erythromycin (100%) and Tetracycline (75%).

Keywords: Urinary tract infection, Antibiotic resistance, Escherichia coli, Staphylococcus aureus

الملخص

تُعد التهابات المسالك البولية من أكثر الأمراض المعدية شيوعًا، إذ تصيب ملايين الأشخاص حول العالم وتُسبب تكاليف رعاية صحية باهظة. هدفت الدراسة إلى تقييم مقاومة المضادات الحيوية الشائعة الاستخدام بين مسببات الأمراض البكتيرية المرتبطة بالتهابات المسالك البولية. أُجري اختبار حساسية المضادات الحيوية باستخدام طريقة انتشار الأقراص، ووفقًا لإرشادات **(CLSI) (2024).** بعد تحديد العينات وعزلها، أظهرت 43 عينة فقط نتائج إيجابية لالتهابات المسالك البولية البكتيرية (28.7%). وكان المسبب الرئيسي لالتهابات المسالك البولية هو الإشريكية القولونية 15 (34.9%)، تليها الكلبسيلا الرئوية 11 (25.6%)، والزائفة الزنجارية 8 (18.6%)، والمتقلبة الرائعة 5 (11.6%)، والمكورات العنقودية الذهبية 4 (9.3%). أظهرت عزلات الإشريكية القولونية معدلات مقاومة متفاوتة لأدوية سيفوتاكسيم (60%)، وسيبروفلوكساسين (53.3%)، وتتراسايكلين (66.7%)، وتريمثوبريم (73.3%)، وتريمثوبريم/سلفاميثوكسازول (66.7%). أما عزلات الكلبسيلا الرئوية فكانت شديدة المقاومة لأدوية سيبروفلوكساسين (63.63%)، وتتراسايكلين (100%)، وتوبراميسين (63.63%)، وتريمثوبريم (81.8%)، وتريمثوبريم/سلفاميثوكسازول (63.63%). أما معظم الزائفة الزنجارية والمتقلبة الرائعة فكانت مقاومة لأدوية تريميثوبريم (100% لكل منهما) وتريمثوبريم/سلفاميثوكسازول (100% و80% على التوالي). وكانت جميع عزلات الزائفة الزنجارية مقاومة للنيتروفورانتوين (100%). أظهرت عزلات المكورات العنقودية الذهبية مقاومة كبيرة لـ تريميثوبريم / سلفاميثوكسازول (75٪)، توبراميسين (100٪)، إريثروميسين (100٪) وتتراسايكلين **(75)** ٪

الكلمات المفتاحية: عدوى المسالك البولية، مقاومة المضادات الحيوية، الإشريكية القولونية، المكورات العنقودية الذهبية

Introduction

Urinary tract infections (UTIs) are common bacterial infections affecting both sexes, with higher prevalence in females [1]. Escherichia coli is the primary causative agent in most cases. Diagnosis is based on symptoms and urinalysis, with urine culture recommended for severe or complicated cases [2]. UTIs are traditionally classified as uncomplicated or complicated, with uncomplicated UTIs occurring in healthy premenopausal women without urinary tract abnormalities [3]. Uncomplicated UTIs are typically easy to treat, while complicated UTIs are more difficult to treat due to the pathogen, the

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patient's anatomy or health, and/or the presence of medical devices like stents or urinary catheters [4]. Despite the fact that both men and women can contract UTIs, the disease is usually associated with women, with 50% of them experiencing symptoms throughout the course of their lives [5]. Bacteria can enter and spread throughout the urinary tract by three different routes: the lymphatic, hematogenous, and ascending pathways. The majority of episodes occur through the ascending pathway, while the hematogenous pathway relatively rarely occurs [6]. Ascending route is often facilitated by urinary catheters where bacteria form biofilms and ascend into the bladder [7]. Both the upper and lower urinary tracts may be affected by a UTI. Cystitis, or bladder infection, is a lower UTI, whereas pyelonephritis, or kidney infection, is an upper UTI [8]. The most prevalent type of infection acquired in healthcare settings is healthcare-associated UTIs (HAUTIs) [9]. Traditional treatment involves short-course antimicrobial therapy, but increasing antibiotic resistance poses challenges [10]. Non-antimicrobial options for prevention and management include cranberry products, estrogen therapy for postmenopausal women, and mechanical barrier devices, though evidence for their efficacy varies [11]. Age, diabetes, spinal cord damage, and catheterization are examples of underlying host variables that might worsen UTI and have an impact on its etiology [12]. Studies in Thi-Qar and Basra governorates reveal high antibiotic resistance rates, with E. coli showing resistance to commonly prescribed antibiotics like ampicillin and ciprofloxacin in Thi- Qar, and co-trimoxazole and cefotaxime in Basra [13] [14]. While Gram-negative bacteria demonstrated the highest resistance to Amoxicillin-clavulanate (100%), Piperacillin (100 %), and both Ceftazidime and Nitrofurantoin (86.36 %), Gram-positive isolates exhibited the highest resistance to Amoxicillin-clavulanate (100%), Ampicillin (100%), Oxacillin (100%) in AL-Nasiriyah City [15] .

Materials and method

Inclusion and exclusion criteria

Patient who diagnosed with UTIs, exhibiting common symptoms such as dysuria, urinary frequency, urgency, suprapubic pain, or flank pain, were included in this study. Patients with recent antibiotic use for UTI within the past 7–14 days were excluded to avoid false-negative cultures.

Samples collection:

A total of 150 Midstream urine samples were collected by clean-catch method from male and female (102 males, 48 females) patients of varying ages ranged between (11-80 year), between September 2024 and February 2025. These samples were obtained from individuals receiving care at hospitals, health centers, and clinics across the northern region of Thi-Qar province (Al Refai, Qalat Suker and Al Neser districts). A structured questionnaire was used to assess all suspected UTI cases. Only one specimen per patient was included.

Isolation and identification:

After being cultivated on blood agar and MacConkey agar, the samples were incubated for 24 hours for positive cases and up to 48 hours for negative cases at 37°C. The bacterial isolates were

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identified based on colony characteristics and Gram staining, with confirmation through biochemical profiling using biochemical reactions. The main groups of Enterobacteriaceae were differentiated by analyzing their biochemical properties and enzymatic reactions to specific substrates. The IMViC tests include indole, methyl red, Voges-Proskauer, and citrate utilization test were used for this purpose [16]. Additionally, Samples were tested for oxidase activity to differentiate oxidase positive Neisseria and Pseudomonas spp and oxidase-negative Enterobacteriaceae [17]. Catalase test used to distinguish Gram positive Staphylococcus spp from Streptococcus spp [18].

Antimicrobial susceptibility test:

Patients with no history of antimicrobial drug use for UTIs in the preceding two weeks were included in the study. To assess antibiotic resistance patterns in bacterial isolates associated with urinary tract infections, all isolates underwent antibiogram testing following the Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines [19]. Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method for Amikacin (30 µg), Gentamicin (10 µg), Tobramycin (10 µg), Ciprofloxacin (5 µg),Trimethoprim (5 µg), Trimethoprim / Sulfamethoxazole (25 µg), Tetracycline (30 µg), Cefotaxime (30µg), Cefoxitin (30 µg), Nitrofurantoin (300 µg), Imipenem (10 µg), Clindamycin (2 µg) and Erythromycin (15 µg).

Statistical Analysis:

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference factors in study percentage[20]. Chi-square test was used in this study .

Results and discussion

From 150 samples were taken from patients who diagnosed with Urinary tract infections, bacterial infections were observed in 43 specimens (28.7%). Furthermore 107 (71.3%) of samples exhibited no growth at all, as shown in Figure (1). These results were in line with those of several other research carried out in Iraq. Mohammed Allami et al. reported that 216 out of 830 midstream urine specimens showed positive bacterial cultures (26%) [21]. A study conducted in Baghdad stated that (26.58 %, 63/237) of patients attending Medical City Hospital were positive for bacterial urinary tract infections [22]. In Ebril by Ekrem Kİreççİ et al, reported that the prevalence of bacterial urinary tract infections was (58.3 %) [23]. Biochemical reactions and differential media showed only five types of bacteria. The most common isolated pathogen was E.coli, accounted for 15 (34.9%), followed by Klebsiella pneumoniae (25.6%), Pseudomonas aeruginosa (18.6%),Proteus mirabilis (11.6%) and S.aureus (9.3%) as described in the Table (1). This study observed that Gram-negative bacteria (90.7%) were the leading cause of Urinary tract infections. In comparison, the Gram-positive bacteria recorded (9.3%) of total cases of UTI. This study disagrees with a study conducted by Naji and Awadh. (2022), who reported that Gram-positive bacteria were the predominant pathogen associated with UTI (66.7%), while the prevalence of Gram-negative bacteria was 33.3% [24].

According to residency, the highest rate of UTI cases was in Qalat Suker district 18 (41.9%), while disturbance of UTI cases in Al Refai and

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Al-Neser districts was (32.5% and 25.6%) as shown in Table (2). Urinary tract infections was higher in females 26/43 (60.5%), compared to males 17/43 (39.5%) as demonstrated in (Figure 2). Women are more likely to experience UTIs than men because women have shorter urethras than men, as well as short proximity to the anus [4]. The current study agrees with Mazin Saleem Salman who found that females showed the highest prevalence rate of UTIs (77%), while males recorded (23%) of overall cases of UTI [25]. This study revealed that UTI cases were slightly higher in Urban than Rural (accounts for 53.5 and 46.5 respectively) as described in Table (3). The highest rate of bacterial isolates among age groups was in the sixth group aged between (61- 70) years, with 10 (23.25 %) isolates, followed by the group aged (21-30) which recorded 9 isolates (20.93%) . the first age group (11-20), showed the lowest frequency with only 2 patients with UTIs, accounting for (4.65%), as shown in Table (4). This study agrees with K. Marshall and D. Hale, who reported that UTIs increases with age, as factors like diabetes, kidney stones, stroke, dementia, and bowel and bladder incontinence may predispose elderly people to experience more UTIs [26]. In contrast, it has been observed that younger people who aged between (21-30) exhibited the highest infection rate (35.4% for females and 29.3% for males), followed by the age group 31-40 years [27].

The resistance rates of E.coli against Amikacin, Imipenem, Nitrofurantoin, Tobramycin, Ciprofloxacin, Cefotaxime, Tetracycline, Trimethoprim, Trimethoprim/sulfamethoxazole were 40%, 40% , 40%, 46.7, 53.3%, 60%, 66.7%, 73.3% and 66.7%, respectively (Table 5). This finding was in agreement with data reported by[28], who stated that uropathogenic E. coli showed high resistance to Trimethoprim/sulfamethoxazole (65%), Cefotaxime (75%), and Tetracycline (57%). A study conducted in Baghdad, which found that uropathogenic E.coli isolates were highly resistant to Trimethoprim (82%), and it had a strong correlation with the presence of dfrA1-gene [29]. According to Louie Mar Gangcuangco et al. the trimethoprim- sulfamethoxazole resistant isolates of E. coli have shown considerably higher resistance to gentamicin, ampicillin, and fluoroquinolones, in comparison to trimethoprim-sulfamethoxazole-resistant susceptible strains [30]. Since pharmacists and prescriptions are not strictly enforced in the country, and people are free to take medications without a prescription, the high rate of resistance to these antibiotics may be due to self-medication [31].

The high resistance rates of Klebseilla pneumoniae isolates to the following antibiotic were: Cefotaxime (54.54%), Imipenem (54.54%), Ciprofloxacin (63.63%), Trimethoprim / Sulfamethoxazole (63.63%), Tobramycin (63.63%), Trimethoprim (81.8%) and Tetracycline (100%), as demonstrated in Table (5). Mohammed et al. (2023) showed that the resistance of K. pneumoniae to Cefotaxime was approximately (96.6%), while Ciprofloxacin and Tetracycline were (41.4% and 75.9%, respectively) [32]. A study carried out From November 2021 to of February 2022, reported that the resistance rates of Klebseilla pneumoniae against Tetracycline and Trimethoprim / Sulfamethoxazole were 78.1% and 71.5%,

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respectively [33], while Shukran A. Mohammed et al. stated that all isolates of Klebseilla pneumonia were resistant to Tetracycline (100%) [34]. The resistance rates of P. aerogenosa isolates to Tobramycin, Ciprofloxacin and Imipenem were 75%, 37.5% and 50%, respectively. Nitrofurantoin, Trimethoprim, and Trimethoprim / Sulfamethoxazole had no activity against Pseudomonas aerogenosa (100%), as shown in Figure (3). In contrast, a study found that Pseudomonas aerogenosa exhibited high resistance to Ciprofloxacin (71.6%) [35]. our observation disagrees with a study, stated that Pseudomonas aerogenosa had a high susceptibility rate to Nitrofurantoin (80%)[36].

Proteus mirabilis exhibited high resistance to Ciprofloxacin (60%), Imipenem (80%), Nitrofurantoin (100%), Tetracycline (80%), Trimethoprim (100%) and Trimethoprim/sulfamethoxazole (80%). The current study was close to [37], which reported that all isolates of Proteus mirabilis were resistant to Tetracycline and Trimethoprim/sulfamethoxazole (100%). In contrast, a study has been observed that Proteus mirabilis had high susceptibility rates against Imipenem and Ciprofloxacin (92% and 75%, respectively) [38]. The susceptibility rates of S. aureus isolates to the following antibiotics were: Amikacin (100%), Nitrofurantoin (100%), Ciprofloxacin (50%), Clindamycin (50%), Cefoxitin (50%), and Gentamicin (75%). All isolates of all isolates of S.aureus (100%) were resistant to Erythromycin and Tobramycin, while 75% of these isolates were resistant to Tetracycline and Trimethoprim / Sulfamethoxazole, as shown in the Table (6). This findings were in agreement with a study conducted in Afghanistan found that isolates of S.aureus exhibited high resistance rates to Erythromycin (60%) [39]. The mechanisms of resistance to aminoglycosides such as tobramycin in S.aureus include reduced uptake, target alterations of the 30S ribosomal subunit, and enzymatic modification of the aminoglycoside drugs [40]. Our finding was consistent with a study, stated that S. aureuas showed significant susceptibility rate to Nitrofurantoin (88.9%) [41].

Conclusion

Bacterial isolates associated with urinary tract infections showed variable resistance to commonly used antibiotics, with high resistance rates of Enterobacteriaceae isolates against Tetracycline, Trimethoprim, and Trimethoprim / Sulfamethoxazole. Nitrofurantoin, Trimethoprim, and Trimethoprim / Sulfamethoxazole were ineffective against Pseudomonas aerogenosa. Erythromycin and Tobramycin had no activity against S. aureus.

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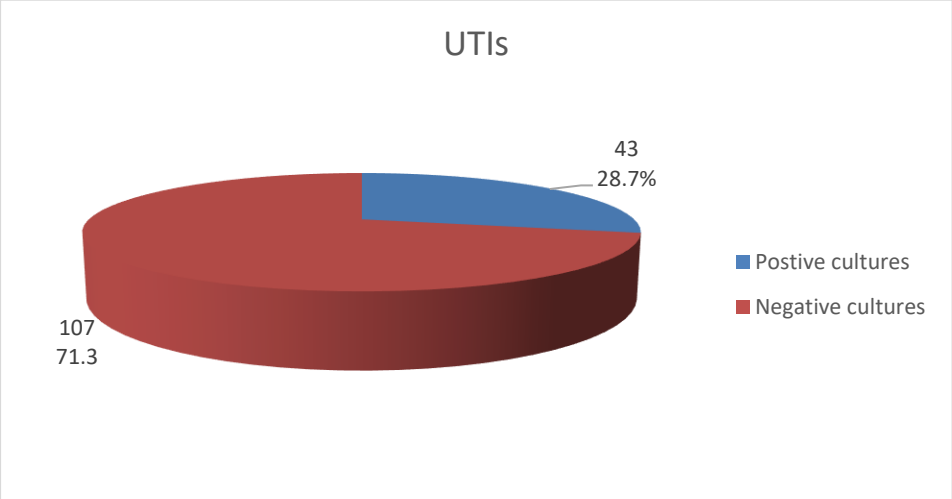

Figure 1: Positive versus negative bacterial culture.

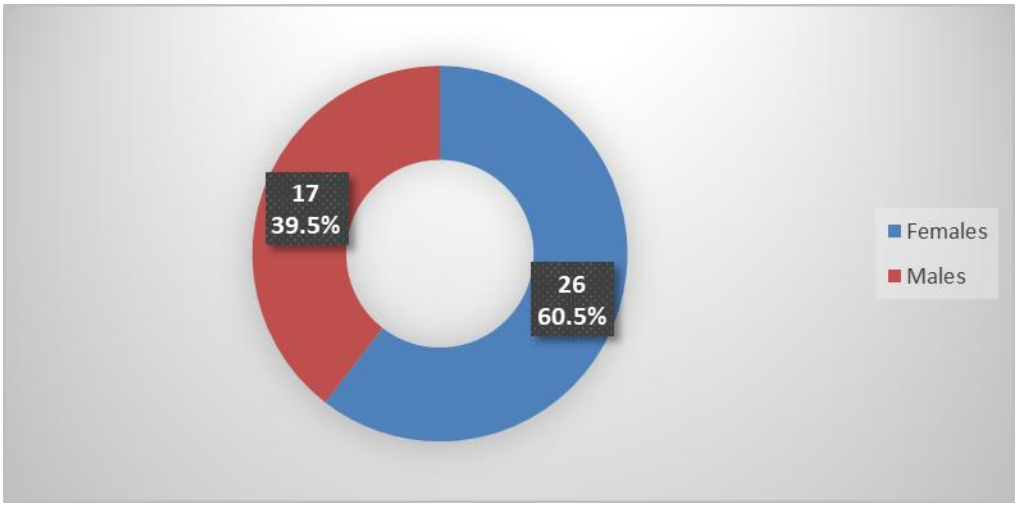

Figure 2: Distribution of UTI patients according to sex.

Table (1): Frequency of isolated pathogens associated with UTIs.

Bacterial isolates	Frequency	
	No.	%
Escherichia coli	15	34.9
Klebsiella pneumonia	11	25.6
Pseudomonas aeruginosa	8	18.6
Proteus mirabilis	5	11.6
Staphylococcus aureus	4	9.3
(P≤0.01).		

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Table (2): Disturbance of UTI according to residency.

Residency	Positive Culture		Total	
	No.	%	No.	%
Al-Neser	11	25.6	50	33.33
AL-Refai	14	32.5	50	33.33
Qalat suker	18	41.9	50	33.33
Total	43	28.7	150	100%
(P≤0.05).				

Table (3): Disturbance of UTI cases according to habitation

Habitation	Positive Culture		Total	
	No.	%	No.	%
Rural	20	46.5	87	58.00
Urban	23	53.5	63	42.00
Total	43	28.7	150	100
(P≤0.05).				

Table (4): Distribution of UTI patients according to age.

Age groups	Positive cultures		Total	
	No.	**%**	**No.**	**%**
11-20	2	4.65	37	24.67
21-30	9	20.93	29	19.33
31-40	5	11.63	23	15.33
41-50	6	13.95	16	10.67
51-60	7	16.28	18	12
61-70	10	23.26	19	12.67
> 70	4	9.30	8	5.33
Total	43	28.7	150	100
(P≤0.05).				

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Table 5: Antibiotic susceptibility test for *Enterobacteriacea.*

List	*E.coli* No.(%)			*K.pneumoniae* No.(%)			*P. mirabilis* No.(%)		
	S	I	R	S	I	R	S	I	R
AK	7(46.7)	2(13.3)	6(40)	5(45.45)	1(9.1)	5(45.45)	3(60)	1(20)	1(20)
CTX	5(33.3)	1(6.7)	9(60)	3(27.3)	2(18.2)	6(54.54)	3(60)	0(0)	2(40)
Fox	8(53.3)	3(20)	4(26.7)	6(54.54)	1(9)	4(36.46)	3(60)	1(20)	1(20)
Cip	6(40)	1(6.7)	8(53.3)	4(36.4)	0(0)	7(63.6)	1(20)	1(20)	3(60)
CN	6(40)	2(13.3)	7(46.7)	5(45.45)	1(9)	5(45.45)	1(20)	2(40)	2(40)
IMI	8(53.3)	1(6.7)	6(40)	3(27.3)	2(18.2)	6(54.54)	0(0)	1(20)	4(80)
F	7(46.7)	2(13.3)	6(40)	7(63.63)	1(9)	3(27.3)	0(0)	0(0)	5(100)
TE	4(26.6)	1(6.7)	10(66.7)	0(0)	0(0)	11(100)	0(0)	1(20)	4(80)
Tob	6(40)	2(13.3)	7(46.7)	2(18.2)	2(18.2)	7(63.63)	3(60)	2(40)	0(0)
TM	3(20)	1(6.7)	11(73.3)	1(9)	1(9)	9(81.8)	0(0)	0(0)	5(100)
SXT	4(26.7)	1(6.7)	10(66.7)	3(27.3)	1(9)	7(63.63)	0(0)	1(20)	4(80)
($P \leq 0.05$).									

No.(%): Number(percentage), S: Sensitive, I: Intermediate, R: Resistant, AK: Amikacin, CTX: Cefotaxime, Fox: Cefoxitin, Cip: Ciprofloxacin, CN: Gentamicin, IMI: Imipenem, F: Nitrofurantoin, TE: Tetracycline, Tob: Tobramycin, TM: Trimethoprim, SXT: Trimethoprim / Sulfamethoxazole. P: p. value.

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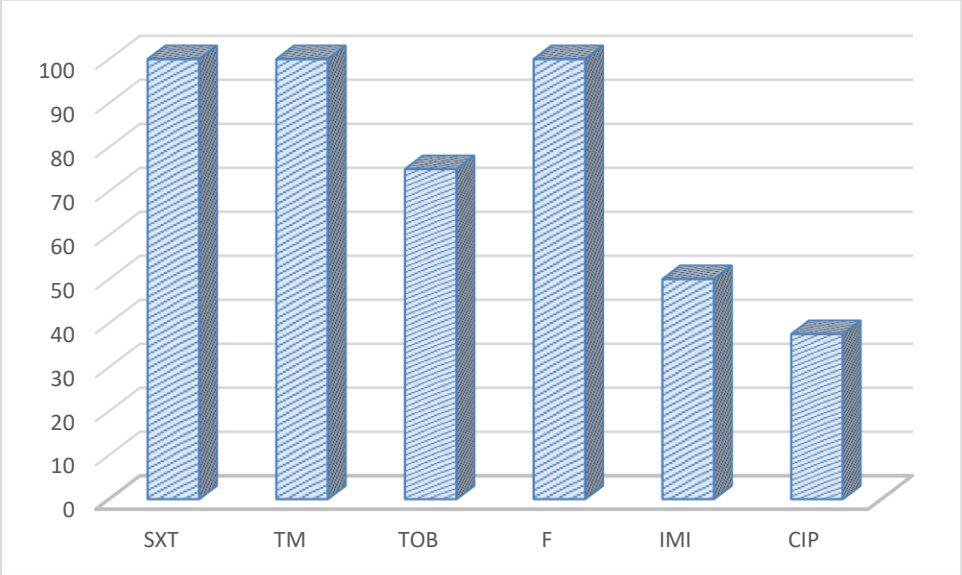

Figure 3: antibiotic resistance rates for *P. aeruginosa*.
SXT: Trimethoprim / Sulfamethoxazole, TM: Trimethoprim, TOB: Tobramycin, F: Nitrofurantoin, IMI: Imipenem, CIP: Ciprofloxacin

Table (6): Antibiotic susceptibility test for *S. aureus*.

Antibiotics list	***S. aureus*** No.(%)		
	Sensitive	Intermediate	Resistant
Amikacin	4 (100)	0(0)	0(0)
Cefoxitin	2(50)	0(0)	2(50)
Ciprofloxacin	2(50)	0(0)	2(50)
Clindamycin	2(50)	1(25)	1(25)
Erythromycin	0(0)	0(0)	4(100)
Gentamicin	3(75)	0(0)	1(25)
Nitrofurnation	4(100)	0(0)	0(0)
Tetracycline	1(25)	0(0)	3(75)
Tobramycin	0(0)	0(0)	4(100)
Trimethoprim / Sulfamethoxazole	1(25)	0(0)	3(75)
(P≤0.05).			

No.(%): Number(percentage), P: p. value.