Middle East Research Journal of Microbiology and Biotechnology
ISSN: **2789-8644 (Print & Open Access)**
Frequency: Bi-Monthly
DOI: https://doi.org/10.36348/merjmb.2026.v06i02.001

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Antibiotic Resistance Patterns of Bacteria Isolated from Patients with Urolithiasis in Tikrit City, Iraq

Mohemid Maddallah Al-Jebouri[1*], Omar Abid Hamood Al-Jebouri[2]
[1]Department of Medical Laboratory Technology, Health and Medical College of Technology, Al-Qalam University, Kirkuk, Iraq
[2]Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq

Abstract: ***Background*:** The emergence of antibiotic resistance in the management of UTI's is a serious public health issue, particularly in the developing world. Studies aimed at gaining knowledge about the type of pathogens responsible for UTI's and their susceptibility patterns may help the clinicians to choose the right empirical treatment. ***Methodology*:** A total of 135 patients with urolithiasis were studied including 91 males and 44 females. Patients aged between 15 to 70 years and submitted for chemical analysis. Species were identified by the conventional methods which emphasized colony morphology, Gram stain biochemical tests and API 20 E identification system. All isolates were subjected to antibiotic disc diffusion and minimal inhibitory concentration tests. ***Results*:** The most common organism isolated was *E.coli* which was detected in 19 patients (35.8%) which followed by *Proteus mirabilis, Staphylococcus species, Pseudomonas aeruginosa, Enterobacter faecalis, Citrobacter freundii and Serratia marcescens* and the percentages of isolation were 15.1, 13.2, 13.2, 7.5, 7.5, 3.8, 1.8 and 1.8% respectively. It was found that the highest sensitivity of organisms was toward amikacin (100%). A moderate-to-strong correlation (R> 0) indicated non-random structure in MIC distribution among bacterial isolates studied. ***Conclusions*:** The present study showed that urine culture was done for all patients and 50 of them were positive. The most common organism isolated was *E.coli* which was detected in 19 patients (35.8%). The regression demonstrates a negative association between standard error and antibiotic sensitivity. Linear Regression Analysis showed a positive slope i.e increasing MIC across observations and this a systematic increase in resistance across certain bacteria–antibiotic combinations.

Keywords: Urinary Tract Infection (UTI), Urolithiasis, Antibiotic Resistance, *Escherichia coli*, Minimum Inhibitory Concentration (MIC).

Research Paper

***Corresponding Author:**
Mohemid Maddallah Al-Jebouri
Department of Medical Laboratory Technology, Health and Medical College of Technology, Al-Qalam University, Kirkuk, Iraq

How to cite this paper:
Mohemid Maddallah Al-Jebouri & Omar Abid Hamood Al-Jebouri; "Antibiotic Resistance Patterns of Bacteria Isolated from Patients with Urolithiasis in Tikrit City, Iraq" Middle East Res J. Microbiol Biotechnol., 2026 May-Jun 6(2): 58-66.

Article History:
| Submit: 06.04.2026 |
| Accepted: 09.05.2026 |
| Published: 14.05.2026 |

INTRODUCTION

Most urinary calculi generally are composed primarily of a poorly soluble salt with a small amount of protein, containing calcium (Ca+2) as a main constituent [1, 2]. The direct cause of calculi is unknown and likely to be multifactorial, but urinary physiological abnormalities can be identified in more than 60% of patients [3-5]. Hypercalciuria is the most common of these abnormalities, increases the risk of stone formation by raising saturation of stone forming salt and reducing the endogenous stone inhibitors [5-7]. The prevalence of urolithiasis is approximately 2-3% of general population [8, 9]. The peak age of males is 30 years old, while in the women has bimodal age distribution with peaks at 35 and 55 years old. One kidney stone form the probability that a second stone will form within 5-7 years in approximately 50% [4-10]. Stone disease is 2-3 times more common in men than in women and it occurs more often in adults than in elderly and more often in elderly than in children. Urolithiasis occurs more frequently in hot dry areas than in temperate regions [11]. The vast majority of UTI's are caused by *Enterobacteriaceae* originated from the gut before entering the urethra. The organisms included in this family are *E.coli, Klebsiella, Proteus, Enterobacter, Citrobacter, Providencia, Serratia and Salmonella* species which are the most common members of this family causing UTI. E.coli is by far the most frequently isolated organisms being responsible for approximately 80% of UTI's. *Pseudomonas* species is also Gram-negative aerobic

bacilli, but it distinct and unrelated to *Enterobacteriaceae.* Most of Pseudomonas organisms recovered from the urine are relatively low virulent and do not tend to invade tissue unless the host defense mechanisms are compromised. The most common Gram-positive organisms found in the urine of UTI's patients are Staphylococci including *S. aureus* and *S. saprophyticus* which cause UTI in young sexually active women mostly. *Enterococcus* species, group B beta hemolytic *Streptococcus agalactiae* can cause UTI in pregnant women. When considering UTI, not only the chosen drug must be active against the infecting organism and relatively nontoxic, but a number of other factors must be considered like mode of excretion of the drug, the tissue concentration obtained and the effect of pH. Antibiotic resistance is a specific type of drug resistance when a microorganism has the ability of withstanding the effects of antibiotics. Antibiotic resistance evolves via natural selection acting upon random mutation, but it can also be engineered by applying an evolutionary stress on a population. Urinary Tract Infections (UTI's) are one of the most prevalent extra-intestinal bacterial infections. Nowadays, it represents one of the most common dis-eases encountered in medical practice affecting people of all ages from the neonate to the geriatric age group [12]. Worldwide, about 150 million people are diagnosed with UTI each year [13]. Most infections are caused by retro-grade ascent of bacteria from the faecal flora via the urethra to the bladder and kidney especially in the females who have a shorter and wider urethra and are more readily transferred by microorganisms [14]. The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria being massaged up the urethra and into the bladder during pregnancy and/or child birth [15, 16]. Majority of UTI's are not life threatening and do not cause any irreversible damage. However, when the kidneys are involved, there is a risk of irreparable tissue damage with an increased risk of bacteremia [17]. The emergence of antibiotic resistance in the management of UTI's is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation. Studies aimed at gaining knowledge about the type of pathogens responsible for UTI's and their susceptibility patterns may help the clinicians to choose the right empirical treatment.

MATERIALS AND METHODS

Source of Specimens

This study was conducted in the Urology Department in Tikrit Teaching Hospital as a part of research programs for higher studies in College of Medicine, University of Tikrit. A total of 135 patients with urolithiasis were studied including 91 males and 44 females. Patients aged between 15 to 70 years and submitted for chemical analysis.

Culture of Urine Specimens

Species were identified by the conventional methods which emphasized colony morphology, Gram stain and biochemical tests (Refer). API 20E system was also utilized according to manufacture instructions [18].

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing of all isolates was per-formed on Mueller-Hinton medium by the Kerby Bauer- method (1996) following the definition of the National Committee of Clinical Laboratory Standard (NCCLS,1999). The medium was allowed to cool at 45℃ and poured into Petri dishes to about 4 mm thickness of medium. The solidified plates were incubated at 37℃ for 15 -30 minutes to let the excess moisture to evaporate (Fisher scientific, USA) [19].

Inoculation and Incubation

The plates were inoculated by dipping a sterile swab into the inoculum, the excess inoculum was removed by pressing and rotating the swab firmly against the sidewall of the tube above the level of fluid, then the swab was rubbed all over the surface of the medium, rotating the plate 3 times at an angle of 60 degree after each ap-plication and finally the swab passed around the edge of agar surface. The plate was left to dry at room temperature with the lid closed for few minutes. After 15 minutes of inoculation, the antibiotic discs were applied and the plates were inverted for incubation to avoid accumulation of moisture on the agar surface [8]. Maximum 10 antibiotic discs were selected and placed onto each plate using flamed forceps for application of the discs on the plate and each disc pressed down gently to ensure even contact with the medium. After overnight incubation at 37℃ the diameter of each zone including the diameter of zone inhibition was measured and recorded in mm and com-pared with the standard inhibition zone. For motile organisms, e.g. Proteus spp. the swarming haze was ignored and zones were measured at the point where growth was obviously inhibited [8].

Minimal Inhibitory Concentration (MIC) Testing

Double dilutions of 5 different antibiotics were incorporated in Mueller-Hinton agar plates and they were prepared. A loopful of each isolated organism culture was inoculated into tubes containing nutrient broth and incubated at 37^0 C for overnight. Dilution of the broth was done up to 100 folds with nutrient broth. All plates were inoculated with diluted broth culture organisms and incubated at 37^0 C for 24 hours. The results were read to the point where there was no visible growth and this point called the MIC. Plates containing no antibiotics were incubated in each batch of incubated plates as control [ref].

Statistical Analyses

Statistical analyses in the present study were done by using Microsoft Office Excel 2007, SPSS version 12(Statistical Package for Social Sciences). The

programs used were F-test, T-test, least significant difference (LSD) and Chi-square [9].

RESULTS

Bacterial isolates

The present study showed that urine culture was done for all patients and 50 of them were positive. The most common organism isolated was *E.coli* which was detected in 19 patients (35.8%) which followed by *Proteus mirabilis, Staphylococcus species , Pseudomonas aeruginosa, Enterobacter faecalis, Citrobacter freundii and Serratia marcescens* and the percentages of isolation were 15.1, 13.2, 13.2, 7.5, 7.5, 3.8, 1.8 and 1.8% respectively (Table 1, Figure 1). The patients revealed mixed growth which were 2 *E.coli* and 2 *P. aeruginosa*, 1 *P. mirabilis and 1 E. faecalis*. Statistically there was no significant difference between males and females in the organisms distribution (P>0.05) using Chi- square test. But the linear regression analysis revealed a correlation coefficient (R) value ≈ 0.89 and the coefficient of determination (R^2) was ≈ 0.79 which indicated Strong positive correlation between male and female infection distribution and almost 79% of variation in female counts is explained by male counts.

Table 1: Distribution of isolated organisms causing urinary tract infection (UTI) among urolithiasis patients

Organism	Male		Female		Total	
	No.	%	No.	%	No.	%
Escherichia coli	9	16.9	10	18.8	19	35.8
Proteus mirabilis	4	7.5	4	7.5	8	15.1
Pseudomonas aeruginosa	2	3.8	5	9.4	7	13.2
Staphylococcus aureus	2	3.8	3	5.7	5	9.4
Enterococcus aerogenes	1	1.8	3	5.7	4	7.5
Klebsiella pneumoniae	2	3.8	2	3.8	4	7.5
Staphylococcus epidermis	1	1.8	1	1.8	2	3.8
Enterococcus faecalis	0	0	1	1.8	1	1.8
Serratia marcescens	0	0	1	1.8	1	1.8
Citrobacter freundii	0	0	1	1.8	1	1.8
Total	**21**	**39.6**	**32**	**60.4**	**53**	**100**

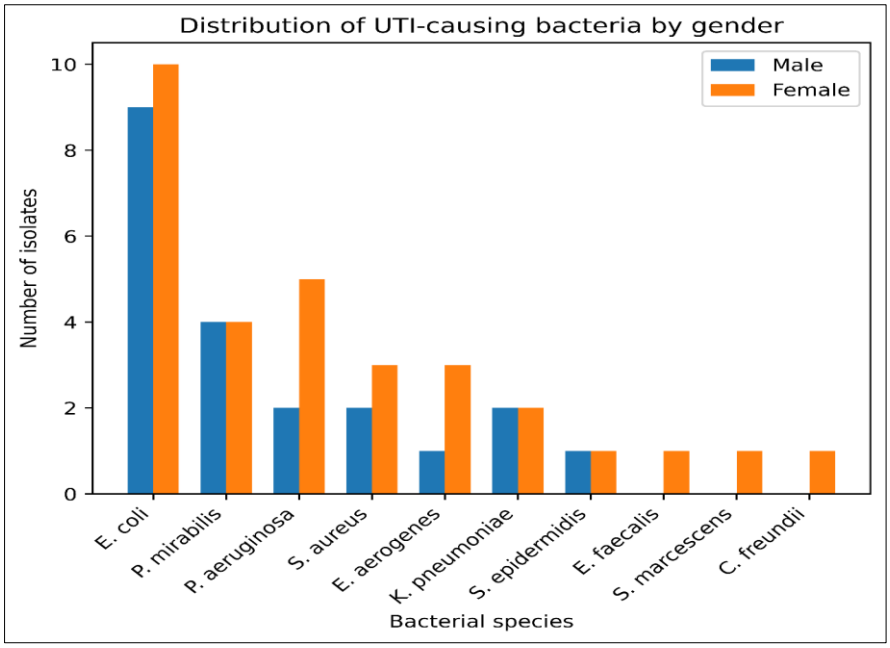

Figure 1: Distribution of different bacterial types isolated from patients with urolithiasis

Antibiotic Susceptibility

The susceptibility of bacterial isolates to different antimicrobial agents was carried out using the antibiotic disc diffusion technique. It was found that the highest sensitivity of organisms was toward amikacin (100%). While the lowest was to ampicillin (16%). Whereas the other antibiotics like ciprofloxacin, cefotaxime, nitrofurantoin, nalidixic acid, cephalexin, amoxicillin, gentamicin and cotrimoxazole were affecting bacteria at different degrees as shown in Tables 2 and 3 and Figures 2 and 3. Comparison of antibiotic effectiveness based on sensitivity testing of antibiotics utilizing Duncan test demonstrated grouping letters (A–D) displayed above bars revealed that group A was the most effective antibiotics (highest sensitivity) and groups B-C showed the Moderate effectiveness but

group D indicated the low effectiveness / resistance dominant(Figure 2). The regression demonstrates a negative association between standard error and antibiotic sensitivity, while the funnel plot indicates acceptable symmetry with no substantial publication bias but moderate heterogeneity across observations (Figure 3). Antibiotic effectiveness was stronger and more consistent in precise observations which likely negative correlation ($R < 0$) and R^2 would indicate moderate-to-strong fit based on visible trend as concluded from Table 3 and Figure 3.

Table 2: Antibiograms of *Enterobacteriaceae* species isolated from urolithiasis patients

Antibiotic	*E.coli*			*Pr. mirabilis*			*Kl. pneumoniae*			*Ent. aerogenes*			*C. freundii*			*S. marcescens*		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin	3	2	14	2	0	6	0	0	4	0	1	3	0	0	1	0	0	1
Amoxycillin	5	4	10	2	2	4	1	0	3	0	1	3	0	0	1	0	0	1
Amikacin	19	0	0	8	0	0	4	0	0	4	0	0	1	0	0	1	0	0
Nalidixic acid	9	7	3	6	2	0	3	1	0	1	2	1	0	0	1	1	0	0
Nitrofurantoin	12	4	3	4	4	0	4	0	0	4	0	0	0	0	1	1	0	0
Cephalexin	6	5	8	3	3	2	0	2	2	0	2	2	1	0	0	0	1	0
Cefotaxime	18	1	0	7	1	0	4	0	0	1	2	1	1	0	0	1	0	0
Cotrimoxazole	5	8	6	0	3	5	0	0	4	0	0	4	0	0	1	0	1	0
Gentamicin	7	5	7	3	1	4	0	2	2	0	1	3	0	1	0	1	0	0
Ciprofloxacin	16	3	0	8	0	0	4	0	0	3	1	0	1	0	0	1	0	0

S, sensitive; I, intermediate; R, resistant

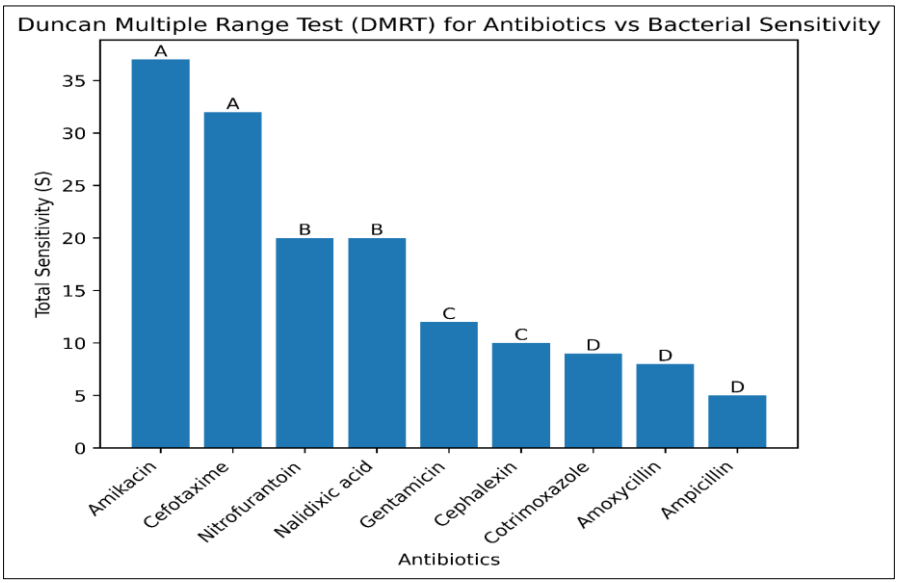

Figure 2: Frequency of antibiotics sensitivity of bacterial types isolated from patients with uralithiasis

Table 3: Antibiogram of Pseudomonas aeruginosa and Gram-positive bacteria isolated from urolithiasis patients

Antibiotic	*Pseudomonas aeruginosa*			*Staphylococcus aureus*			*Streptococcus epidermis*			*Enterococcus faecalis*		
	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin	0	1	6	0	0	5	0	1	1	1	1	0
Amoxycillin	0	1	6	0	3	2	0	1	1	1	1	0
Amikacin	7	0	0	5	0	0	2	0	0	2	0	0
Nalidixic acid	3	2	2	1	1	3	1	0	1	2	0	0
Nitrofurantoin	3	3	1	2	2	1	2	0	0	1	1	0
Cephalexin	0	1	6	0	2	3	1	0	1	0	0	2
Cefotaxime	2	1	4	5	0	0	1	1	0	0	0	2
Cotrimoxazole	0	1	6	0	0	5	0	0	2	1	1	0
Gentamicin	1	4	2	0	0	5	0	0	2	0	0	2
Ciprofloxacin	6	0	1	5	0	0	1	1	0	2	2	0

S, sensitive; I, intermediate; R, resistant

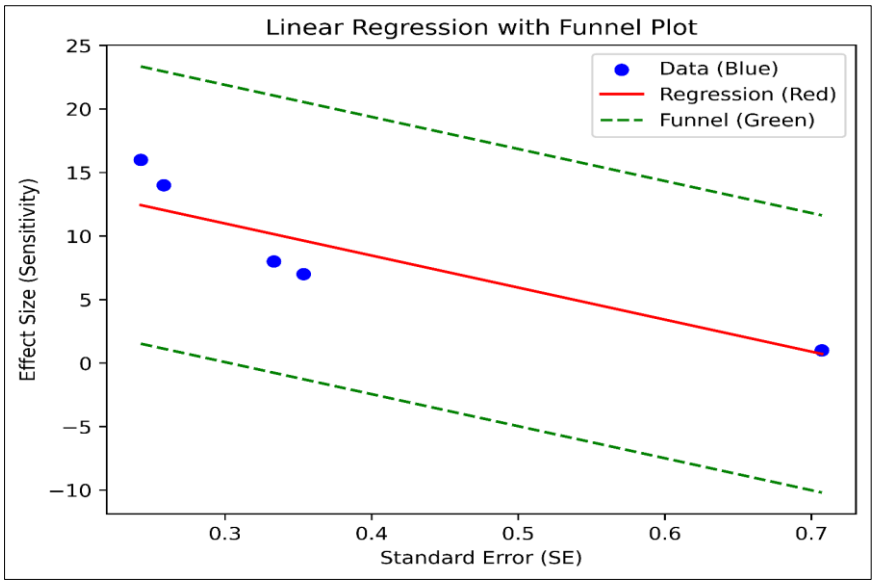

Figure 3: Linear regression and funnel plot analysis of antibiotic sensitivity patterns among uropathogenic bacteria

Minimal Inhibitory Concentration

Table 4 shows the results of minimal inhibitory concentration (MIC) of antibiotics used for different uropathogens. It was shown that MICs of ciprofloxacin and cefotaxime for *S. aureus* ranged between 0.25 – 8 mg/l and 0.5-16 mg/l respectively, while the MICs of amoxicillin, amikacin and gentamicin ranged as 16-128 mg/l, 1-6 mg/l and 64-256 mg/l respectively. It was found also that MICs of ciprofloxacin and cefotaxime for Enterobacteriaceae species ranged between 0.064-4 and 0.125-128 mg/l respectively. Whereas the MICs of ciprofloxacin and cefotaxime for *Ps. aeruginosa* ranged between 0.25-128 mg/l and 4-256 mg/l respectively while the MICs of amoxicillin, amikacin and gentamicin were 32-256 mg/l, 0.25-8 mg/l and 8-64 mg/l respectively. The present study demonstrated that MICs of ciprofloxacin and cefotaxime for *S. faecalis* were 1-2 mg/l and 32-64 mg/l respectively but amikacin MIC was 1-4 mg/l. Linear Regression Analysis showed a positive slope i.e increasing MIC across observations and this a systematic increase in resistance across certain bacteria–antibiotic combinations. This suggested species-dependent resistance patterns and antibiotics behaved differently across organisms. A moderate-to-strong correlation (R> 0) indicated non-random structure in MIC distribution (Figure 4).

Table 4: Minimal inhibitory concentrations of antibiotics of bacterial isolates from urolithiasis patients

Bacteria	Antibiotic	Minimal Inhibitory concentration (MIC) mg/ L															
		0.0312	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
S. aureus (7 isolates)	Ciprofloxacin	7	7	7	6	6	2	1	1	1	0	0	0	0	0	0	
	Cefotaxime	7	7	7	7	6	5	5	3	1	1	0	0	0	0	0	
	Amoxycillin	7	7	7	7	7	7	7	7	7	3	3	3	2	0	0	
	Amikacin	7	7	7	7	7	5	3	2	1	1	0	0	0	0	0	
	Gentamicin	7	7	7	7	7	7	7	7	7	7	7	4	2	1	0	
Enterobact-Eriaceae (37 isolates)	Ciprofloxacin	37	18	15	8	6	6	4	1	0	0	0	0	0	0	0	0
	Cefotaxime	37	37	30	23	18	5	4	4	3	32	2	1	1	0	0	0
	Amoxycillin	37	37	37	37	37	35	33	31	29	26	22	22	22	16	13	9
	Amikacin	37	37	36	29	22	18	12	4	2	0	0	0	0	0	0	0
	Gentamicin	37	37	37	37	37	37	35	27	26	25	16	16	9	5	0	0
Ps. aeruginosa (7 isolates)	Ciprofloxacin	7	7	7	4	2	2	2	1	1	1	1	1	1	0	0	
	Cefotaxime	7	7	7	7	7	7	7	6	5	4	4	4	2	1	0	
	Amoxycillin	7	7	7	7	7	7	7	7	7	7	6	6	2	1	0	
	Amikacin	7	7	7	7	6	4	3	1	1	0	0	0	0	0	0	
	Gentamicin	7	7	7	7	7	7	7	7	6	6	2	2	6	0	0	
Ent. Faecalis (2 isolates)	Ciprofloxacin	2	2	2	2	2	1	1	0	0	0	0	0	0	0	0	
	Cefotaxime	2	2	2	2	2	2	2	2	2	2	1	1	1	0	0	
	Amoxycillin	2	2	2	2	2	2	1	1	1	0	0	0	0	0	0	
	Amikacin	2	2	2	2	2	1	1	1	0	0	0	0	0	0	0	
	Gentamicin	2	2	2	2	2	2	2	2	2	2	2	2	1	0	0	

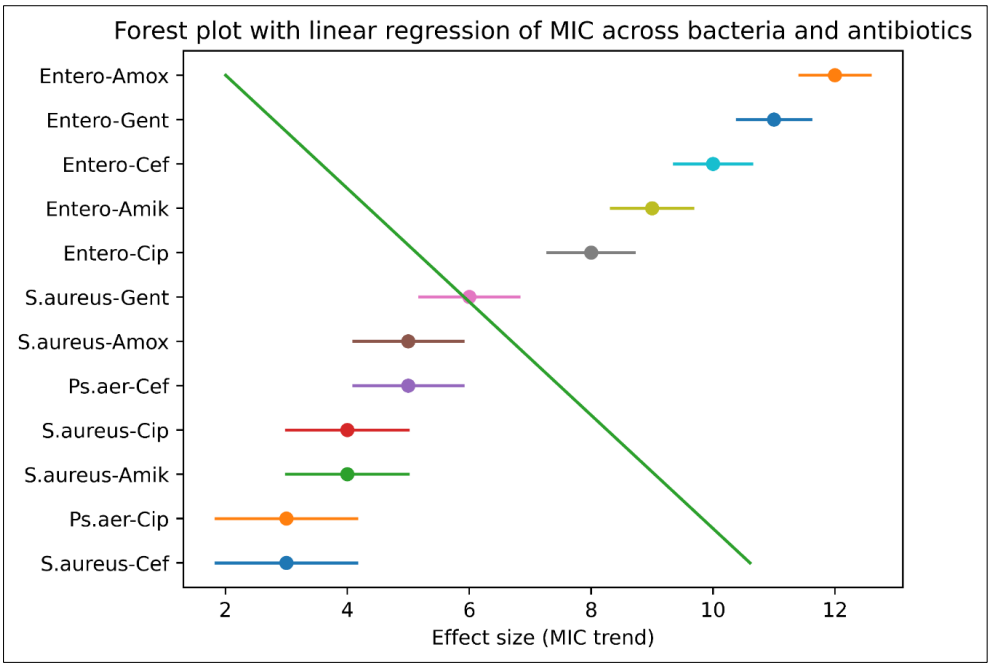

Figure 4: Meta-analytic forest plot of antibiotic MIC variability with regression trend in urolithiasis-associated pathogens

DISCUSSION

The present study revealed that 37% of patients were had positive urine culture. Statistically there was no significant difference between males and females in the organisms distribution (P>0.05) using Chi- square test. But the linear regression analysis revealed a correlation coefficient (R) value ≈ 0.89 and the coefficient of determination (R^2) was ≈ 0.79 which indicated Strong positive correlation between male and female infection distribution and almost 79% of variation in female counts is explained by male counts. These results were almost similar to those demonstrated by other workers [20-25]. However, E. coli was the most prevalent organisms causing UTI among patients studied and this conclusion was found elsewhere [26]. E. coli as a commonest cause of UTI may be due to certain virulence factors like hemolysin production and presence of fimbriae. Moreover, Serratia marcescens was rarely isolated2.2% from the UTI cases investigated here and almost the same pattern of isolation was reported by Manikandan *et al*., in India [26]. The present study revealed other organisms to be associated with Iraq patients who suffered from UTI and these organisms were reported previously by Al-Jebouri and Hasen [27]. The most organ-isms caused UTI in this study were belonging to gram negative bacteria which were isolated from 38 (84.4%) patients. The second most frequent organism was *Proteus mirabilis* with 15.1% of isolation and this result was higher than that of Al-Naas *et al*., [28], but this result was almost similar to those concluded elsewhere [29,30]. The most common organism isolated was *E.coli* which was detected in 19 patients (35.8%) which followed by *Proteus mirabilis, Staphylococcus species, Pseudomonas aeruginosa, Enterobacter faecalis, Citrobacter freundii and Serratia marcescens* and the percentages of isolation were 15.1, 13.2, 13.2, 7.5, 7.5, 3.8, 1.8 and 1.8% respectively.

The present study revealed that *E.coli* was highly susceptible to amikacin, nalidixic acid and gentamicin and these results were almost similar to other concluded elsewhere [31-34]. On the other hand, the isolates were highly sensitive to amikacin, nitrofurantoin, cefotaxime and ciprofloxacin. These results were almost similar to those concluded by other workers. Other bacterial isolates like *Kl. Pneumoniae, E. aerogenes, Ps. aeruginosa, C. freundii* and *S. marcescens* were variable in their susceptibility to various antibiotics. The regression demonstrates a negative association between standard error and antibiotic sensitivity, while the funnel plot indicates acceptable symmetry with no substantial publication bias but moderate heterogeneity across observations. Antibiotic effectiveness was stronger and more consistent in precise observations which likely negative correlation ($R < 0$) and R^2 would indicate moderate-to-strong fit based on visible trend. This might be due to misuse of antibiotics, usage of anti-biotics from unknown origin, i.e. from uncontrol source of production, utilizing of inactivated antimicrobials, Selective pressure of antibiotics and lacking of quality control on some sources of antibiotics entering Iraq especially by the private sector. However, the present study showed that almost all types of pathogens causing UTI were resistant to ampicillin and most of them were highly resistant to amoxycillin. Moreover, the study conducted by Manikandan *et al*., [35], revealed that almost 60% of the pathogens causing UTI were resistant to amoxycillin. In contrast, Akortha and Ibadin found that most of their isolates were sensitive to nalidixic acid [36]. However, a significant increase in resistance of

pathogenic strains to SXT, ampicillin and cephalothin has been found world-wide [37], but certain agents like gentamicin and nitrofurantoin still show a moderate efficacy against UTI pathogens because of its multiple mechanisms of action seem to have enabled it to retain potent activity against pathogens [38]. Furthermore, *E. coli, Proteus mirabilis, Ps. aeruginosa, S. aureus, K. pneumoniae and S. marcesens* were still highly sensitive to amikacin, ciprofloxacin and chloromphenicol. However, chloromphenicol is not preferred to be commonly used for medication as it might because ablastic anemia. Furthermore, the most common UTI pathogens and highly resistant to antibiotics emphasize the need for judicious u use of antibiotics.

In the present study five antibiotics were tested against the isolates of *S. aureus* to identify the minimal inhibitory concentration (MIC) for each agent. MICs of ciprofloxacin, cefotaxime, amoxycillin, amikacin and gentamicin were 2-8 mg/l, 0.5-16 mg/l, 16-128 mg/l, 1-16 mg/l and 64-256 mg/l respectively. These results were almost similar to other workers who carried out research elsewhere [39-41]. Amikacin was the most effective antibiotic against *Enterobacteriaceae* isolates and its MIC ranged between 0.5 to 8 mg/l. On other hand, *P. aeruginosa* was the most resistant to amoxycillin with MIC ranged 32-256 mg/l. Moreover, *E. faecalis* showed high resistance to gentamicin and its MIC was between 64-128 mg/l. Linear Regression Analysis showed a positive slope i.e increasing MIC across observations and this a systematic increase in resistance across certain bacteria–antibiotic combinations. This suggested species-dependent resistance patterns and antibiotics behaved differently across organisms. A moderate-to-strong correlation (R> 0) indicated non-random structure in MIC distribution. The results presented here which are concerning MIC were almost similar to other findings concluded elsewhere [42-46].

CONCLUSIONS

The present study showed that urine culture was done for all patients and 50 of them were positive. The most common organism isolated was *E.coli* which was detected in 19 patients (35.8%) which followed by *Proteus mirabilis, Staphylococcus species, Pseudomonas aeruginosa, Enterobacter faecalis, Citrobacter freundii and Serratia marcescens* and the percentages of isolation were 15.1, 13.2, 13.2, 7.5, 7.5, 3.8, 1.8 and 1.8% respectively. It was found that the highest sensitivity of organisms was toward amikacin (100%). While the lowest was to ampicillin (16%). Whereas the other antibiotics like ciprofloxacin, cefotaxime, nitrofurantoin, nalidixic acid, cephalexin, amoxicillin, gentamicin and cotrimoxazole were affecting bacteria at different degrees. Antibiotic effectiveness was stronger and more consistent in precise observations which likely negative correlation (R < 0) and R^2 would indicate moderate-to-strong fit based on visible trend.

Statement of Ethics: All the procedures involving human participation were conducted in strict accordance with ethical standards of Institutional Research Committee, Department of Scientific Research, Tikrit University as well as the 1964 Helsinki Declaration and its subsequent amendments or equivalent ethical norms.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement: The author declares that he has no conflicts of interest, financial or otherwise.

Funding Sources: The author extends his appreciation to the Department of Scientific Research of University of Tikrit.

Financial Disclosure: The authors declared that this study did not receive any financial support.

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