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Investigation of the Most Common Specimens in the Pediatric Hospital in Kirkuk and the Epidemiology of Predominant Bacteria Using Multiple Antibiotic Resistance Indices

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Abstract

Background: Urinary tract infections (UTIs) are among the most prevalent infectious diseases in children and are a leading cause of antibiotic use and pediatric hospitalization.

Objectives: This study aimed to investigate the most common specimens and determine the frequency of Multiple Antibiotic Resistance Indexes (MARI) for predominant bacteria in the pediatric hospital of Kirkuk.

Methods: A total of 299 different samples were collected from the pediatric hospital in Kirkuk province between May 1st and October 1st. The samples were cultured on blood agar, mannitol agar, and MacConkey agar and incubated for 24 hours at 37°C. Identification was based on morphological and microscopic examination, as well as the API kit.

Results: The results showed that most of the samples were urine, with 221 samples collected. Of these, 66 (28.50%) showed positive growth, and 155 (71.49%) showed negative growth. Blood samples were the second most common, with 51 samples collected, of which 4 (9.61%) were positive, and 47 (90.38%) were negative. Stool samples amounted to 13, with 2 (15.38%) showing positive growth, and 11 (84.61%) showing negative growth. Cerebrospinal fluid (CSF), throat swab, and vaginal swab samples amounted to 11, 2, and 1, respectively, with no bacterial growth observed in them. The majority of isolates were from urine, with *Escherichia coli* being the most common species (28 isolates, 42.42%). Other species isolated included *Klebsiella pneumoniae* (13 isolates, 19.69%), *Staphylococcus* spp. (12 isolates, 19.05%), *Enterobacter* spp. (7 isolates, 10.61%), and *Pseudomonas* spp. (4 isolates, 6.06%). *Proteus* spp. and *Streptococcus* spp. each accounted for 1 isolate (1.25%). The bacteria isolated from blood were *Acinetobacter* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Klebsiella* spp., each representing 1 isolate (1.25%) of the total. Two (100%) *E. coli* isolates were obtained from stool samples. *E. coli* isolates from urine were resistant to ampicillin (Am), tetracycline (Te), amoxicillin/clavulanic acid (AMC), and doxycycline (Do). *Klebsiella* spp. were resistant to Am, *Staphylococcus* spp. to erythromycin (E) and azithromycin (AZM), *Enterobacter* spp. to amikacin (AK) and AMC, and *Pseudomonas aeruginosa* to gentamicin (CN), all exhibiting 100% resistance. The MARI was greater than 0.2 in 27 isolates of *E. coli* (96.42%), 13 isolates of *Klebsiella* spp. (100%), 11 isolates of *Staphylococcus* spp. (91.66%), 7 isolates of *Enterobacter* spp. (100%), and 4 isolates of *P. aeruginosa* (100%).

Conclusions: The current study concludes that urinary tract infection is the most common pathological condition among the young age group. Urine is one of the most frequently examined samples, with *E. coli* being the most predominant pathogen. The study also highlights the prevalence of antibiotic-resistant bacteria, as evidenced by the elevated MARI in isolates of *E. coli*, *K. pneumoniae*, *Enterobacter* spp., and *Pseudomonas* spp.

Keywords: Predominant Bacteria, Multiple Antibiotic Resistance Index, Clinical Specimens

1. Background

Urinary tract infections (UTIs) are among the most prevalent infectious diseases in children and are a leading cause of antibiotic use and pediatric hospitalization (1, 2). Urinary tract infections also represent a significant challenge for pediatricians, healthcare providers, and the management of children in emergency departments (1, 3, 4). Bacteria are the primary cause of UTIs, with fungi and some viruses

being rare contributors. Uropathogenic *Escherichia coli* is the most frequent cause of UTIs, followed by *Staphylococcus* species, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterococcus faecalis* (4).

Escherichia coli strains that cause UTIs have defense mechanisms, including a glycosylated polysaccharide capsule that protects against phagocytosis and the immune complement system (2). *Escherichia coli* is one of the most significant causative agents of UTIs, cholecystitis, bacteremia, meningitis, and traveler's diarrhea (5). More than 40% of the bacteria responsible for UTIs are resistant to some of the antibiotics used, leading to patient relapse, treatment failure, progression to more severe diseases (such as bacteremia), which requires hospitalization, and, ultimately, death (3).

Gram-negative bacteremia remains a leading cause of mortality and morbidity, with its incidence rising globally (6). The presence of resistant genes on extrachromosomal DNA, primarily found in the *Enterobacter*iaceae family, is mainly responsible for the occurrence of antibiotic resistance in gram-negative bacteria that cause infections in hospitals and the community (7).

The second most common cause of death among young children is diarrhea, often resulting from pathogens transmitted from the mouth to the feces (8). *Escherichia coli* (enteropathogenic, enteroinvasive, and enteroaggregative strains) is one of the causes of diarrhea in children under five years of age (9).

2. Objectives

Given the increasing number of pediatric patients admitted to the children's hospital in Kirkuk, especially those with UTIs who fail to respond to treatment, the study aimed to identify the most frequent specimens and investigate the epidemiology of predominant bacteria using the Multiple Antibiotic Resistance Index (MARI).

3. Methods

3.1. Sample Collection

A total of 299 samples were collected from males and females aged less than one to 15 years at the pediatric hospital in Kirkuk Governorate, during the period from May 1, 2022, to October 1, 2023.

3.2. Culturing Samples

The samples were cultured and examined under a microscope using the API20E and API Staph systems after being incubated for 24 hours at 37°C on blood agar, mannitol salt agar, and MacConkey agar.

3.3. Bacterial Sensitivity Study

Bacterial sensitivity was determined using the Kirby-Bauer disk diffusion method, where the inhibition zone was measured using a ruler (in millimeters).

3.4. Multiple Antibiotic Resistance Index

Multiple Antibiotic Resistance Index was calculated and interpreted as described by (10), applying the formula a/b, where "b" represents the total number of antibiotics tested and "a" represents the number of antibiotics to which a single isolate is resistant (11).

3.5. Ethical Approval

Ethical approval was obtained from the Scientific Research Ethics Committee of the Ministry of Health - Kirkuk Health Department under Order 1263, dated January 2022.

4. Results

A total of 299 samples were collected from suspected patients, as diagnosed by the examining physician, at the pediatric hospital in Kirkuk Governorate, between May 1, 2022, and October 1, 2023. As shown in Table 1, most of the samples were urine, with a total of 221 samples, of which 66 (28.50%) showed positive growth and 155 (71.49%) showed negative growth. Blood samples followed, with 51 samples, 4 (9.61%) of which were positive growth and 47 (90.38%) were negative growth. Stool samples accounted for 13 samples, 2 (15.38%) of which were positive growth and 11 (84.61%) were negative growth. The numbers of Cerebrospinal fluid (CSF), throat swab, and vaginal swab samples were 1, 2, and 11, respectively, with no bacterial growth observed in any of them (Table 2).

4.1. Resistance of the Most Isolated Bacteria from Urine to Antibiotics

Figure 1 shows the antibiotic resistance profile of *E. coli* bacteria isolated from urine. The bacteria exhibited 100% resistance to the antibiotics ampicillin (Am),

Table 1. Percentage of Bacterial Growth According to Sources of Isolation [a]

Isolation Source	Total Number	Bacterial Growth	
		Positive Growth	Negative Growth
Urine	221	66 (28.50)	155 (71.49)
Blood	51	4 (9.61)	47 (90.38)
Stool	13	2 (15.38)	11 (84.61)
CSF	11	-	11 (100)
Throat swab	2	-	2 (100)
Vaginal swab	1	-	1 (100)

Abbreviation: CSF, cerebrospinal fluid.

[a] Values are expressed as No. (%).

Table 2. Percentage of Isolates According to Sources of Isolation

Source of Isolation (No.) and Bacterial Species	No. (%)
Urine (66)	
Escherichia coli	28 (42.42)
Klebsiella pneumoniae	13 (19.696)
Staphylococcus spp.	12 (19.047)
Enterobacter spp.	7 (10.606)
Pseudomonas spp.	4 (6.06)
Proteus spp.	1 (1.515)
Streptococcus spp.	1 (1.515)
No identification	6 (9.09)
Blood (4)	
Acinitobacter spp.	1 (25)
Enterobacter spp.	1 (25)
Pseudomonas spp.	1 (25)
Klebsiella spp.	1 (25)
Stool (2)	
E. coli	2 (100)
CSF (0)	-
Throat swab (0)	-
Vaginal swab (0)	-

Abbreviation: CSF, cerebrospinal fluid.

tetracycline (Te), amoxicillin/clavulanic acid (AMC), and doxycycline (Do). The resistance percentages for the following antibiotics were as follows: Azithromycin (AZM) (76.47%), ciprofloxacin (CIP) (28.57%), trimethoprim-sulfamethoxazole (SXT) (78.57%), cephalexin (CN) (52.63%), amikacin (AK) (66.66%), cefixime (CFM) (69.23%), levofloxacin (LEV) (25%), ceftazidime (CAZ) (75%), aztreonam (ATM) (56%), cefepime (FEP) (81.48%), nalidixic acid (NA) (58.33%), nitrofurantoin (F) (26.66%), and imipenem (IPM) (7.14%).

The bacteria showed resistance to AZM, CIP, SXT, AM, AMC, CAZ, ATM, FEP, DO, and F, with resistance percentages of 91.66%, 14.28%, 5%, 100%, 91.66%, 81.81%, 72.72%, 72.72%, 85.71%, and 55.55%, respectively (Figure 2).

The isolates showed 100% resistance to erythromycin (E) and AZM, and resistance to CIP, CN, DO, vancomycin (VA), and rifampin (RA) at percentages of 37.5%, 70%, 87.5%, 60%, and 14.28%, respectively (Figure 3).

The isolates exhibited 100% resistance to AK and AMC, and resistance to CIP, SXT, CFM, CAZ, ATM, and cefepime (FEP) at percentages of 25%, 16.66%, 60%, 57.14%, 42.85%, and 14.28%, respectively (Figure 4).

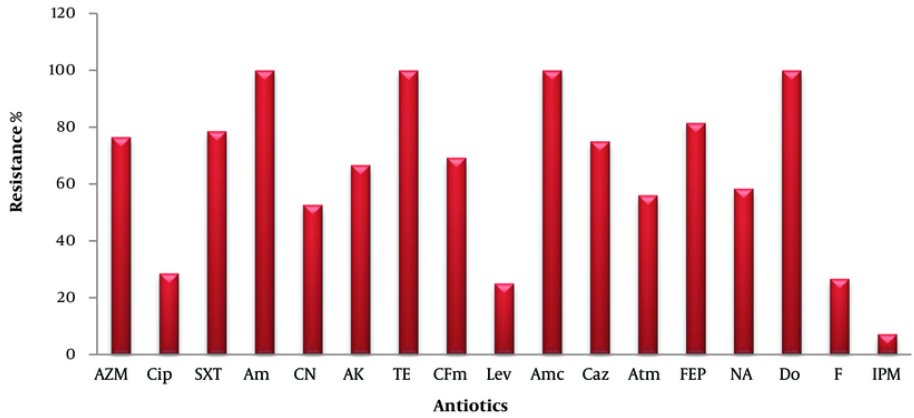

Figure 1. Resistance to antibiotics in *Escherichia coli* isolated from urine. AZM, azithromycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; AM, ampicillin; CN, cephalexin; AK, amikacin; TE, tetracycline; CFM, cefixime; LEV, levofloxacin; AMC, amoxicillin-clavulanate; CAZ, ceftazidime; ATM, aztreonam; FEP, cefepime; NA, nalidixic acid; DO, doxycycline; F, nitrofurantoin; IPM, imipenem.

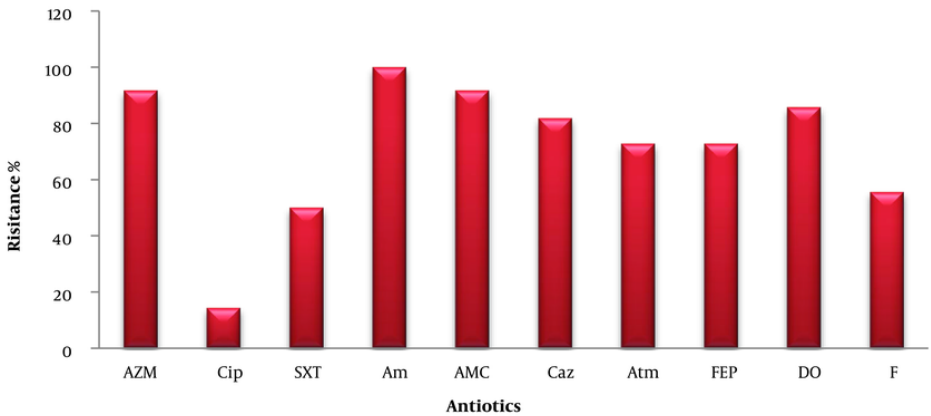

Figure 2. Resistance to antibiotics in *Klebsiella* spp. isolated from urine. AZM, azithromycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; AM, ampicillin; AMC, amoxicillin-clavulanate; CAZ, ceftazidime; ATM, aztreonam; FEP, cefepime; DO, doxycycline; F, nitrofurantoin.

The isolates were resistant to CN by 100%, CIP, CAZ, FEP, and IPM by 75%, and AZM, SXT, AK, LEV, and F by 50% (Figure 5).

4.2. Multiple Antibiotic Resistance Index for Bacterial Isolates from Urine

Table 3 shows the MARI for bacterial isolates from urine. The MARI was greater than 0.2 in 27 isolates of *E. coli* (96.42%), 13 isolates of *Klebsiella* spp. (100%), 11 isolates of *Staphylococcus* spp. (91.66%), 7 isolates of *Enterobacter* spp. (100%), and 4 isolates of *Pseudomonas aeruginosa* (100%).

5. Discussion

Overprescription of antibiotics is common in primary healthcare facilities. To reduce the likelihood of antibiotic resistance developing, health authorities should strictly regulate or outright prohibit the overprescription of antibiotics (12).

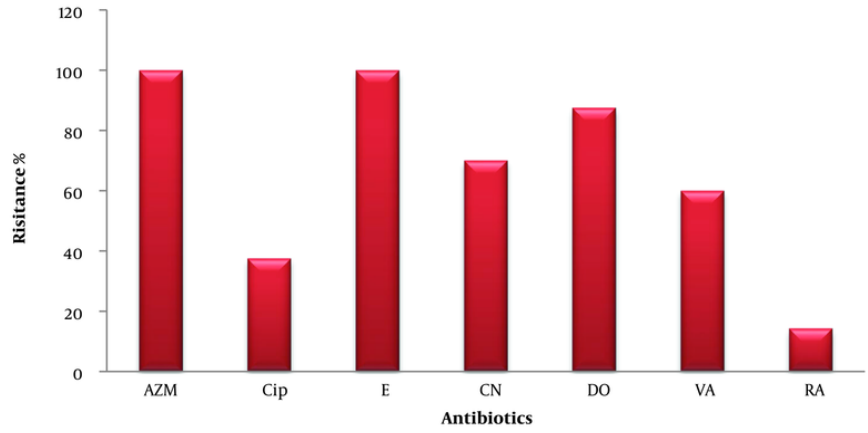

Figure 3. Resistance to antibiotics in *Staphylococcus* spp. isolated from urine. AZM, azithromycin; CIP, ciprofloxacin; E, erythromycin; CN, cephalexin; DO, doxycycline; VA, vancomycin; RA, rifampin.

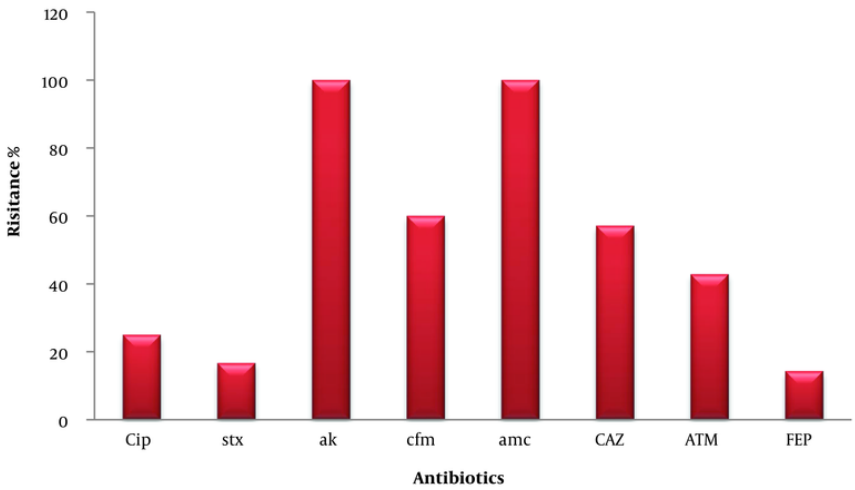

Figure 4. Resistance to antibiotics in *Enterobacter* spp. isolated from urine. CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; AK, amikacin; CFM, cefixime; AMC, amoxicillin-clavulanate; CAZ, ceftazidime; ATM, aztreonam; FEP, cefepime.

It is reported that *E. coli* (64.5%), *Klebsiella* species (11.6%), and *Enterococcus* species (6.1%) were the most common bacteria isolated from urine samples (13). This was supported by (14), which indicated that gram-negative bacteria were the most frequently isolated species in UTIs. A study highlighted the predominance of *E. coli* bacteria in patients suffering from UTIs (15).

The results indicated that bacteria isolated from urine samples exhibited resistance to many antibiotics, classifying them as multidrug-resistant (MDR) bacteria. This was confirmed by (16), who recorded the resistance of gram-negative bacteria to each of the following antibiotics: Gentamicin, Am/sulbactam, CIP, IM, FEP, LEV, ATM, and AK, with resistance rates of 69.56%, 56.52%, 43.47%, 0%, 52.17%, 82.60%, 60.87%, and 39.13%, respectively.

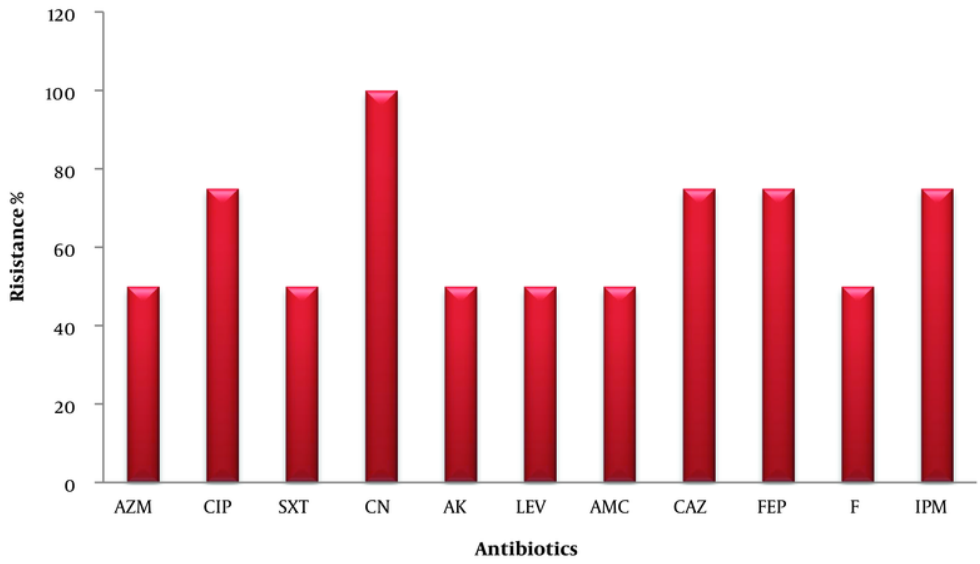

Figure 5. *Enterobacter* species isolated from urine that are resistant to antibiotics. AZM, azithromycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CN, cephalexin; AK, amikacin; LEV, levofloxacin; AMC, amoxicillin-clavulanate; CAZ, ceftazidime; FEP, cefepime; F, nitrofurantoin; IPM, imipenem.

Table 3. Multiple Antibiotic Resistance Index of Bacterial Isolates from Urine [a]

MARI	*Escherichia coli*	*Klebsiella* Species	*Staphylococcus* Species	*Enterobacter* Species	*Pseudomonas aeruginosa*
< 0.2	1 (3.57)	0 (0)	1 (8.33)	0 (0)	0 (0)
0.2 -0.6	18 (64.28)	8 (61.53)	6 (50)	6 (85.71)	0 (0)
0.7 - 0.9	7 (25)	5 (38.46)	3 (25)	0 (0)	4 (100)
1 <	2 (7.14)	0 (0)	2 (16.66)	1 (14.28)	0 (0)

[a] Values are expressed as No. (%).

Similarly, (17) found that *E. coli* and *Klebsiella* species were resistant to a range of antibiotics, including sulfonamides (56%, 41%), fluoroquinolones (69%, 53%), third-generation cephalosporins (69%, 58%), macrolides (70%, 76%), and penicillin (85%, 95%), respectively.

According to the study by (18), a significant proportion of antibiotic resistance was observed in *K. pneumoniae* and *E. coli*. *K. pneumoniae* demonstrated high resistance to streptomycin (88%), E (88%), cloxacillin (96%), while *E. coli* exhibited resistance to all three antibiotics.

The antibiotics IM (95%), STX (69.8%), ATM (60.5%), chloramphenicol (45.3%), and meropenem (27.9%) were more effective against *Pseudomonas* isolates (19).

Regarding the resistance of *Pseudomonas* bacteria, the results of (19) indicated that *Pseudomonas* isolates exhibited greater resistance to IM (95%), STX (69.8%), ATM (60.5%), chloramphenicol (45.3%), and meropenem (27.9%).

In contrast, (20) reported that the antibiotic ceftazidime had the highest resistance rate (63%) against *P. aeruginosa* isolates, with resistance to other antibiotics ranging between 7% and 35%.

Regarding the antibiotic resistance of *Staphylococcus* bacteria, (21) indicated that all *Staphylococcus* isolates were 100% resistant to AZM and E, and 95.56% resistant to cefixime, 50% resistant to Am, and 95% resistant to amoxicillin. Meanwhile, (22) indicated that all *Staphylococcus aureus* isolates were 100% resistant to Am

and penicillin, 97.6% resistant to AK, and 90% resistant to ciprofloxacin and gentamicin.

According to (23), the development of quinolone resistance in *Enterobacter*iaceae is a complex and multifaceted process. The primary resistance mechanisms include one or more genetic mutations at the target site that alter the drug's affinity for binding to target enzymes, overexpression of the AcrAB-TolC MDR efflux pumps, and decreased expression of porins and plasmid-coded resistance proteins, such as the protection protein Qnr.

A study assessed gene sequencing in *E. coli* bacteria and indicated the presence of the genes orf00490, orf00819, orf001916, and orf01200, which regulate the expression of the enzyme fumarate reductase subunit D (frdD) as well as the cell division protein FtsI (penicillin-binding protein 3) (24). Additionally, the outer membrane porin protein OmpD is controlled by the genes orf00490, orf00819, and orf001916.

The occurrence of several mutations in these genes leads to bacterial resistance to many antibiotics, while the gene orf04094 expresses histidine kinase, orf02235 expresses multidrug resistance, and orf03479 expresses valine-glycine repeat G, which is excreted through the type VI secretion system (T6SS), one of the extracellular substances that contribute to antibiotic resistance.

The resistance of *P. aeruginosa* to cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones is primarily due to horizontal gene transfer, involving integrons, plasmids, and transposons (25).

It is noted from the results that the MARI of bacteria isolated from urine was mostly greater than 0.2, indicating the extent of the epidemic of each of the bacteria (*E. coli*, *K. pneumoniae*, *Enterobacter* spp., *Pseudomonas* spp.).

This is supported by (18), which found that *E. coli* had the highest MAR index, reaching 1.00 in 17 isolates that showed resistance to 14 antibiotics. Meanwhile (26), indicated in their study that 44% of *Staphylococcus* spp. isolates and 50% of *E. coli* isolates had MAR indices greater than 0.2.

In a study, the MARI of *Pseudomonas* was 0.85 (27), while (18) showed in their study that the MARI in *Pseudomonas* isolates ranged between 0.0 and 0.8.

As for *Enterococcus* bacteria (28), indicated in their study that the MARI for *Enterococcus* ranged between 0.08 and 0.83, while (20) showed that the MARI for *P. aeruginosa* ranged between 0.23 and 0.38.

Multiple Antibiotic Resistance Index is a useful tool for assessing the vulnerability of humans to the risk of resistant bacteria, as well as the extent of environmental danger due to antibiotic-resistant bacteria (10). This is especially important because antibiotic-resistant environmental bacteria can evolve into pathogens through genetic association (29) and phenotypic diversity between environmental and clinical bacteria (30). Additionally (31), indicated that a rise in MARI to 0.82, 0.73, and 0.64 signifies significant contamination with Vibrio parahaemolyticus bacteria. The MARI for *Pseudomonas* putida isolated from fish was 0.76, indicating high antibiotic use in fish farms (32). The rise of MARI above 0.2 signifies the extensive use of antibiotics in the aquatic environment. This variation in the MARI of isolated bacteria is attributed to mobile genetic elements, particularly Class 1 integrons, which provide a significant opportunity for the spread of antibiotic resistance among fish.

While (33) indicated that *Enterobacter* with an MARI above 0.3 was observed to possess outer membrane proteins as well as other virulence factors, such as Type 1 fimbriae, biofilm production, and serum resistance.

This is supported by (34), which noted that there is a difference in MARI between bacterial genera. *Pseudomonas aeruginosa* had a higher MARI than *Klebsiella* spp. and *Proteus* spp., which in turn had a higher MARI than *Enterobacter* spp. The study also pointed out that 25% of the isolates posed a significant risk to humans and animals, with the majority being *P. aeruginosa*, which poses a higher threat as a potential pathogen compared to *Enterobacter* spp., based on MARI and virulence factors.

It was indicated that bacterial isolates with an MARI greater than 0.3 and a virulence factor above 0.5 pose a significant threat, while isolates with an MARI less than 0.3 and a virulence factor less than 0.5 represent a medium risk (34). Isolates with low risk have both an MARI less than or equal to 0.3 and a virulence factor greater than or equal to 0.5. Non-risk isolates are those with an MARI greater than 0.3 and a virulence factor greater than 0.5.

It is noted from the results that the values of MARI vary between genera as well as between the same bacterial species. This may be due to many factors, as indicated by (35-37), who found that differences in MARI

values depend on the source of the sample, geographical location, and the method of testing.

5.1. Conclusions

According to the current study, UTIs are the most common pathological cases among young people, and urine is one of the most frequently examined samples. *Escherichia coli* is the most common bacterium, and antibiotic-resistant bacteria spread through the increasing MARI of isolated strains, including *Enterobacter* species, *Pseudomonas* species, *E. coli*, and *K. pneumoniae*.

Footnotes

Authors' Contribution: A. H. Sh. Al-W.: Visualization, reviewing, editing, conceptualization, and methodology; L. A. Z: Writing, investigation, software, validation, and original draft preparation.

Conflict of Interests Statement: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: Ethical approval was obtained from the Scientific Research Ethics Committee of the Ministry of Health, Kirkuk Health Department, by Order 1263 in January 2022.

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