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Bacterial Co-Infections and Antibiotic Resistance in Urinary Tract Infection of Covid-19 Patients in Erbil City

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ABSTRACT

The COVID-19 pandemic has raised concerns over secondary infections because it has limited treatment options and empiric antimicrobial treatment poses serious risks of aggravating antimicrobial resistance. Many studies have shown that COVID-19 patients are predisposed to developing secondary infections. The prevalence of urinary tract infections (UTI) was evaluated in 248 patients admitted to different hospitals in Erbil city, Iraq. Results showed 138 (55.65%) patients were positive for bacterial growth, of which 72 isolates of Gram-negative and 66 isolates of Gram-positive bacteria were isolated and identified from the urine of COVID-19 patients depending on the 16S rRNA gene using PCR. The predominant obtained bacteria were Staphylococci species, of which isolates belong to 6 species, and the predominant Gram-negative species was *Pseudomonas* spp. which belongs to 4 species. Also, their susceptibility to 15 antibiotics was tested and it was found that most of the Gram-negative isolates were highly resistant to ampicillin 66(91.66%), amoxicillin 60(83.33%), cephalexin 52(72.22%) and cefixime 50(69.44%). While the most effective antibiotic was imipenem, and with percentage of 26(36.11%), it also showed variable sensitivity to other antibiotics. For Gram-positive bacteria, the highest resistance was against cefixime 40(60.60%), ampicillin 28(42.42%), and 18(27.27%) for both amoxicillin and ciprofloxacin, while the most effective antibiotic was gentamicin, which showed effectiveness against all the Gram-positive isolates. Another part of the study concerned with detection of the existence of ESBLs genes responsible for antibiotic resistance. The results revealed that all the isolates possessed the 16S rRNA gene, whereas *bla*TEM and *bla*CTX-M were found to be the most possessed genes in all the isolates, with a percentage of (88.33%).

1. Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections. UTIs are currently one of the most common diseases that can affect any individual, from newborns to adults. UTIs affects nearly 150 million people worldwide each year. UTIs affect men and women of all ages and vary dramatically in their presentation and impact.

They are also considered the second most common reason after respiratory diseases for consulting and prescribing antibiotics (O'Brien *et al.*, 2016). UTIs are caused by the abnormal growth of pathogens in any part of the urinary tract, such as the kidney, urethra, bladder, and prostate (Prakash and Saxena, 2013). UTIs carry an acute risk of morbidity and mortality due to uro-sepsis, renal abscess formation, and acute kidney injury. By the end of 2019, a new disease began to spread globally caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which began in Wuhan, Hubei Province, China (Salata *et al.*, 2020, Gong *et al.*, 2020). Coronavirus is a β -enveloped non-segmented positive-sense RNA virus that belongs to the family Coronaviridae and the order Nidovirales, which is broadly distributed in humans (Richman *et al.*, 2020) Although the prevalence and incidence of bacterial co-infection in patients infected with SARS-CoV-2 is not well understood, in addition to the ineffectiveness of antibiotics in the treatment of COVID-19, they are still prescribed in patients with suspected or documented COVID-19 for a variety of reasons, such as difficulty in ruling out bacterial co-infection, and the possibility of bacterial secondary infection during the course of the treatment (Alhazzani *et al.*, 2020, Huttner *et al.*, 2020, Cox *et al.*, 2020).

However, this assumption raises concerns about antibiotic overuse and subsequent harm associated with bacterial resistance. Therefore, the aims of this study are to identify the causative bacteria for urinary tract infection in patients of COVID-19 by using PCR for screening for 16S rRNA gene from the isolated bacteria, and also to determine the incidence of bacterial co-infection, urinary tract infection, and the bacterial susceptibility against the common antibiotics

used during the illness course and their effect on the rise of bacterial resistance against antibiotics.

2. Materials and methods

2.1 Study design

Sampling for this study was done at Lalav, Rizgari Teaching, Emarate, and West Erbil Emergency hospitals in Erbil city. The study was performed from (September, 2021 to January, 2022), where 122 females and 126 males of COVID-19 patients were included Figure 1. Using quantitative RT-PCR (qRT-PCR) on throat swab samples, all patients were found to be positive for SARS-CoV-2. The clinical laboratory findings for all COVID-19 patients were reviewed. All data was collected and recorded on a customized data collection form.

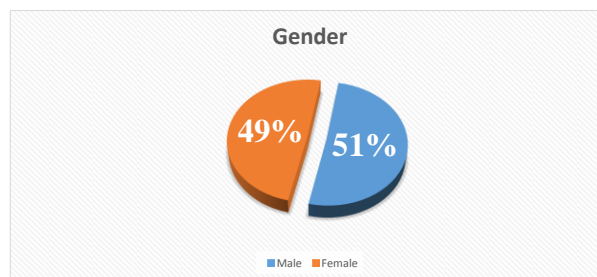


Figure 1. percentage of gender of collected samples.

2.2 Sample collection

A total of 248 urine samples were collected "mid-stream" from COVID-19 patients. All the samples were collected in sterile urine cups. For microbiological tests, all specimens were transferred to the laboratory. All samples were first centrifuged at 5000 RPM for 5 minutes (Chen *et al.*, 2021), then the supernatant was discarded and precipitants were inoculated on blood and MacConkey agar.

2.3 Identification of bacteria in the specimens

All the bacteria were identified depending on their morphological characteristics, biochemical tests, and confirmed by the molecular method (Atlas *et al.*, 1995, Shoaib *et al.*, 2020). The morphological characteristics of Gram-negative and Gram-positive bacterial colonies were studied on blood agar, MacConkey and nutrient agar, after performing the Gram-stain technique (Hall, 2013).

2.4 Antibiotic susceptibility test

Using the Kirby Bauer method as described, the antibiotic susceptibility of all the isolated bacteria was tested against 15 antibiotics (CLSI,2017).

2.5 Molecular study

2.5.2 Isolation of DNA from bacterial cell

The method that performed for isolation of DNA from bacterial cells was done by using Presto™ Mini gDNA (Taiwan) bacterial kit.

2.5.2 Primer

Conventional PCR (Eppendorf/ Germany) was used to detect the 16S rRNA gene in all isolated bacteria. MacroGen (Germany) supplied the primers listed in table1. MacroGen synthesized the primer as a lyophilized powder; consequently, the concentration is expressed in pmol. Primers were prepared as a working stock before being added to the PCR reaction volume. (Table 1).

Table (1) Primers sequences and their product size in present study.

Primers		Primer sequences	Product size	References
27F	Forward	AGAGTTTGATCMTGGCTCAG	1400	(Heuer et al., 1997)
1492R	Reverse	TACGGYTACCTTGTTACGACTT		
laTEM	Forward	TCGCCGCATACACTATTCTCAGAATGA	445	(Boyd et al. 2004)
	Reverse	ACGCTCACCGGCTCCAGATTTAT		
blaSHV	Forward	ATG CGT TATATT CGC CTG TG	747	(Paterson et al., 2003)
	Reverse	TGCTTTGTTATTCGGGCCAA		
laCTX-M	Forward	ATGTGCAGYACCAGTAARGTKATGGC	593	(Boyd et al. 2004)
	Reverse	TGGGTRAARTARGTSACCAGAAAYCAGCG G		

2.5.3 DNA amplification and Description of PCR master kit

The Master Mix kit contains Taq DNA Polymerase, dNTPs, MgCl₂, and buffer in a pre-mixed, freeze-dried pellet form. After adding (1.3 µl) of each forward and reverse primer and (2.5 µl) of DNA template to the AccuPower PCR tube, (20 µl) of distilled water was added to the AccuPower PCR tube. Vortexing subsequently dissolved the lyophilized blue pellet. PCR was performed on samples, and the samples were run through the thermal cycler for 35 cycles, as shown in (Table 2).

Table (2) PCR protocol and thermocycling conditions (Heuer et al., 1997).

Gene Name	Initial denaturation	Cycles	Denaturation	Annealing	Elongation	Final Elongation
16S rRNA	96 °C/4min	35	94°C/30sec	57°C/30sec	72°C/1min	72°C/10min

2.5.4 Detection of DNA content by agarose gel electrophoresis

The gel electrophoresis technique was used to separate the DNA molecules according to their size in a 1.5% agarose gel as described

by (Zhang et al., 2018, Alizadeh et al., 2021), after the determining of concentration and purity of DNA using Nanodrop spectrophotometer (Berim and Ruckenstein, 2016).

2.5.5 Sequencing of 16SrRNA PCR products

The sequencing of PCR products was performed in Korea. The purified PCR product, as described by (Leonard et al., 1998), was sent in a 1.5 ml Eppendorf tube following its purification from agarose, along with 12 µl of forward primer in a separate tube. The BigDye® Terminator V3.1 Cycle Sequencing Kit (Applied Biosystem) was used for the sequencing reactions, a High-performance 4-capillary 3130 Genetic Analyzer pop7 polymer was used for separation, and the Geneious programme (version 9.0.5) was used to analyse the sequenced genes.

2.5.6 Detection of ESBL (extended spectrum beta-lactamase) phenotype and genotype

All the specimens were tested for their ability to

produce ESBL by using the Double Disc Synergy Test (DDST) using a disc of Amoxicillin-Clavulanate (AMC 20/10 µg) along with Ceftriaxone (CRO 10 µg) and Aztreonam (ATM 30 µg), where the bacteria were swabbed on a mueller-hinton agar plate as proposed by (CLSI). The Amoxicillin-Clavulanate disc was placed in the centre of the plate and 20mm away from Ceftriaxone and Aztreonam. An extension of the growth inhibition zone peripheral disc towards the centrally placed Amoxicillin-Clavulanate disc indicates ESBL production. Enhancement of the zone of inhibition against amoxicillin-clavulanic acid by either of the two antimicrobial discs indicated the presence of extended-spectrum beta-lactamases (Elhariri *et al.*, 2017). For the ESBL genotype detection, the isolates were examined for the existence of *bla*SHV, *bla*CTX-M and *bla*TEM genes using multiplex PCR, as described by Pishtiwan and Khadija (2019), with slight modification. All PCR reactions were performed by using 3 µl DNA template (density of 10 ng/µl), 2 µl of each primer (Table 1), 25µl of the Master Mix consisting of 3 mM MgCl₂, 0.2% Tween® 20, 20 mM Tris-HCl pH 8.5, (NH₄)₂SO₄, 0.4 mM of each dNTP, and 0.2 units/µl Ampliqon Taq DNA polymerase, and 11 µl water nuclease free) in a final volume of 50 µl. Following are the conditions for polymerase chain reaction amplification: Denaturation for 10 minutes at 95°C, 30 denaturation cycles for 30 seconds at 94°C, annealing for 30 seconds at 60°C, extension for 2 minutes at 72°C, and a final extension for 10 minutes at 72°C. Size separation PCR amplicons were used to detect respective genes via agarose gel electrophoresis (Pishtiwan and Khadija, 2019).

3. Results

3.1 Frequency of Gram-positive and Gram-negative bacteria in COVID-19 patients

One hundred thirty-eight (55.65%) isolates of Gram-positive and Gram-negative bacteria were isolated from 248 COVID-19 patients, which included 82 (67.21%) of Gram negative and positive bacteria from 122 females and 56 (44.44%) from 126 males. All the isolates were identified based on the 16S rRNA (Universal gene, exist in all bacteria, (Kirchman *et al.*, 2010,

Hermans *et al.*, 2017). gene using PCR and sequencing. The results of this study showed that 8 genera and 17 species of Gram-positive bacteria and 7 genera and 12 species of Gram-negative bacteria were isolated (Figures 2,3). Of the bacteria that were isolated, Staphylococcus species was the most common 36 (10 of *S.aureus*, 10 of *S.hominis*, 6 of *S.haemolyticus*, 6 of *S.epidermidis*, 2 of *S.saprophyticus*, 2 of *S.xylosus*), followed by Pseudomonas species 28 (14 of *Pseudomonas* spp. , 4 of *P.aeruginosa*, 4 of *P.putida*, 2 of *P.plecoglossicida*, 4 of *P.kuykendallii*), 14 of Klebsiella species (10 of *K.pneumoniae* , 4 of *K.aerogenes*, 12 *E.coli* , 10 of Enterococcus species (4 of *E.faecium*, 6 of *E.faecalis*), 8 of *P.mirabilis*, 6 of Bacillus species (4 of *B.licheniformis*, 2 of *B.korlensis*), 6 of Corynebacterium species (2 of *Corynebacterium* spp., 2 of *C.singulare*, 2 of *C.amycolatum*), 6 of *A.baumannii*, 2 of *M.morganii*, 2 of *A.spanius*, 2 of *P.ginsengagrvi*, 2 of *M.orygis*, 2 of *B.simplex*, and 2 of *S.agalactiae* strains. These findings corroborated those of (Sanjee *et al.*, 2017, Urmi *et al.*, 2019, Al-Naqshbandi *et al.*, 2019). The data presented in the Creta *et al.* (2021) review showed that patients with COVID-19 may exhibit signs, symptoms, and radiological and laboratory findings that indicate involvement of the lower urinary tract and male genital system. In another study held in India on hospitalized COVID-19 patients, nearly 12% of collected samples showed a positive growth of bacteria on MacConkey and blood agar plates, and the isolated bacteria were both Gram-negative and Gram-positive with more prevalence of Gram-negative bacteria (Das *et al.*, 2022).

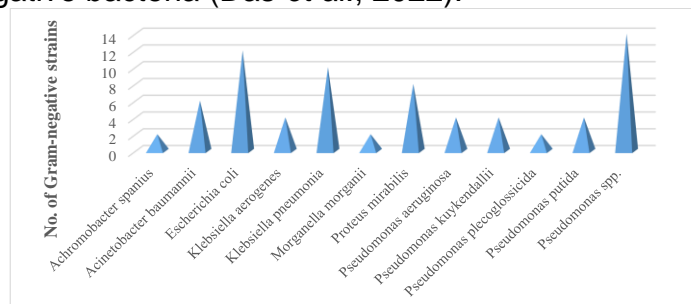


Figure 2. Number of isolated Gram-negative bacteria in urinary tract infection of Covid-19 patients.

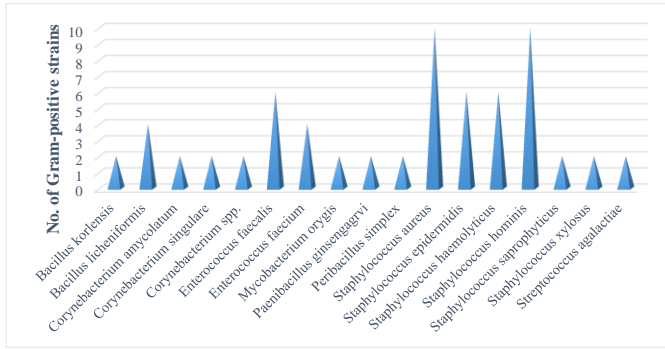


Figure 3. Number of isolated Gram-positive bacteria in urinary tract infection of Covid-19 patients.

3.2 Antibiotic susceptibility

The results of the antibiotic susceptibility test demonstrated that the susceptibility of the isolated bacteria to the tested antibiotics differed, as shown in Table (3,4) and it was found that most of the Gram-negative bacteria were highly resistant to ampicillin with percentage 66(91.66%), and followed by amoxicillin 60(83.33%), cephalexin 52(72.22%), and cefixime 50(69.44%), while most of Gram-positive bacteria found to be resistant to cefixime, ampicillin with a percentage of 40(60.60%), 28(42.42%) respectively, followed by amoxicillin, and ciproflaxin with a percentage 18(27.27%) for both antibiotics. While the most effective antibiotics on Gram-negative bacteria were imipenem, norfloxacin, trimethoprim, and gentamicin with percentage 26(36.11%), 28(38.88%), 30(41.66%), and 32(44.44%) respectively, and for Gram-positive bacteria, all the isolates were sensitive for gentamicin followed by imipenem, and nitrofurantoin with a percentage 0(0.0%), 4(6.06%) and 6(9.09%) respectively, these results were in similar to results obtained by (Folliero *et al.*, 2020, Ali *et al.*, 2021). On the other hand, Gram-negative bacteria showed variable resistance to other antibiotics such as amoxicillin-clavulanic acid, and tetracycline with percentages of 48(66.66%), 46(63.88%) respectively, while levofloxacin, nitrofurantoin, and ofloxacin showed the same effect with a percentage of 44(61.11%) for all, whereas ciproflaxin and ceftriaxone showed a resistance with a percentage of 42(58.33%), 40 (55.55%), respectively. Also Gram-positive bacteria showed a variable resistance to the antibiotics used in the study, such as levofloxacin 14(21.21%), and 12(18.18%) for all of amoxicillin-

clavulanic acid, cephalexin, and ofloxacin. Ceftriaxone was 10(15.15%), whereas norfloxacin, tetracycline, and trimethoprim had the same effect with a percentage of 8(12.12%) for all. All the Gram-positive bacteria were sensitive to gentamicin. These results were agreed with the results of (Fenta *et al.*, 2020). However, in Gram-negative bacteria, *E.coli* had the highest resistance percentage against the antibiotics used in the study among all Gram-negative isolated bacteria, and showed a resistance degree pattern to number of antibiotics includes amoxicillin, amoxicillin-clavulanic acid, ampicillin, cephalexin, ciproflaxin, levofloxacin, ofloxacin with percentage of 100%, and norfloxacin, tetracycline, trimethoprim with percentage of 50%, and cefixime, ceftriaxone, imipenem, nitrofurantoin with a percentage of 33.3%, while the highest sensitivity was against gentamicin with a percentage 16.6%, these results matched with results reported by (Rossignol *et al.*, 2017). Moreover, in Gram-positive bacteria, *S.aureus* had the highest resistance percentage against the antibiotics used in the study among all Gram-positive isolated bacteria and showed a resistance degree pattern to a number of antibiotics including ciproflaxin, levofloxacin, ofloxacin as a highest percentage of resistance about 60%, and amoxicillin-clavulanic acid, cefixime, ceftriaxone, norfloxacin, tetracycline, and trimethoprim with a percentage of 40%, while the cephalexin percentage was 20%. Nevertheless, Onwubiko and Sadiq (2011) demonstrated the resistance degree pattern of *S.aureus* to a variety of antibiotics, including penicillin, amoxicillin, tetracycline, amoxicillin/clavulanate, ciproflaxin, ceftriaxone, and ofloxacin, with percentages of (92.9%), (69.3%), (68.8%), (63.0%),(31.1%), (28.6%), and (23.4%), respectively. In addition, Parashar (2014) demonstrated that *S. haemolyticus* exhibited high levels of resistance to penicillin (91.3%), amoxicillin (88.9%), gentamicin (75%), and cefotaxime (76.6%). Indeed, the proportion of antibiotic resistance varied from study to study and country to country, as well as by sample size, specimen type, patient, sampling method, and time period.

Table (3) Number and percentage of antimicrobial resistance of isolated Gram-negative bacteria.

Name of Antibiotic	Symbol	Isolated bacteria											Total 72 %	
		<i>E. coli</i>	<i>K. pneumoniae</i> n=10 %	<i>K. aerogenes</i> n=4 %	<i>Levintibilly</i> n=4 %	<i>M. morganii</i> n=2 %	<i>Pseudomonas</i> spp.	<i>P. plecoglossicida</i>	<i>P. aeruginosa</i> n=4 %	<i>Levintida</i> n=4 %	<i>P. karyendallii</i> n=4 %	<i>Asparitus</i> n=2 %		<i>A. baumannii</i> n=6 %
Amoxicillin	AX	1	4	4	8	2	14	2	4	0	4	0	6	60(83.33)
Amoxicillin-Clavulanic acid	AMC	1	2	4	0	0	14	2	4	4	0	0	6	48(66.66)
Ampicillin	AM	1	10	4	8	0	14	2	4	0	4	2	6	66(91.66)
Cefixime	CFM	4	2	4	8	2	14	2	4	0	4	0	6	50(69.44)
Ceftriaxone	CRO	4	2	4	0	0	14	2	4	0	4	0	6	40(55.55)
Cephalexin	CL	1	2	4	8	0	14	2	4	0	0	0	6	52(72.22)
Ciprofloxacin	CIP	1	2	0	6	0	14	2	0	0	0	0	6	42(58.33)
Gentamicin	CN	2	2	0	2	0	14	2	0	4	0	0	6	32(44.44)
Impipenem	IPM	4	2	0	0	0	14	2	0	0	0	0	4	26(36.11)
Levofloxacin	LEV	1	2	0	8	0	14	2	0	0	0	0	6	44(61.11)
Nitrofurantoin	F	4	6	0	8	2	14	2	4	0	0	0	4	44(61.11)
Norfloxacin	NOR	6	2	0	0	0	14	2	0	0	0	0	4	28(38.88)
Ofloxacin	OFX	1	2	0	8	0	14	2	0	0	0	0	6	44(61.11)
Tetracycline	TE	6	8	0	8	0	14	2	4	0	0	0	4	46(63.88)
Trimethoprim	TMP	6	2	4	8	0	0	0	4	0	4	0	2	30(41.66)
Total		120(81%)	50(3.33%)	28(1.86%)	80(5.33%)	6(0.4%)	196(13.06%)	28(1.86%)	36(2.4%)	8(0.53%)	20(1.33%)	2(0.13%)	78(5.2%)	22.4%

Table (4) Number and percentage of antimicrobial resistance of isolated Gram-positive bacteria.

Name of Antibiotic	Symbol	Isolates															Total 66%	
		<i>E.faecalis</i> n=6 %	<i>E.faecium</i> n=4 %	<i>S.haemolyticus</i> n=6 %	<i>S.hominis</i> n=10%	<i>S.saprophyticus</i> n=2 %	<i>S.sciureus</i> n=2 %	<i>S.sciureus</i> n=10%	<i>B.licheniformis</i> n=4 %	<i>Corynebacterium</i> spp.	<i>C.singulare</i> n=2 %	<i>Campylobacter</i> n=2 %	<i>M.orgyis</i> n=2 %	<i>B.hortensis</i> n=2%	<i>B.simplex</i> n=2 %	<i>P.glaucogalacti</i> n=2 %		<i>S.galactiae</i> n=2 %
Amoxicillin	AX	0	4	6	6	0	0	0	0	0	0	0	0	0	2	0	0	18(27.27%)
Amoxicillin-Clavulanic acid	AMC	0	0	0	0	6	0	0	4	0	0	0	0	0	2	0	0	12(18.18%)
Ampicillin	AM	2	0	4	8	6	0	2	0	2	0	0	0	0	2	2	0	28(42.42%)
Cefixime	CFM	2	0	6	10	6	2	2	4	0	0	5	0	0	2	2	2	40(60.60%)
Ceftriaxone	CRO	0	0	0	0	2	0	0	4	2	0	0	0	0	2	0	0	10(15.15%)
Cephalexin	CL	2	0	2	2	0	0	0	2	0	0	2	0	0	0	0	0	12(18.18%)
Ciprofloxacin	CIP	2	0	6	4	0	0	0	6	0	0	0	0	0	0	0	0	18(27.27%)
Gentamicin	CN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0(0.00%)
Impipenem	IPM	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4(6.06%)
Levofloxacin	LEV	2	0	2	2	0	0	0	6	2	0	0	0	0	0	0	0	14(21.21%)
Nitrofurantoin	F	0	0	0	0	0	0	0	0	4	0	0	0	0	2	0	0	6(9.09%)
Norfloxacin	NOR	0	0	2	0	0	0	0	4	2	0	0	0	0	0	0	0	8(12.12%)
Ofloxacin	OFX	0	4	2	0	0	0	0	6	0	0	0	0	0	0	0	0	12(18.18%)
Tetracycline	TE	0	0	2	2	0	0	0	4	0	0	0	0	0	0	0	0	8(12.12%)
Trimethoprim	TMP	0	0	2	0	0	0	0	4	0	0	0	0	0	2	0	0	8(12.12%)
Total		10(0.66%)	12(0.8%)	34(2.26%)	34(2.26%)	20(1.33%)	2(0.133%)	4(0.26%)	44(2.93%)	12(0.8%)	0(0%)	2(0.13%)	0(0%)	14(0.93%)	4(0.26%)	2(0.13%)	2(0.13%)	6.46%

3.3 ESBL (extended spectrum beta-lactamase) phenotypes and genotypes detection

All the isolates were tested for phenotypic ESBL by using the Double Disk Synergy Test (DDST), and the results showed that (69.23% were ESBL producers and (30.76%) were ESBL non-producers, as shown in (table 5). Regarding the genotypes, the PCR was used to detect three genes (*bla*TEM, *bla*CTX-M, and *bla*SHV) responsible for the resistance of the isolates against three different antibiotics: ampicillin, amoxicillin, and cephalexin for Gram-negative bacteria, and amoxicillin, ampicillin, and cefixime for Gram-positive bacteria. As shown in Figure 4, the results revealed that all of the isolates possessed at least one of the three genes. These findings corroborated the findings of Rani *et al.* (2016) where ESBLs are common in most of bacteria that are resistant to Cephalosporin antibiotics, where nearly about 40% of bacteria isolated from urine were resistant to Cephalosporins. ESBL is widely distributed in Gram-negative bacteria particularly in *K.pneumoniae* and *E.coli*, and for Gram-positive mostly in *P.aeruginosa* (Rolston *et al.*, 2017, Akpaka *et al.*, 2021). Because the prevalence of ESBLs is constantly changing, hospitalized patients all over the world are at risk of developing resistance to the most commonly used antibiotics (Castanheira *et al.*, 2021). In many laboratories with current clinical methods, they failed to detect the ESBLs, which are crucial for the β -lactam therapy, which led to inappropriate description of antibiotics, so depending on the PCR method makes it more sensitive and specific (Krishnamurthy *et al.*, 2013). For example, in our study, the phenotype (DDST) ESBL of *S.aureus*, *P.aeruginosa*, *E.faecium*, *P.plecoglossicida*, *B.licheniformis*, *A.baumannii*, and *B.simplex* showed a negative result in Figure 5, whereas they showed a positive result when PCR was used for detection in Figure 6. Furthermore, detecting beta-lactamase is required in order to conduct an appropriate epidemiological investigation into antimicrobial resistance. CTX-M-type ESBLs appeared to be the most common type of ESBL globally, with a higher incidence in most locations

than SHV and TEM ESBLs (Rawat and Nair, 2010). Among the three ESBL genotypes included in our study, the most prevalent were found to be (88.33%) for both *bla*TEM and *bla*CTX-M, whereas *bla*SHV was (53.84%). These findings agreed with results of another study held in Erbil city (Pishtiwan and Khadija, 2019).

Table (5) ESBLs producers and non-producers

Species	ESBL	
	Producer	Non-producer
<i>Achromobacter spanius</i>	Positive	-----
<i>Acinetobacter baumannii</i>	-----	Negative
<i>Bacillus korensis</i>	Positive	-----
<i>Bacillus licheniformis</i>	-----	Negative
<i>Corynebacterium singulare</i>	Positive	-----
<i>E.faecalis</i>	Positive	-----
<i>E.faecium</i>	-----	Negative
<i>K.aerogenes</i>	Positive	-----
<i>K.pneumoniae</i>	Positive	-----
<i>Morganella morganii</i>	Positive	-----
<i>Mycobacterium orygis</i>	Positive	-----
<i>P.aeruginosa</i>	-----	Negative
<i>P.kuykendallii</i>	Positive	-----
<i>P.mirabilis</i>	Positive	-----
<i>P.plecoglossicida</i>	-----	Negative
<i>P.putida</i>	Positive	-----
<i>Paenibacillus ginsengagrvi</i>	Positive	-----
<i>Peribacillus simplex</i>	-----	Negative
<i>Pseudomonas spp.</i>	-----	Negative
<i>S.agalactiae</i>	Positive	-----
<i>S.aureus</i>	-----	Negative
<i>S.epidermidis</i>	Positive	-----
<i>S.haemolyticus</i>	Positive	-----
<i>S.hominis</i>	Positive	-----
<i>S.saprophyticus</i>	Positive	-----
<i>S.xylosus</i>	Positive	-----
	(69.23%)	(30.76%)

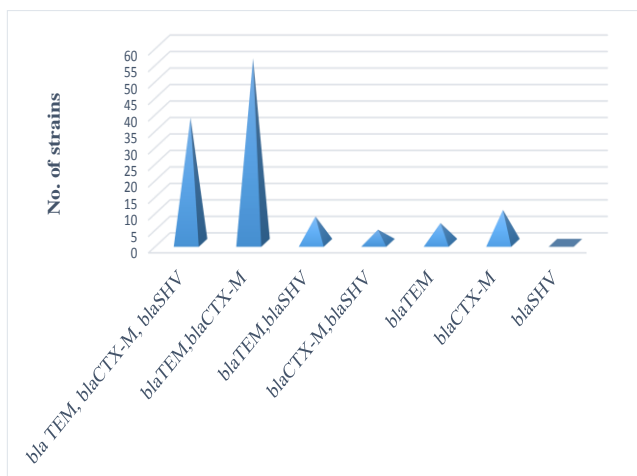


Figure 4. The overall incidence of ESBL genotypes in the tested isolates.

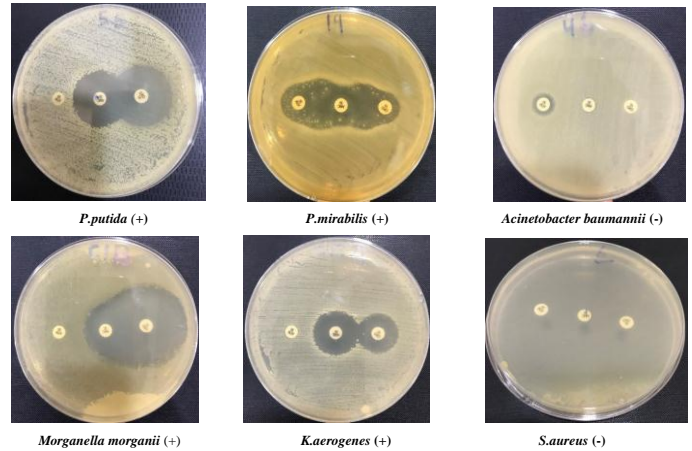


Figure 5. Phenotypes of ESBL (+ Positive / - Negative)

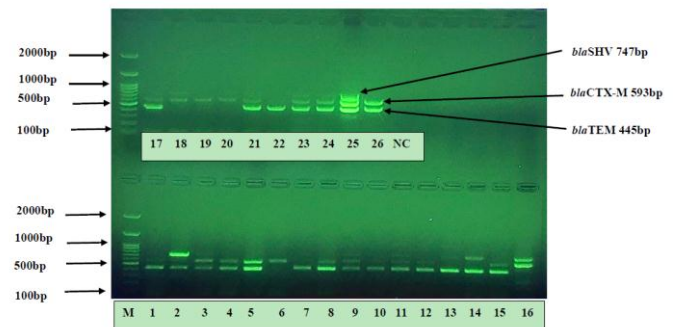


Figure 6. PCR product of *bla*TEM, *bla*SHV, *bla*CTX-M in isolates. Lane M: ladder (100 bp). Lane NC: Negative control. Lanes 1: *P.plecoglossicida*, 2: *K.pneumoniae*, 3: *B.licheniformis*, 4: *P.mirabilis*, 5: *P.aeruginosa*, 6: *Acinetobacter baumannii*, 7: *P.putida*, 8: *E.faecium*, 9: *Pseudomonas spp.*, 10: *S.haemolyticus*, 11: *S.hominis*, 12: *S.epidermidis*, 13: *B.simplex*, 14: *E.faecalis*, 15: *Morganella morganii*, 16: *Paenibacillus ginsengagrvi*, 17: *Mycobacterium orygis*, 18: *C.singulare*, 19: *S.agalactiae*, 20: *Bacillus korensis*, 21: *S.aureus*, 22: *Achromobacter spanius*, 23: *P.kuykendallii*, 24: *S.saprophyticus*, 25: *K.aerogenes*, 26: *S.xylosus*.

4 Discussion

As suggested by the studies, the outbreak of COVID-19, which was originally transmitted from animals, has created a new public health concern (Sharifipour *et al.*, 2020). Recently, more attention has started to be paid to patients with prolonged stays in hospitals as a result of hospital acquired infections. In addition, all the hospitalized patients started to be given empiric antibiotics. In this study, we studied bacterial UTIs in COVID-19 patients during the period between (September, 2021 and January, 2022) in different hospitals in Erbil city. The age of hospitalized patients in this study ranged from 24–84 years old, and most of them had at least one or more chronic diseases such as hypertension, diabetes mellitus, kidney diseases, or cardiovascular diseases, as recorded in CDC guidance (Control and Prevention, 2020, Clancy and Nguyen, 2020). Despite the fact that studies from the United Kingdom, China, Spain, and Italy

revealed a low incidence of bacterial infection, wide-spectrum antibiotics are still prescribed (Hughes *et al.*, 2020, Soriano *et al.*, 2021, Nebreda-Mayoral *et al.*, 2022). While another study held in Kirkuk city, Iraq revealed that 94% of COVID-19 patients had a bacterial co-infection (Wali *et al.*, 2021). It was difficult to compare our results with the findings of other studies to figure out the rate of UTIs in COVID-19 patients because they did not specifically analyse this type of infection or did not use the same terms of infections (Hughes *et al.*, 2020, Vaughn *et al.*, 2021). Our study adds to this literature by introducing the common causative bacteria for UTI, estimating co-infection in patients while they are receiving medical treatment in hospitals, and determine the effectiveness of antibiotics used to avoid using wide-spectrum antibiotics. Most of the studies that dealt with bacterial co-infection in COVID-19 patients focused on co-infection in the respiratory system, so there are fewer resources that provide information about bacterial co-infection of UTIs. In our study, we focused on identifying the causative bacteria of UTIs and their sensitivity to the most commonly given antibiotics to patients with COVID-19 in hospital. In the current study, the incidence of UTIs in females was higher than in males due to the anatomical structure of the urogenital system with a percentage of (59.67%) and (40.32%) in males. These results matched with (Gudiol *et al.*, 2021, Vaughn *et al.*, 2021). Regarding the prevalence of UTIs described in EPINE-HULP 2019, the percentage of patients who had UTIs was 23.6% (55.65% in our work). The frequency of microorganisms identified shows significant differences between our study and EPINE-HULP 2019: Staphylococci, which comprised about 36 (26.08%), followed by 28 (20.28%) *Pseudomonas* spp., also 14 (10.14%) isolates of *Klebsiella* spp. In addition, *E.coli* comprised 12 (8.69%), and 8 (5.79%) of *Proteus mirabilis*, while 6 (4.34%) of each of *Acinetobacter baumannii*, *Corynebacterium* spp., and *Enterococcus faecalis*. Furthermore, 4 (2.89%) of *Enterococcus faecium* and *Bacillus licheniformis* were included. Moreover, 2 (1.44%) isolates were isolated from the following species of bacteria: *Achromobacter spanius*, *Morganella*

morganii, *Paenibacillus ginsengarvi*, *Mycobacterium orygis*, *Bacillus korensis*, *Peribacillus simplex*, and *Streptococcus agalactiae*, while in EPINE-HULP 2019, the most frequent was *E.coli* 11 (39.29%), followed by *P.aeruginosa* 2 (7.14%), *Klebsiella* spp. 2 (7.14%) and *E.faecalis* 1 (3.57%). The frequency of a particular species varies from one study to another, in a study held by Bardi *et al.* (2021), the most frequent microorganisms were *E. faecalis* and *E. faecium*, but not in the findings of Garcia-Vidal *et al.* (2021), whose main causative agent were Gram-negative bacilli. Regarding the antibiotic susceptibility, Gram-negative bacteria showed higher resistance against the used antibiotics than Gram-positive bacteria, where (22.4%) of Gram-negative were resistant, while only (6.46%) of Gram-positive were resistant. The highest percentage of resistance of Gram-negative showed by *Pseudomonas* ssp. and *S.haemolyticus* from Gram-positive bacteria, these results matched with An Italian retrospective analysis of 32 COVID-19 ICU patients showed that about 80% of multi-drug resistant bacteria isolated were Gram-negative bacilli (Karruli *et al.*, 2021). Most of the Gram-positive bacteria in this study were resistant to ampicillin and cefixime, while getamicin and imipenim were the most effective antibiotics on Gram-positive bacteria. In Gram-negative bacteria, most of isolates were resistant to ampicillin and amoxicillin, while imipenem and norfloxacin were the most effective antibiotics.

5 Conclusion

Urinary tract infections are considered one of the most common bacterial infections. With the spread of SARSCoV2, more patients were in need of hospitalization for proper treatment. This action increases the chance of having bacterial co-infections. According to the present study, approximately 50% of COVID-19 patients suffered from UTIs and the predominant isolated Gram-positive bacteria was Staphylococcus species, while in Gram-negative bacteria it was *Pseudomonas* species, so hospitalized patients need to be checked for UTIs even if no symptoms are showing. In addition, empirical antibiotic choice by clinicians should be based on the awareness of the local predominance of

bacterial profiles and antibiotic sensitivities rather than on universal or even national guidelines. Based on the results shown in the present study, *bla*TEM and *bla*CTX-M were highly prevalent among other types of ESBLs.

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