



Molecular Characterization of *Enterobacter cloacae* Isolated from Urinary Tract Infections

Elahe Barzam Dehkordi¹, Elahe Tajbakhsh^{1,*} and Hassan Momtaz¹

¹Department of Microbiology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

*Corresponding author: Department of Microbiology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Email: elahetajbakhsh@gmail.com

Received 2022 January 18; Revised 2022 May 07; Accepted 2022 June 01.

Abstract

Background: Urinary tract infections represent a major expensive, common public health problem worldwide due to their high prevalence and the difficulties associated with their management.

Objectives: This study aimed to characterize the *Enterobacter cloacae* strains isolated from urinary tract infections in the medical diagnostic laboratories of Shahrekord, Iran.

Methods: Urine samples from patients with urinary tract infections from the Shahrekord medical diagnostic laboratories located in Chaharmahal and Bakhtiari Province, Iran, were collected from June 2019 to February 2020. When the samples were cultured, the different isolates of *E. cloacae* were identified by biochemical tests. Biofilm production capacity was evaluated. Bacterial susceptibility to antibiotics was determined using the Kirby Bauer method, and antibiotic resistance genes were researched by the multiplex PCR technique.

Results: In this study, 65 isolates of *E. cloacae* were obtained. The highest percentage of resistance was observed for co-trimoxazole (84.62%), ampicillin (76.93%), tetracycline (73.85%), and above half of the *E. cloacae* strain isolates (53.85%) were strongly involved in biofilm production. Some genes, including *qnr A*, *qnr B*, *qnr S*, *tet A*, *tet B*, *sul I*, *bla CTXM*, *bla SHV*, *and(2)Ia*, *ant(3)Ia*, and *aac(3)IIa*, were detected in the genome of these isolates.

Conclusions: The strains are multi-resistant, and their resistance has already reached the carbapenem class. This requires further investigation, and urgent measures must be adopted.

Keywords: Urinary Tract Infections, *Enterobacter cloacae*, Public Health, Resistance Genes, Iran

1. Background

Urinary tract infection (UTI), which involves many organs of the body, is one of the most common diseases in hospitals and the community (1). They can affect adults, youth, and children (2, 3). Urinary tract infections affect women much more frequently than men because of the anatomical configuration of the female genital tract, particularly the proximity of the urinary meatus to the anus (4). Urinary tract infections represent a major expensive (5) and common public health problem worldwide due to their high prevalence, difficulties associated with their management, and their complications (6). This concerns all outpatients and inpatients (7). Every year, above 200 million people are globally diagnosed with UTI, accounting for the global economy above \$6 billion (6, 7).

The most isolated species is Gram-negative (enterobacteria) (3), such as *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp. (8), *Pseudomonas aeruginosa*, and *Serratia*

marcescens with high resistance rates (9). Among the strains, *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* are three significant pathogens involved in UTIs with important resistance phenotypes (10). These bacterial species have identified the most developed resistance mechanisms in enterobacteria. The World Health Organization (WHO) continues to raise the alarm about the severe handicap of antimicrobial resistance in managing infections such as UTIs. Some evidence shows an increasing resistance to conventional drugs among urinary tract pathogens (11, 12). The high rates of resistance to antimicrobials often used to treat common infections such as UTIs indicate the limited number of effective ways to control these infections. The situation is more alarming because infections induced by resistant bacteria often lead to a prolonged disease state and an increased mortality rate (9).

According to recent statistics reported by the WHO, the rate of resistance to ciprofloxacin used to treat UTIs ranged

from 8.4% to 92.9% in 33 countries (13). *Enterobacter cloacae* isolates involved in UTIs have been characterized as multidrug-resistant for many years (14). Due to various intrinsic and acquired resistance mechanisms, it possesses; *E. cloacae* presents a real therapeutic challenge (7). Many studies have indicated antimicrobial resistance in *E. cloacae* strains isolated from UTIs (7, 15). The antimicrobial resistance of bacteria is one of the major problems in the world (3). However, few studies have focused on detecting the genetic elements of resistance in this bacterium (16). The present study was to fill this gap and provide usable scientific data on the resistance level of *E. cloacae* isolates in Shahrekord, Iran. This study would improve the treatment and management of UTIs because of using the multidrug-resistant strains of *E. cloacae*.

2. Objectives

This study aimed to provide information about the molecular profile of *E. cloacae* strains isolated from UTIs.

3. Methods

3.1. Study Setting

In this cross-sectional study, about 786 urine samples from male and female patients of different age ranges and history of UTI, who referred to the medical diagnostic laboratories in Shahrekord were collected from June 2019 to February 2020. Informed consent was obtained from the patients before collecting the samples. Moreover, ethical approval was obtained from the ethics committee. Bacterial strains were identified based on their culture and biochemical characteristics (6).

3.2. Antibiotic Susceptibility Study

The antibiotic sensitivity of the isolated strains was determined using the Kirby Bauer disk diffusion method. The antibiotic disc (Padtanteb, Iran) containing the following antibiotics was used: Amikacin (30 µg/AN30), kanamycin (K/30 µg), erythromycin (E15/15 µg), tetracycline (TE30/30 µg), gentamicin (GM10/10 µg), chloramphenicol (C) ampicillin (AM10/10 µg), ceftriaxone (CRO30/30 µg), ciprofloxacin (CP5/30 µg), cephalothin (CF30/30 µg), imipenem (IPM10/10 µg), nitrofurantoin (FM300/10 µg), nalidixic acid (NA30/30 µg), norfloxacin (NOR10/30 µg), and co-trimoxazole (SXT25/30 µg). The obtained inhibition zone diameters (IZDs) were recorded and interpreted according to the CLSI guidelines (6).

3.3. DNA Extraction and PCR Reactions

The DNA extraction kit (CinnaGen, Iran) was used according to the manufacturer's instructions to obtain genomic DNA from bacterial colonies. The selected isolates were subjected to multiplex PCR using previously described *Enterobacter* primers (CinnaGen, Iran) and conditions set by Tajbakhsh et al. (17) (Table 1).

Table 1. Primer Sequence for *Enterobacter*

Primer Sequences (5'-3')
F: ATGCTCTGGAAACTGCCTGATG
R: CGGGTAACGTCAATAGACAAGG

3.4. Detection of Antibiotic Resistance Genes

PCR was performed for antibiotic resistance genes using the primers (CinnaGen, Iran) described in Table 2 (18-22). Hybridization temperatures and PCR conditions vary from gene to gene. The PCR conditions for each gene are presented in Table 3. DNA sequencing was performed with the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>) to confirm resistance gene variants.

3.5. Biofilm Formation Assay

The ability of the isolates to form biofilms was investigated by a method previously described by Naves et al. (23). It is based on the assay in minimum and enriched media of *E. cloacae* strains and the mathematical determination of the patterns and quantity of biofilm formed.

3.6. Statistical Analysis

Descriptive analyses, including the mean value and variability (standard deviation) and descriptive tables, were used. Chi-squared and Fisher's exact tests were used to evaluate the significance of differences between the different parameters evaluated with EpiData software ($P < 0.05$).

4. Results

This study aimed to characterize the molecular level of *E. cloacae* strains involved in the urinary tract of infected patients. To this end, 65 *E. cloacae* were isolated from 786 urine samples obtained from medical diagnostic laboratories, Shahrekord, Iran. Of the 65 *E. cloacae* isolates, 55 isolates were female, and 10 isolates were male. For each isolate, characteristic tests were performed.

Table 2. Primer Sequence and Amplicon Size

Gene and Primer Sequence (5'-3')	Size of Product (bp)
qnr A	360
F: CAGCAAGAGGATTCTCACG	
R: AATCCGGCAGCACTATTACTC	
qnr B	488
F: GGCTGTCAGTCTATGATCG	
R: GAGCAACGATGCCTGGTAG	
qnr S	428
F: GCAAGTTCATTGAACAGGGT	
R: TCTAAACCGTCGAGTTCGGCG	
ant (2)Ia	572
F: CGCCGTGGGTCGATGTTGATG	
R: TTTTCCGCCCCGAGTGAGGTG	
ant (3)Ia	245
F: TCGACTCAACTATCAGAGG	
R: ACAATCGTGACTTCTACAGCG	
aac(3)IIa	563
F: GCAATAACGGAGGCGCTCAAAA	
R: TTCCAGGCATCGCATCTCATACG	
Sul 1	433
F: CGGCGTGGGCTACCTGAACG	
R: GCCGATCGCGTGAAGTTCCG	
Bla SHV	231
F: AAG ATC CAC TAT CGC CAG CAG	
R: ATT CAG TTC CGT TTC CCA GCG G	
Bla CTM	850
F: GGT TAA AAA ATC ACT GCG TC	
R: TTG GTG ACG ATT TTA GCC GC	
tet A	927
F: GTAATTCGAGCACTGTCGC	
R: CTGCCTGGACAACATTGCTT	
tet B	659
F: TTGGTTAGGGCAAGTTTTG	
R: GTAATGGGCAATAACACCG	

4.1. Antibiotic Susceptibility Study

The results of the sensitivity test of the strains to different antibiotics are presented in Table 4. This table shows that half of the *E. cloacae* isolates are multidrug-resistant to ampicillin, tetracycline, amikacin, kanamycin, erythromycin, chloramphenicol, gentamicin, co-trimoxazole, and nalidixic acid, which are antibiotics

from various classes. Resistance to imipenem, ceftriaxone, ciprofloxacin, cephalothin, and norfloxacin was also observed for above 30% of the strains. Only 20% of the strains were sensitive to imipenem. The highest resistance percentage was observed for co-trimoxazole (84.62%), ampicillin (76.93%), and tetracycline (73.85%), respectively. On the other hand, the resistance percentages were only 23.08% for Nitrofurantoin and 30.77% for imipenem and cephalothin.

4.2. Biofilm Production Study

Regarding the biofilm production by *E. cloacae*, above half of the *E. cloacae* strain isolates (53.85%) were strongly involved in biofilm production. Moreover, 53.85% had high biofilm production activity, 30.77% had moderate production, and 15.38% had low production.

4.3. Antibiotic Resistance Based on Biofilm Production

The relationship between biofilm production and the antibiotic resistance of *E. cloacae* strains is presented in Table 5. According to this table, the majority of strains with a high capacity for biofilm production were resistant to ampicillin (100%), amikacin (94.29%), tetracycline (97.14%), erythromycin (97.14%), co-trimoxazole (100%) ($P < 0.05$), and the half of these strains were resistant to most of the used antibiotic. Moreover, half of the strains with moderate biofilm production reaction showed resistance to several classes of antibiotics, including ampicillin (75%), tetracycline (55%), amikacin, erythromycin (50%), co-trimoxazole (55%), ciprofloxacin (70%), and ceftriaxone (85%). Antibiotic resistance was observed for strains with low biofilm production capacity, however, to a lesser extent.

4.4. Detection of Antibiotic Resistance Genes

Resistance genes were searched to understand the phenotypic data related to strain resistance (Table 6). This table showed that 60% of the strains that developed resistance to antibiotics such as norfloxacin, nalidixic, and ciprofloxacin, as the antibiotics of the quinolone and fluoroquinolone family, possessed the resistance genes *qnrA*, *qnrB*, *qnrS* with the characteristic of quinolones ($P < 0.05$). Similarly, above 60% of the strains resistant to ampicillin, cephalothin, and imipenem had resistance genes belonging to the beta-lactamase family, including *bla_{SHV}* and *bla_{CTX-M}*. Thus, these results show that the different resistance genes present in the genomes of *E. cloacae* strains were expressed. These strains are multi-resistant, and these resistances already reach the carbapenem class. This requires important surveillance.

Table 3. PCR Conditions for Antibiotic Resistance Genes

Gene	PCR Program	PCR Condition
<i>qnr A</i>	1 cycle: 94°C–6 min; 33 cycles: 95°C–70 s, 55°C–65s, 72°C–90 s, 1 cycle: 72°C–8 min.	5 μ L PCR buffer 10X, 2.5 mm MgCl ₂ , 200 μ M dNTP (Fermentas), 0.5 μ m of each primers F & R.
<i>qnr B</i>		
<i>qnr S</i>		
<i>ant (2)Ia</i>	1 cycle: 95°C–4 min; 30 cycles: 95°C–45s, 58°C–60s, 72°C–40s, 1 cycle: 72°C–5min.	
<i>ant (3)Ia</i>		
<i>aac(3)IIa</i>	1 cycle: 94°C–5 min; 32 cycles: 94°C–60 s, 55°C–60 s, 72°C–2 min, 1 cycle: 72°C–10 min.	
<i>Sul I</i>	1 cycle: 94°C–5 min; 32 cycles: 94°C–60s, 60°C–60s, 72°C–2 min, 1 cycle: 72°C–5min.	
<i>Bla SHV</i>	1 cycle: 95°C–4min; 30 cycles: 94°C–45s, 59°C–60s, 72°C–40s, 1 cycle: 72°C–5min.	
<i>Bla CTX-M</i>		
<i>tet A</i>	1 cycle: 95°C–5min; 32 cycles: 94°C–60s, 59°C–60s, 72°C–2min, 1 cycle: 72°C–10min.	
<i>tet B</i>		

Table 4. Antibiotic Susceptibility of *Enterobacter cloacae* Strain Isolates^a

Antibiotic	R	I	S
Ampicillin	50 (76.93)	10 (15.38)	5 (7.69)
Tetracycline	48 (73.85)	11 (16.92)	6 (9.23)
Amikacin	45 (69.23)	15 (23.08)	5 (7.69)
Kanamycin	40 (61.54)	15 (23.08)	10 (15.38)
Erythromycin	43 (66.16)	12 (18.46)	10 (15.38)
Chloramphenicol	40 (61.54)	10 (15.38)	15 (23.08)
Gentamicin	35 (53.84)	15 (23.08)	15 (23.08)
Co-Trimoxazole	55 (84.62)	5 (7.69)	5 (7.69)
Nalidixic Acid	45 (69.23)	15 (23.08)	5 (7.69)
Ciprofloxacin	30 (46.15)	15 (23.08)	20 (30.77)
Norfloxacin	25 (38.46)	20 (30.77)	20 (30.77)
Cephalothin	20 (30.77)	15 (23.08)	30 (46.15)
Ceftriaxone	25 (38.46)	20 (30.77)	20 (30.77)
Imipenem	20 (30.77)	25 (38.46)	20 (30.77)
Nitrofurantoin	15 (23.08)	17 (26.15)	33 (50.77)

^a Values are expressed as No. (%).

5. Discussion

The present study aimed to specify the molecular characterization of *E. cloacae* strains involved in UTIs. Out of the 65 *E. cloacae* strain isolates, above half of the strains were resistant to several classes of antibiotics. This reflects the bacterial multi-resistance characterizing the strains involved in UTIs (24). The bacterial multi-resistance include aminoglycosides, tetracyclines, quinolones, penicillin, macrolides, and sulfonamides. The management processes are thus limited (25). Thus, this constitutes a break

from the cure by antibiotic therapy. The resistance to beta-lactam antibiotics, especially carbapenems, shows that the antibiotics of last resort are minimal. In the case of UTIs with multidrug-resistant bacteria, physicians will be faced with a significant lack of solutions in a short period.

This issue shows the real public health problem with the antibiotic resistance of uropathogenic bacteria. This confirms the finding of earlier studies by Hryniewicz et al. (11) on the increasing extent of resistance among pathogens in UTIs to conventional drugs. A few years ago, during epidemiological surveillance, there was a significant decrease in the antibiotic susceptibility of strains involved in UTIs. These observations would be due to the acquisition and transmission of resistance genes between bacteria through plasmids by conjugation or transformation. Self-medication and non-compliance with dosage would also be factors, which could have considerably increased resistance in *E. cloacae* strains. However, in contrast to the findings of our study, in which the sensitivity to imipenem of the strains was only 20%, the sensitivity was above 80% in their study (12). According to Matsumoto and Muratani (26), imipenem has high antimicrobial activity against fresh urinary isolates such as *E. cloacae*. This strain is and remains an important opportunistic and multi-resistant pathogen for humans, which can create the epidemics of nosocomial infections (16). Nevertheless, the minimum inhibitory concentrations of different antibiotics tested should have been determined.

All isolated *E. cloacae* strains were biofilm producers, and above half of the isolates were strongly associated with biofilm production. Moreover, these findings corroborate with those reported by Sabir et al. (27). They also found that *E. cloacae* species had the highest biofilm pro-

Table 5. Antibiotic Resistance Evaluation Based on Biofilm Reaction^a

Antibiotic	Biofilm Reaction						P-Value
	High n = 35		Moderate n = 20		Weak n = 10		
	R	S	R	S	R	S	
Ampicillin	35 (100)	0 (0)	15 (75)	5 (25)	5 (50)	5 (50)	0.000 ^b
Tetracycline	34 (97.14)	1 (2.86)	11 (55)	9 (45)	3 (30)	7 (70)	0.000 ^c
Amikacin	33 (94.29)	2 (5.71)	10 (50)	10 (50)	6 (60)	4 (40)	0.001 ^b
Kanamycin	30 (85.71)	5 (14.29)	15 (75)	5 (25)	6 (60)	4 (40)	0.243 ^b
Erythromycin	34 (97.14)	1 (2.86)	10 (50)	10 (50)	6 (60)	4 (40)	0.000 ^b
Chloramphenicol	29 (82.86)	6 (17.14)	13 (65)	7 (35)	7 (70)	3 (30)	0.298 ^b
Gentamicin	25 (71.43)	10 (28.57)	12 (60)	8 (40)	6 (60)	4 (40)	0.624 ^c
Co-Trimoxazole	35 (100)	0 (0)	11 (55)	9 (45)	7 (70)	3 (30)	0.000 ^b
Nalidixic Acid	32 (91.83)	3 (8.57)	15 (75)	5 (25)	8 (80)	2 (20)	0.256 ^b
Ciprofloxacin	10 (28.57)	25 (71.43)	14 (70)	1 (30)	5 (50)	5 (50)	0.000 ^c
Norfloxacin	20 (57.14)	15 (42.86)	12 (60)	8 (40)	7 (70)	3 (30)	0.765 ^c
Cephalothin	19 (54.29)	16 (45.71)	16 (80)	4 (20)	7 (70)	3 (30)	0.147 ^c
Ceftriaxone	16 (45.71)	19 (54.29)	17 (85)	3 (15)	6 (60)	4 (40)	0.017 ^c
Imipenem	20 (57.14)	15 (42.86)	15 (75)	5 (25)	7 (70)	3 (30)	0.388 ^b
Nitrofurantoin	13 (37.14)	22 (62.86)	13 (65)	7 (35)	5 (50)	5 (50)	0.136 ^c

^a Values are expressed as No. (%).^b Fisher's exact test^c Chi-squared test

duction (87.5%) among pathogens isolated from catheter-associated UTIs with bacterial biofilm. Biofilm formation is one important characteristic of *E. cloacae* and other *Enterobacteriaceae*, which has been used in the rapid bioremoval of hydrocarbons in diesel fuel. In other words, the presence of this species in the urinary tract is a real threat to the patient. This study demonstrated the predominant role of biofilm production in increasing the resistance trait developed by *E. cloacae*. It showed that biofilm production increases antibiotic resistance in *E. cloacae* isolates.

Moreover, this finding is in line with the findings of Sabir et al. and Maheshwari et al. (27, 28). Sabir et al. (27) showed that biofilm-producing strains were highly resistant to antibiotics due to their slow penetration, a resistant phenotype, and an altered microenvironment. Maheshwari et al. (28) also documented that the presence of biofilm was a factor affecting the several-fold increase of antibiotic resistance. The biofilm formation represents a means of protection for *E. cloacae* and defense against antibiotics.

The different resistance genes were expressed in the genomes of *E. cloacae* strains isolated from our samples. The bla SHV genes were more frequent (64%, 80%, 60%) than

the bla CTX-M genes (54%, 95%, 45%). Furthermore, this result does not confirm the findings of Cabral et al. (29), who claimed that the CTX-M genes were most expressed. According to a study conducted in Brazil in 2017, the bla CTX-M was more frequent in isolates from infection sites (16). As the phenotypic expression is only the result of the composition of the genetic material, the PCR results can be simply understood. Furthermore, no relationship was detected between quinolone resistance genes and the presence of beta-lactam genes. Azargun et al. (30) reported a significant association between the prevalence of plasmid resistance to quinolones and ESBL-producing isolates. No carbapenem resistance gene (NDM, VIM, OXA-48) was searched, while the susceptibility test considered carbapenem class antibiotics. This means that this study was limited in terms of molecular characterization. Since we are witnessing the emergence of the mcr-1 positive strains of *Enterobacteriaceae* worldwide, this gene could have been searched to study its epidemiology (31).

5.1. Conclusions

UTIs are a genuine public health concern due to the involvement of multi-resistant strains such as *E. cloacae*.

Table 6. Frequency of Antibiotic Resistance Genes by Type of Antibiotic

Antibiotic/ Gene	<i>qnrA</i>	<i>qnrB</i>	<i>qnrS</i>	<i>tet A</i>	<i>tet B</i>	<i>sulI</i>	<i>ant (2)Ia</i>	<i>ant (3)Ia</i>	<i>aac(3)IIa</i>	<i>blaSHV</i>	<i>Bla CTX-M</i>
NA; n = 45; (P-value = 0.000*)	27	9	5								
	60	20	11.11								
CP; n = 30; (P-value = 0.000*)	20	8	2								
	66.67	26.67	6.67								
NOR; n = 25; (P-value = 0.000*)	15	6	1								
	60	24	4								
TE; n = 48; (P-value = 0.275*)				35	30						
				72.92	62.5						
SXT; n = 55						40					
						72.72					
K; n = 40; (P-value = 0.822*)							18	17			
							45	42.5			
AN; n = 45; (P-value = 0.67*)							20	18			
							44.44	40			
GM; n = 35									15		
									42.86		
AM; n = 50; (P-value = 0.309*)										32	27
										64	54
CF; n = 20; (P-value = 0.151)										16	19
										80	95
IMP; n = 20; (P-value = 0.342*)										12	9
										60	45

The potentials to produce biofilm and antibiotic resistance genes are essential elements giving this strain a multi-resistant and defensive strength. Accordingly, the management of these infections is difficult since antibiotic therapy is severely limited.

Footnotes

Authors' Contribution: Study concept and design: E. B. D., and E. T.; data analysis and interpretation: E. B. D., and E. T.; manuscript drafting: E. B. D., and E. T.; critical revision of the manuscript for main intellectual content: H. M.

Conflict of Interests: The author(s) declared no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

Data Reproducibility: The data presented in this study are openly available in one of the repositories or will be available on request from the corresponding author by this journal representative at any time during submission

or after publication. Otherwise, all consequences of possible withdrawal or future retraction will be with the corresponding author.

Ethical Approval: The Research and Ethics Committee of the Islamic Azad University, Shahrekord Branch, approved the experiments (IR.IAU.SHK.REC.1400.074, link: ethics.research.ac.ir/EthicsProposalView.php?id=246903).

Funding/Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Informed Consent: Informed consent was obtained from the patients before obtaining the samples.

References

- Mach F, Marchandin H, Bichon F. [Treatment and prevention of urinary tract infections]. *Actual Pharm.* 2020;**59**(598):48-52. French. doi: [10.1016/j.actpha.2020.06.023](https://doi.org/10.1016/j.actpha.2020.06.023).
- Ishikawa K, Matsumoto T, Yasuda M, Uehara S, Muratani T, Yagisawa M, et al. The nationwide study of bacterial pathogens associated with urinary tract infections conducted by the Japanese

- Society of Chemotherapy. *J Infect Chemother*. 2011;**17**(1):126–38. doi: [10.1007/s10156-010-0174-1](https://doi.org/10.1007/s10156-010-0174-1). [PubMed: [21174142](https://pubmed.ncbi.nlm.nih.gov/21174142/)].
3. Rajabi Z, Soltan Dallal MM. Study on bacterial strains causing blood and urinary tract infections in the neonatal intensive care unit and determination of their antibiotic resistance pattern. *Jundishapur J Microbiol*. 2015;**8**(8). e19654. doi: [10.5812/jjm.19654v2](https://doi.org/10.5812/jjm.19654v2). [PubMed: [26468359](https://pubmed.ncbi.nlm.nih.gov/26468359/)]. [PubMed Central: [PMC4601293](https://pubmed.ncbi.nlm.nih.gov/PMC4601293/)].
 4. Folliero V, Caputo P, Della Rocca MT, Chianese A, Galdiero M, Iovene MR, et al. Prevalence and antimicrobial susceptibility patterns of bacterial pathogens in urinary tract infections in university hospital of Campania "Luigi Vanvitelli" between 2017 and 2018. *Antibiotics (Basel)*. 2020;**9**(5). doi: [10.3390/antibiotics9050215](https://doi.org/10.3390/antibiotics9050215). [PubMed: [32354050](https://pubmed.ncbi.nlm.nih.gov/32354050/)]. [PubMed Central: [PMC7277346](https://pubmed.ncbi.nlm.nih.gov/PMC7277346/)].
 5. Sybesma W, Zbinden R, Chanishvili N, Kutateladze M, Chkhotua A, Ujmajuridze A, et al. Bacteriophages as potential treatment for urinary tract infections. *Front Microbiol*. 2016;**7**:465. doi: [10.3389/fmicb.2016.00465](https://doi.org/10.3389/fmicb.2016.00465). [PubMed: [27148173](https://pubmed.ncbi.nlm.nih.gov/27148173/)]. [PubMed Central: [PMC4826877](https://pubmed.ncbi.nlm.nih.gov/PMC4826877/)].
 6. Delcaru C, Podgoreanu P, Alexandru I, Popescu N, Marutescu L, Bleotu C, et al. Antibiotic resistance and virulence phenotypes of recent bacterial strains isolated from urinary tract infections in elderly patients with prostatic disease. *Pathogens*. 2017;**6**(2). doi: [10.3390/pathogens6020022](https://doi.org/10.3390/pathogens6020022). [PubMed: [28561794](https://pubmed.ncbi.nlm.nih.gov/28561794/)]. [PubMed Central: [PMC5488656](https://pubmed.ncbi.nlm.nih.gov/PMC5488656/)].
 7. Gajdacs M, Urban E. Resistance trends and epidemiology of citrobacter-enterobacter-serratia in urinary tract infections of inpatients and outpatients (RECESUTI): A 10-year survey. *Medicina (Kaunas)*. 2019;**55**(6). doi: [10.3390/medicina55060285](https://doi.org/10.3390/medicina55060285). [PubMed: [31216725](https://pubmed.ncbi.nlm.nih.gov/31216725/)]. [PubMed Central: [PMC6630883](https://pubmed.ncbi.nlm.nih.gov/PMC6630883/)].
 8. Guermazi-Toumi S, Boujlel S, Assoudi M, Issaoui R, Tlili S, Hlaïem ME. Susceptibility profiles of bacteria causing urinary tract infections in Southern Tunisia. *J Glob Antimicrob Resist*. 2018;**12**:48–52. doi: [10.1016/j.jgar.2017.09.004](https://doi.org/10.1016/j.jgar.2017.09.004). [PubMed: [28918351](https://pubmed.ncbi.nlm.nih.gov/28918351/)].
 9. Rosana Y, Ocviyanti D, Halim M, Harlinda FY, Amran R, Akbar W, et al. Urinary tract infections among Indonesian pregnant women and its susceptibility pattern. *Infect Dis Obstet Gynecol*. 2020;**2020**:9681632. doi: [10.1155/2020/9681632](https://doi.org/10.1155/2020/9681632). [PubMed: [32372856](https://pubmed.ncbi.nlm.nih.gov/32372856/)]. [PubMed Central: [PMC7191430](https://pubmed.ncbi.nlm.nih.gov/PMC7191430/)].
 10. Xu J, He F. Genomic analysis of two bacterial strains co-isolated from a urinary tract infection: NDM-1-producing Enterobacter cloacae accompanied by extended-spectrum beta-lactamase-producing Escherichia coli. *J Glob Antimicrob Resist*. 2019;**17**:198–200. doi: [10.1016/j.jgar.2019.04.007](https://doi.org/10.1016/j.jgar.2019.04.007). [PubMed: [31005730](https://pubmed.ncbi.nlm.nih.gov/31005730/)].
 11. Hryniewicz K, Szczypta K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *J Antimicrob Chemother*. 2001;**47**(6):773–80. doi: [10.1093/jac/47.6.773](https://doi.org/10.1093/jac/47.6.773). [PubMed: [11389109](https://pubmed.ncbi.nlm.nih.gov/11389109/)].
 12. Jimenez-Guerra G, Borrego-Jimenez J, Gutierrez-Soto B, Exposito-Ruiz M, Navarro-Mari JM, Gutierrez-Fernandez J. Susceptibility evolution to antibiotics of Enterobacter cloacae, Morganella morganii, Klebsiella aerogenes and Citrobacter freundii involved in urinary tract infections: an 11-year epidemiological surveillance study. *Enferm Infect Microbiol Clin (Engl Ed)*. 2020;**38**(4):166–9. doi: [10.1016/j.eimc.2019.07.010](https://doi.org/10.1016/j.eimc.2019.07.010). [PubMed: [31606242](https://pubmed.ncbi.nlm.nih.gov/31606242/)].
 13. World Health Organization. [Record number of countries contribute data revealing disturbing rates of antimicrobial resistance]. Geneva, Switzerland: World Health Organization; 2020. French. Available from: <https://www.who.int/fr/news/item/01-06-2020-record-number-of-countries-contribute-data-revealing-disturbing-rates-of-antimicrobial-resistance>.
 14. Kaminska W, Patzer J, Dzierzanowska D. Urinary tract infections caused by endemic multi-resistant Enterobacter cloacae in a dialysis and transplantation unit. *J Hosp Infect*. 2002;**51**(3):215–20. doi: [10.1053/jhin.2002.1236](https://doi.org/10.1053/jhin.2002.1236). [PubMed: [12144801](https://pubmed.ncbi.nlm.nih.gov/12144801/)].
 15. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, et al. Epidemiology of Carbapenem-Resistant Enterobacteriaceae Infections: Report from the China CRE Network. *Antimicrob Agents Chemother*. 2018;**62**(2). doi: [10.1128/AAC.01882-17](https://doi.org/10.1128/AAC.01882-17). [PubMed: [29203488](https://pubmed.ncbi.nlm.nih.gov/29203488/)]. [PubMed Central: [PMC5786810](https://pubmed.ncbi.nlm.nih.gov/PMC5786810/)].
 16. Davin-Regli A, Pages JM. Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment. *Front Microbiol*. 2015;**6**:392. doi: [10.3389/fmicb.2015.00392](https://doi.org/10.3389/fmicb.2015.00392). [PubMed: [26042091](https://pubmed.ncbi.nlm.nih.gov/26042091/)]. [PubMed Central: [PMC4435039](https://pubmed.ncbi.nlm.nih.gov/PMC4435039/)].
 17. Tajbakhsh E, Tajbakhsh S, Khamesipour F. Isolation and molecular detection of gram-negative bacteria causing urinary tract infection in patients referred to Shahrekord hospitals, Iran. *Iran Red Crescent Med J*. 2015;**17**(5). e24779. doi: [10.5812/ircmj.17\(5\)2015.24779](https://doi.org/10.5812/ircmj.17(5)2015.24779). [PubMed: [26082853](https://pubmed.ncbi.nlm.nih.gov/26082853/)]. [PubMed Central: [PMC4464383](https://pubmed.ncbi.nlm.nih.gov/PMC4464383/)].
 18. Banihashem M, Majidpour A, Boustanshenas M, Mazar-Atabaki S. Tetracycline resistance associated with tet genes or integrons among Enterobacter cloacae strains isolated from patients with urinary tract infections. *Infection Epidemiology and Microbiology*. 2020;**6**(1):1–9. doi: [10.29252/iem.6.1.1](https://doi.org/10.29252/iem.6.1.1).
 19. Ciesielczuk H, Hornsey M, Choi V, Woodford N, Wareham DW. Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. *J Med Microbiol*. 2013;**62**(Pt 12):1823–7. doi: [10.1099/jmm.0.064428-0](https://doi.org/10.1099/jmm.0.064428-0). [PubMed: [24000223](https://pubmed.ncbi.nlm.nih.gov/24000223/)].
 20. Ghotaslou R, Yeganeh Sefidan F, Akhi MT, Asgharzadeh M, Mohammadzadeh Asl Y. Dissemination of genes encoding aminoglycoside-modifying enzymes and arma among Enterobacteriaceae isolates in northwest Iran. *Microb Drug Resist*. 2017;**23**(7):826–32. doi: [10.1089/mdr.2016.0224](https://doi.org/10.1089/mdr.2016.0224). [PubMed: [28151044](https://pubmed.ncbi.nlm.nih.gov/28151044/)]. [PubMed Central: [PMC5665088](https://pubmed.ncbi.nlm.nih.gov/PMC5665088/)].
 21. Kern MB, Klemmensen T, Fridtjof-Møller N, Espersen F. Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. *J Antimicrob Chemother*. 2002;**50**(4):513–6. doi: [10.1093/jac/dkf164](https://doi.org/10.1093/jac/dkf164). [PubMed: [12356795](https://pubmed.ncbi.nlm.nih.gov/12356795/)].
 22. Salimian Rizi K, Najari Peerayeh S, Bakhshi B, Rahbar M. Prevalence of integrons and antimicrobial resistance genes among clinical isolates of Enterobacter spp. from hospitals of Tehran. *Int J Enteric Pathog*. 2015;**3**(1). doi: [10.17795/ijep22531](https://doi.org/10.17795/ijep22531).
 23. Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, Blanco J, et al. Measurement of biofilm formation by clinical isolates of Escherichia coli is method-dependent. *J Appl Microbiol*. 2008;**105**(2):585–90. doi: [10.1111/j.1365-2672.2008.03791.x](https://doi.org/10.1111/j.1365-2672.2008.03791.x). [PubMed: [18363684](https://pubmed.ncbi.nlm.nih.gov/18363684/)].
 24. Ahmed SS, Shariq A, Alsalloum AA, Babikir IH, Alhomoud BN. Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *Int J Health Sci (Qassim)*. 2019;**13**(2):48–55. [PubMed: [30983946](https://pubmed.ncbi.nlm.nih.gov/30983946/)]. [PubMed Central: [PMC6436442](https://pubmed.ncbi.nlm.nih.gov/PMC6436442/)].
 25. Bader MS, Loeb M, Brooks AA. An update on the management of urinary tract infections in the era of antimicrobial resistance. *Postgrad Med*. 2017;**129**(2):242–58. doi: [10.1080/00325481.2017.1246055](https://doi.org/10.1080/00325481.2017.1246055). [PubMed: [27712137](https://pubmed.ncbi.nlm.nih.gov/27712137/)].
 26. Matsumoto T, Muratani T. Newer carbapenems for urinary tract infections. *Int J Antimicrob Agents*. 2004;**24 Suppl 1**:S35–8. doi: [10.1016/j.ijantimicag.2004.03.001](https://doi.org/10.1016/j.ijantimicag.2004.03.001). [PubMed: [15364304](https://pubmed.ncbi.nlm.nih.gov/15364304/)].
 27. Sabir N, Ikram A, Zaman G, Satti L, Gardezi A, Ahmed A, et al. Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance. *Am J Infect Control*. 2017;**45**(10):1101–5. doi: [10.1016/j.ajic.2017.05.009](https://doi.org/10.1016/j.ajic.2017.05.009). [PubMed: [28629757](https://pubmed.ncbi.nlm.nih.gov/28629757/)].
 28. Maheshwari M, Ahmad I, Althubiani AS. Multidrug resistance and transferability of blaCTX-M among extended-spectrum beta-

- lactamase-producing enteric bacteria in biofilm. *J Glob Antimicrob Resist*. 2016;**6**:142-9. doi: [10.1016/j.jgar.2016.04.009](https://doi.org/10.1016/j.jgar.2016.04.009). [PubMed: [27530857](https://pubmed.ncbi.nlm.nih.gov/27530857/)].
29. Cabral AB, Maciel MAV, Barros JF, Antunes MM, Barbosa de Castro CMM, Lopes ACS. Clonal spread and accumulation of beta-lactam resistance determinants in *Enterobacter aerogenes* and *Enterobacter cloacae* complex isolates from infection and colonization in patients at a public hospital in Recife, Pernambuco, Brazil. *J Med Microbiol*. 2017;**66**(1):70-7. doi: [10.1099/jmm.0.000398](https://doi.org/10.1099/jmm.0.000398). [PubMed: [27902398](https://pubmed.ncbi.nlm.nih.gov/27902398/)].
30. Azargun R, Sadeghi MR, Soroush Barhaghi MH, Samadi Kafil H, Yeganeh F, Ahangar Oskouee M, et al. The prevalence of plasmid-mediated quinolone resistance and ESBL-production in Enterobacteriaceae isolated from urinary tract infections. *Infect Drug Resist*. 2018;**11**:1007-14. doi: [10.2147/IDR.S160720](https://doi.org/10.2147/IDR.S160720). [PubMed: [30087570](https://pubmed.ncbi.nlm.nih.gov/30087570/)]. [PubMed Central: [PMC6061675](https://pubmed.ncbi.nlm.nih.gov/PMC6061675/)].
31. Zeng KJ, Doi Y, Patil S, Huang X, Tian GB. Emergence of the Plasmid-Mediated *mcr-1* Gene in Colistin-Resistant *Enterobacter aerogenes* and *Enterobacter cloacae*. *Antimicrob Agents Chemother*. 2016;**60**(6):3862-3. doi: [10.1128/AAC.00345-16](https://doi.org/10.1128/AAC.00345-16). [PubMed: [26976876](https://pubmed.ncbi.nlm.nih.gov/26976876/)]. [PubMed Central: [PMC4879368](https://pubmed.ncbi.nlm.nih.gov/PMC4879368/)].