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# Isolation, Identification and antibiotic resistance profile distribution of clinical E. coli in Iraqi patients

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	Introduction: Escher	richia coli is a Gram-negative, rod-shaped bacterium that belongs to			
	the Enterobacteriace	eae family and the Gammaproteobacteria class. E. coli is one of the			
	most prevalent organisms that cause bacterial illnesses. Globally, the rise of multidrug-				
	resistant E. coli poses a significant risk to public health. Antimicrobial resistance in E.				
	coli is causing havoc in the world's healthcare system				
	Aim this study: The goal of this study was to find out how resistant clinical isolates of E.				
	coli were to antibiotics.				
	Methods: Between November 2021 and January2022, a total of 67 clinical samples were				
H	obtained from patients, including urine, wound, ear, feces, and sputum samples. The				
AC	Vitek-2 compact system was then used to confirm E. coli and test susceptibility to				
TR	various antibiotics.				
ABS	Result: E. coli was discovered to be extremely susceptible to ertapenem, imipenem and				
	amikacin (97.0%), but resistant to ampicillin (94%), and through this study, different				
	resistance patterns to E. coli appeared to us, ranging from MDR, XDR, and PDR.				
	Conclusion: E. coli isolated from different clinical specimens exhibited varying antibiotic				
	sensitivity patterns, with high resistance to conventional antibiotics. Ertapenem,				
	imipenem, and amikacin were found to be the most effective antibiotics against E. coli				
	isolates. Clinical isolates of E. coli, on the other hand, had high resistance to ampicillin,				
	Trimethoprim/ sulfamethoxazole and Ceftazidime Therefore, it is advised that				
	physicians conduct	antibiotic sensitivity testing to choose the most effective			
	medications.				
ŀ	Keywords:	E. coli, Antibiotic, resistance, vitek2			

# **1-Introduction**

*Escherichia coli* is a Gram-negative, rodshaped bacterium that belongs to the Enterobacteriaceae family and the Gammaproteobacteria class. One of the most well-studied microorganisms is *E. coli* (1,2). This bacterium's pathogenicity is attributed to the presence of various virulence factors (3). these virulence factors contribute to the establishment of pathogenic resistance against immune defense (4). Antimicrobial resistance is a major public health issue all over the world (5). Inappropriate antibiotic use by humans, factories, and farms, poor hygiene and sanitation, and ineffective infection prevention and control in healthcare settings are all thought to be important factors in the emergence and spread of antibiotic-resistant bacteria (6). Multidrug-resistant (MDR) and ESBL-producing E. coli, which can cause lifethreatening infections, are a good example of antibiotic resistance (7). Antimicrobial resistance in E. coli is causing havoc in the world's healthcare system. This complicates treatment outcomes, raises treatment costs, and limits therapeutic options, all of which contribute to the global spectral of a postantimicrobial age in which some of the most effective drugs lose their efficacy. Numerous prior researches have demonstrated that the bacterium is growing increasingly resistant to commonly used antibiotics (both newer and older medications). Antimicrobial resistance of *E. coli* is reported to be a significant factor in the failure of infectious illness treatment in impoverished nations (8). The World Economic Forum declared in 2016 that multidrug resistance (MDR) is "one of the great health challenges of our time," and that without immediate action, global deaths from MDR might reach 10 million by 2050. In the clinic, there is a clear need for new antibiotics with novel modes of action (9).

#### 2-Materials and Methods 2-1-Collection of samples

Sixty-seven isolates of *E. coli* were obtained from two hundred and fifty specimens collected from different infections in humans: ear infection, urinary tract infection, sputum, burns, wounds and feces, which were collected from patients of different ages for the period from November 2021 to January2022, in Al Fallujah General Hospital and Fallujah Women and Children Hospital. The isolates were identified grown on Eosin methylene blue as a selective medium for *E. coli*, along with the other media such as MacConkey agar and blood agar.

# 2-2-Isolation and Identification of *E. coli*:

The microbial isolates of pathogenic microorganisms used in this study were incubated under aerobic conditions on brain heart infusion broth (BHI) and incubated overnight at  $37^{\circ}$ C, after which the samples were cultured by loopful on solid media Eosin methylene blue, macConkey agar, blood agar and incubated at  $37^{\circ}$ C for 24 hours. Initial identification of *E. coli* isolates was based on visual characteristics on solid media Eosin methylene blue, macConkey agar, blood agar The Vitek-2 technology was used to confirm the *E. coli* that had been identified.

# 2-3-Antibiotic Susceptibility Testing

The antibiotic susceptibility of the isolates was determined using the Vitek 2 System

# **3-Results**

Two hundred and fifty samples of ear infection, urinary tract infection, wounds, feces, burns were collected from Fallujah teaching hospital and Fallujah Women and Children Hospital, where the ages of the patients ranged between (4-55) years. Among 250 samples were of them ,200 samples were positive growth of which 67 samples grew on the medium of the Eosin methylene blue, while the were negative growth, and rest after morphological, microscopic and biochemical tests were done and Vitek 2 System implementation was done, 67 isolates were obtained from multiple source *E*. coli. Distribution of *E. coli* isolates in the table (3-1). The percentage of samples distributed as follows: ear infections 3%, urinary tract infections 73.1%, wound infections 14.9%, diarrhea 7.5%, sputum 1.5%

Source of sample	No. of sample
Ear	2
Urine	49
Wounds	10
Feces	5
Sputum	1

(3-1) Distribution of *E. coli* isolates according to the source of isolation.

The identification of *E. coli* was primarily based on culture and the Vitek 2 compact system. When cultured on MacConkey agar, the isolate produced vivid pink colonies as illustrated in Figure (3-1) A, while on EMB media, the colonies produced a green metallic shine as illustrated in Figure (3-1) B. The isolates were also grown on blood agar to see if they could lyse red blood cells and produce hemolysis as illustrated in Figure (3-1) C.



Figure (3-1) bacterial growth on three different types of mediums A: On MacConkey agar, you can see the bright pink colonies. B: On EMB media, sheen green metallic colonies. C: Hemolysis on blood agar plates, Colonies that are circular, convex, and smooth.

According to the results of antimicrobial susceptibility testing performed by the Vitek2 Compact System, E. coli isolates exhibited the highest levels of resistance to trimethoprim/sulfamethoxazole (89.5 %). followed by ampicillin at an (94%) rate. In the case of ciprofloxacin, a (59.7%) resistance rate was observed. Ceftriaxone and aztreonam resistance rates were (58%), (58%), cefepime resistance rates were approximately (56.7%), gentamycin resistance rates were (22%), and tobramycin resistance rates was (15%). The level lowest of resistance to both Nitrofurantoin and Amikacin was found to be (2.9%), (2.9%). On the other hand, the resistance of *E. coli* to carbapenems antibiotics (imipenem, ertapenem) was (2.9%). Table (3-2) shows the findings of the *E. coli* antimicrobial susceptibility test. In this study it was found that thirty-one (46.3%) isolates multi drug resistance (MDR), twenty-five (37.3%) isolates were within the extensive drug resistance (XDR), and eleven 16.4% isolates were within the figure (3-2)

Antimicrobial agents	Resistance	Intermediate	Sensitive	
	percentage	percentage	percentage	
	R%	I%	S%	
Amikacin	2(2.9%)	0	65(97.0%)	
Ampicillin	63 (94%)	0	4(5.9%)	
Cefepime	38(56.7%)	0	29(43.2%)	
Ceftazidime	45(67.1%)	0	22(32.8%)	
Ceftriaxone	39(58%)	0	28(41.7%)	
Ciprofloxacin	40(59.7%)	0	27(40.2%)	
Ertapenem	2(2.9%)	0	65 (97.01%)	
Gentamycin	15(22%)	0	52(77.6%)	
Imipenem	2(2.9%)	0	65 (97.01%)	
Levofloxacin	43(64.1%)	0	24(35.8%)	

Tabla	(2-2)	• Antimicro	hial raci	stanco n	attorne	of F	coli
Table	3-2	j: Anumicro	idial resi	stance p	batterns (	01 <i>E.</i>	COII.

Nitrofurantoin	2(2.9%)	0	65(97.0%)
Trimethoprim/	60(89.5%)	0	7(10.4%)
sulfamethoxazole			
Piperacillin/tazobactam	5(7.4%)	0	62(92.5%)
Cefoxitin	10(14.9%)	0	57(85%)
Cefazolin	43(64.1%)	0	24(35.8%)



Figure (3-2) Shows the patterns of resistance of *E. coli* to antibiotic

# Discussion

The results reached by (10), and (11) in which they showed that the ratio of resistance to gentamycin (19.6%), and amikacin (2.1%) was close to the low level of resistance to aminoglycoside obtained in this study. In this study, the resistance of E. coli to ampicillin was (94%), which is close to the results obtained by (12), where the resistance to ampicillin in their study was( 93.3%). While this result was in contrast to what was reached by (13), the sensitivity ratio for ampicillin was (49.5%). Furthermore, (14) found that nitrofurantoin resistance was (5.9%), which was consistent with this study's findings. In another study reached by (15), it was found that the resistance of *E. coli* to nitrofurantoin was (32.0%). The proportion of resistance to cefepime in a prior study by (16) was (64.8%). (17) found that about 65% of the bacteria were resistant to ceftriaxone, but (18,19) found that resistance to cefepime, ceftriaxone, was lower at (35.9%), (36.6%), these results were relatively close to the results obtained in this study. Also this study found resistance to the antibiotic ceftazidime, which was similar to what (20) discovered when he found that 65.5% of the isolates were resistant to

ceftazidime. In contrast, (21) reported that 36% of *E. coli* isolates were resistant to ceftazidime. While the rate of resistance to levofloxacin was 64.1% and this is consistent with a study conducted by the (22), where he found a high resistance to levofloxacin 74.8%. by *E. coli*. Piperacillin/tazobactam resistant was determined (7.4%), this ratio is consistent with the findings (21) found that the resistance to Piperacillin/tazobactam was low to E. coli (5.1%), while the (23) found that the resistance to Piperacillin/tazobactam was (56.4%). The resistance of E. coli to cefoxitin and cefazolin was (14.9%) and (64.1%), respectively, this result does not match what Mark found that the percentage of resistance to cefoxitin and cefazolin was (6%), (13%), respectively. Resistance to trimethoprim/sulfamethoxazole was observed at a high rate of (89.5 %) in this investigation. In contrast to this finding, (24) discovered a (39.7%) resistance rate to trimethoprim/sulfamethoxazole. *E. coli* isolates were resistant to imipenem by (2.9%). This is consistent with the results of (25) which showed that the rates resistance of imipenem by E. coli were (3%). On the other side, in a study conducted by (26), it was found that E. coli bacteria were resistant to ertapenem

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(100%), this is inconsistent with what we found in this study, where the percentage of resistance of *E. coli* to ertapenem was (2.9%).

# Conclusion

In this study, *E. coli* isolates showed a high rate of resistance to different antibiotics (MDR, XDR, PDR), which could indicate: the bacteria's potential to generate a resistance system quickly as well as the ability to acquire it from other strains and sources. In terms of the environment. The investigation also discovered that different isolates have different resistance patterns. The isolates of E. coli showed high resistance toward Ampicillin and Trimethoprim/sulfamethoxazole, whereas the most effective antibiotics against isolates were Carbapenems and Amikacin.

# References

- 1. Jang J, Hur H, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S. Environmental Escherichia coli: ecology and public health implications—a review. Ι Appl Microbiol. 2017;123(3):570-81.
- 2. Fatima R, Aziz M. Enterohemorrhagic Escherichia Coli (EHEC). StatPearls; StatPearls Publ Treasure Island, FL, USA. 2019;
- Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic Escherichia coli (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Front Microbiol. 2017;8:1566.
- 4. Meena M, Swapnil P, Zehra A, Aamir M, Dubey MK, Patel CB, et al. Virulence factors and their associated genes in microbes. In: New and future developments in microbial biotechnology and bioengineering. Elsevier; 2019. p. 181–208.
- 5. Bryce A, Hay AD, Lane IF, Thornton H V, Wootton M, Costelloe C. Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by Escherichia coli and association with routine use of

antibiotics in primary care: systematic review and meta-analysis. bmj. 2016;352.

- Boonyasiri A, Tangkoskul T, Seenama C, Saiyarin J, Tiengrim S, Thamlikitkul V. Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. Pathog Glob Health. 2014;108(5):235–45.
- 7. Pormohammad A, Pouriran R, Azimi H, Goudarzi M. Prevalence of integron classes in Gram-negative clinical isolated bacteria in Iran: a systematic review and meta-analysis. Iran J Basic Med Sci. 2019;22(2):118.
- 8. Tuem KB, Gebre AK, Atey TM, Bitew H, Yimer EM, Berhe DF. Drug resistance patterns of Escherichia coli in Ethiopia: a meta-analysis. Biomed Res Int. 2018;2018.
- 9. Koo HB, Seo J. Antimicrobial peptides under clinical investigation. Pept Sci. 2019;111(5):e24122.
- 10. Demir T, Buyukguclu T. Evaluation of the in vitro activity of fosfomycin tromethamine against Gram-negative bacterial strains recovered from community-and hospital-acquired urinary tract infections in Turkey. Int J Infect Dis. 2013;17(11):e966–70.
- Abujnah AA, Zorgani A, Sabri MAM, El-Mohammady H, Khalek RA, Ghenghesh KS. Multidrug resistance and extended-spectrum β-lactamases genes among0 Escherichia coli from patients with urinary tract infections in Northwestern Libya. Libyan J Med. 2015;10(1).
- 12. Al-Taai HRR. Antibiotic resistance patterns and adhesion ability of uropathogenic Escherichia coli in children. Iraqi J Biotechnol. 2018;17(1).
- 13. Chervet D, Lortholary O, Zahar J-R, Dufougeray A, Pilmis B, Partouche H. Antimicrobial resistance in community-acquired urinary tract infections in Paris in 2015. Med Mal Infect. 2018;48(3):188–92.

- Haji SH, Jalal ST, Omer SA, Mawlood AH. Molecular detection of SHV-Type ESBL in E. coli and K. pneumoniae and their antimicrobial resistance profile. Zanco J Med Sci (Zanco J Med Sci). 2018;22(2):262–72.
- 15. Taher I, Almaeen A, Aljourfi H, Bohassan E, Helmy A, El-Masry E, et al. Surveillance of antibiotic resistance among uropathogens in Aljouf region northern Saudi Arabia. Iran J Microbiol. 2019;11(6):468.
- 16. Hussein NR, Daniel S, Salim K, Assafi MS. Urinary tract infections and antibiotic sensitivity patterns among women referred to Azadi teaching hospital, Duhok, Iraq. Avicenna J Clin Microbiol Infect. 2017;5(2):27–30.
- 17. Assafi MSA, Ibrahim NMR, Hussein NR, Taha AA, Balatay AA. Urinary bacterial profile and antibiotic susceptibility pattern among patients with urinary tract infection in duhok city, kurdistan region, Iraq. Int J Pure Appl Sci Technol. 2015;30(2):54.
- 18. Yilmaz EŞ, Aslantaş Ö. Phylogenetic group/subgroups distributions, virulence factors, and antimicrobial susceptibility of Escherichia coli strains from urinary tract infections in Hatay. Rev Soc Bras Med Trop. 2020;53.
- 19. Ho HJ, Tan MX, Chen MI, Tan TY, Koo SH, Koong AYL, et al. Interaction between antibiotic resistance, resistance genes, and treatment response for urinary tract infections in primary care. J Clin Microbiol. 2019;57(9):e00143-19.
- 20. Jahani S, Ghamgosha M, Shakiba A, Hassanpour K, Taheri RA, Farnoosh G. Assessment of third generation cephalosporin (Ceftazidime and ceftriaxone) resistant escherichia coli strains isolated from zahedan hospitals by tracing the TEM gene. J Appl Biotechnol Reports. 2017;4(1):547-52.
- 21. Tabasi M, Karam MRA, Habibi M, Yekaninejad MS, Bouzari S.

Phenotypic assays to determine virulence factors of uropathogenic Escherichia coli (UPEC) isolates and their correlation with antibiotic resistance pattern. Osong public Heal Res Perspect. 2015;6(4):261–8.

- Stone GG, Hackel MA. Antimicrobial activity of ceftazidime-avibactam and comparators against levofloxacinresistant Escherichia coli collected from four geographic regions, 2012– 2018. Ann Clin Microbiol Antimicrob. 2022;21(1):1–9.
- 23. Kumar PM, Jaiswal NK, Singh BK, Sharan S, Kumar R, Tiwari SK. Antimicrobial susceptibility patterns of uropathogenic Escherichia coli and their prevalence among people in and around Dhanbad, Jharkhand. East J Med Sci. 2017;1–3.
- 24. Lai C-C, Chen Y-S, Lee N-Y, Tang H-J, Lee SS-J, Lin C-F, et al. Susceptibility rates of clinically important bacteria collected from intensive care units against colistin, carbapenems, and other comparative agents: results from Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). Infect Drug Resist. 2019;12:627.
- 25. Shams S, Hashemi A, Esmkhani M, Kermani S, Shams E, Piccirillo A. Imipenem resistance in clinical Escherichia coli from Qom, Iran. BMC Res Notes. 2018;11(1):1–5.
- 26. Ghazali MF, Chai MH, Sukiman MZ, Mohamad NM, Ariffin SMZ. Prevalence of carbapenem-resistant Escherichia coli (CREC) within farm animals in Malaysia. Int J Infect Dis. 2020;101:534–5.