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| RESEARCH ARTICLE

Prevalence of Colistin Pan Resistance among Multidrug-Resistant and Extensively Drug-Resistant *Escherichia Coli O157:H7*

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| ABSTRACT
Antimicrobial resistance is an important problem threatening human health. With the appearance of colistin-resistant bacteria, the World Health Organization and Centers for Disease Control and Prevention are declaring a global emergency and an alarming disaster that goes back to the time before antibiotics. The usage of colistin rises as a result of the global growth of Enterobacteriaceae, which produces carbapenemase and certainly causes the emergence of resistance to investigate the prevalence of colistin resistance among multidrug-resistant and extensively drug-resistant *E. coli*. The cross-sectional study included 140 swab samples and 200 urine samples that were collected from patients attending Al Imam Al Hussein Medical City in Karbala. The identification of bacterial isolates and the pattern of antibiotic resistance were determined using the fully automated VITEK 2 compact system in addition to the manual antibiotic resistance testing confirmation. The isolates were highly resistant to Ticarcillin (94.4%), Trimethoprim/ Sulfamethoxazole (91.1%) and Piperacillin (87.3%). In contrast, colistin had the lowest (4.2%) out of the total multi-drug resistant (MDR) strains that formed (46.4%) and the extensively-drug resistant (XDR) strains (25.4). Antimicrobial resistance is one of the biggest health problems facing people today. In Iraq, the appearance of colistin resistance (2.8%) among extensively drug-resistant Escherichia coli O157:H7 may lead to failure of treatment, especially among burn and UTI patients. It is urgently recommended to lower the occurrence of antibiotic resistance through cautious antibiotic usage and stringent infection control protocols, which are priorities.

| KEYWORDS
Escherichia coli; Colistin; Multidrug-Resistant; Extensively Drug-Resistant; Pan Drug-Resistant.

| ARTICLE INFORMATION
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1. Introduction

Drug resistance has been a significant issue since the discovery of antibiotics and the declaration made by Nobel Prize winner Alexander Fleming (Magalhães *et al*. 2021). Several definitions have been used to describe different groups of antibiotic-resistant bacteria (Nabal Díaz et al. 2019). According to the definition recognized as most acceptable, the one determined by the Centers for Disease Control and Prevention [CDC] and the European Centre for Disease Prevention and Control [ECDC], acquired antibiotic resistance patterns have been divided into multidrug-resistant [MDR], extensively drug-resistant [XDR], and pan-drug-resistant [PDR] strains. PDR was defined as the lack of sensitivity to every antimicrobial agent in every category. In contrast, XDR was characterized by the absence of sensitivity to at least one antimicrobial agent in all categories, with the exception of two or fewer, meaning that the bacteria are still susceptible to only one or two categories. MDR is described as the developed resistance to one or more antimicrobial agents in three or more categories (Magiorakos *et al*. 2011). Colistin is known to be the last choice and an essential tool in the battle against microorganisms resistant to many drugs (Nation et al., 2009). It is regarded as one of the most widely used antibiotics of choice for the management of gram-negative bacilli [GNB] infections that are both MDR and XDR

(Karaaslan *et al.* 2016). Colistin was extracted from the polymyxin containing plant *Paenibacillus polymyxa* (Poirel et al. 2017). The emergence of colistin-resistant *E. coli O157:H7* bacteria in recent years is a cause for concern (Bastidas-caldes et al., 2023). E. coli is the predominant bacterial pathogen associated with illnesses acquired in both hospitals and the community, often seen in cases of urinary tract and bloodstream infections (Aghapour *et al*. 2019). The worldwide rise of colistin resistance in several countries throughout Asia, Europe, and some African nations has become a significant cause for worry. Colistin resistance dissemination is characterized by its capacity to spread horizontally by conjugative plasmids either vertically through chromosomal- mutation (Schwarz and Johnson, 2016). In 2015, China recognized the emergence of colistin resistance, mainly due to the presence of a gene called mobile colistin resistance (mcr-1) (Aghapour *et al*. 2019). Colistin resistance arises from alterations in the bacterial lipopolysaccharide (LPS), leading to an augmented positive charge of the LPS. This diminishes its affinity for colistin (Forde et al.,2018). Furthermore, the mcr-1 gene has the ability to disseminate to other bacteria through horizontal gene transfer (Yamamoto *et al.,* 2022). The proliferation of colistin resistant strains of *E. coli* hampers the availability of effective treatment choices, resulting in clinical complexities like pneumonia or sepsis, prolonged stays in hospitals, increased costs for treatment and additionally elevated death rates (Wangchinda et al., 2018). Multiple factors contribute to the development of colistin resistance in *E. coli.* The indiscriminate and erroneous use of antibiotics in both the field of human health and veterinary practice has had a noteworthy repercussion. The misuse of colistin in agriculture to enhance animal growth has led to the emergence of resistance strains. Global dissemination has also been impacted by inadequate infection control protocols, below-standard sanitation practices, and the migration of both people and animals over international boundaries (Dawadi et al., 2021). Previous studies conducted in Iraq investigated colistin resistance. One study conducted in 2019 in Al Hilla City showed that the frequency of colistin resistance in E. coli bacteria was 11.3% (Hasan and Alwan, 2019). Another study carried out in Al-Qadisiyah in 2022 examined the same problem. The results indicate that *E. coli* bacteria exhibit the highest level of resistance to colistin (8.5%) compared to other species within the *Enterobacteriaceae* family (Ameen, 2022). However, no study was conducted in Karbala to detect colistin resistance among clinical isolates. So, the current study aimed to investigate the prevalence of colistin pan-resistance among MDR and XDR *E.coli O157:H7* isolated from patients in Karbala, Iraq.

2. Methodology

2.1 Study Design and Population

This research is cross-sectional and conducted at Imam AL-Hussein Medical City in Karbala, Iraq. The study was conducted from July 1st to December 2nd, 2023.

2.2 Sample collection

Inpatients and outpatients with clinical signs of urinary tract infection (UTI) (dysuria, loin pain, fever, frequent urination, feeling the urge to pee even while the bladder is empty) had 200 midstream urine samples taken in a clean (sterile) container. Additionally, 140 swab samples were collected from inpatients with burns immediately after they were cleaned and debrided. All samples were appropriately marked, showing the source, date, time of collection and patient's age and sex.

2.3 Cultivation and Isolation Bacteria

Urine and swab samples were cultured on MacConkey agar to inhibit the growth of gram-positive bacteria. Identification of *E coli* serotype O157:H7 was done by culturing isolates on sorbitol MacConkey agar (Himedia). After that, incubate at 37°C for 24 hours. Following the incubation period, the cultures were examined to see whether there was significant growth (Thangavelu et al., 2022).

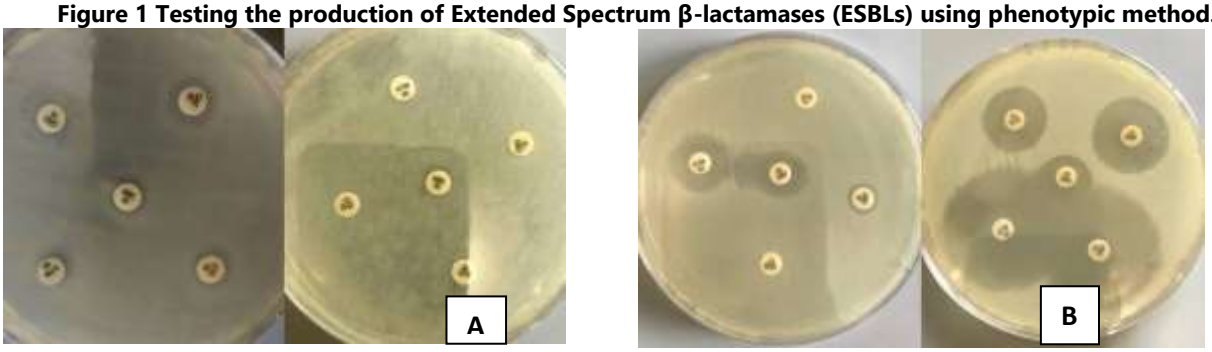
2.4 Identification and Antimicrobial Resistance

The identification and antimicrobial resistance (minimum inhibitory concentrations) of *E. coli* testing by using a fully automated VITEK 2 compact system Gram-negative ID card and an AST (N222) card. The procedure of work was according to the standard operating procedure for VITEK 2 COMPACT. AST data were interpreted using the Clinical and Laboratory Standards Institute (CLSI) standards according to the manufacturer's instructions from BioMérieux, France, and the Advanced Expert System.

2.5 Phenotypic detection of extended spectrum β-lactamases (ESBLs) production

All the potential ESBLs producing *E. coli* isolates were tested according to Batchoun *et al*. 2009. 30µg antibiotic discs of Cefotaxime, Ceftazidime, Ceftriaxone, and Azetreonam surrounded by a central disc containing amoxicillin-clavulanic acid (20µg amoxicillin plus 10µg clavulanic acid), 30mm centre to centre on Mueller Hinton agar plates seeded with organisms being tested for ESBLs production. Plates on Mueller-Hinton agar plates inoculated with bacteria are being investigated for Extended-Spectrum Beta-Lactamase (ESBL) production. Re-incubated aerobically at 37°C for 18 hours. ESBLs positive test was indicated by an increase in zones of inhibition towards amoxicillin-clavulanic acid antibiotic discs. Interpretation of results was performed according to the guideline of CLSI 2023, with positive and negative results reading in **(Figure 1)**.

Figure 1 Testing the production of Extended Spectrum β-lactamases (ESBLs) using phenotypic method.



Phenotype- screening and assurance of extended-spectrum β-lactamases (ESBLs) synthesis. (A); a positive screening test for ESBL production detected according to the CLSI screening technique with 3rd-generation cephalosporins and aztreonam discs and amoxicillin-clavulanic acid; (B): Phenotypically confirmed negative ESBLs producing E. coli.

2.6 Statistical Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software version 26. P value < 0.05 was considered as a significant threshold to calculate the statistical significance.

3. Results

A total of 340 patient samples were investigated for the growth of *E. coli*. Isolates were (85.9%) isolated from 200 urine samples of urinary tract-infected inpatients and outpatients, in addition to (14.1%) of *E. coli* isolated from 140 swab samples obtained from inpatients suffering from burns with different burn degrees **(Table 1).**

Table 1 . Numbers and percentage of *E. coli* found in various clinical samples

Specimen	Specimen Type	Number of specimens/ 340	*E.coli* No. (%)
Urinary tract infection	Urine	200(58.8%)	61 (85.9 %)
Burn	Swab	140(41.2%)	10 (14.1%)
Total		340(100%)	71 (100%)

In the isolated samples, the patients ranged in age from 2- 68 years. 35.2% (25/71) of the samples were male, and the age range of 41–68 years old had the highest percentage of *E. coli* isolates, 22 of which were *E. coli* serotype *O157:H7*. There were 64.8% (46.8/71) *E. coli* isolates from females, the most isolates in the 21–60 age group, 43 of which were serotype *O157:H7* (Table 2).

Table 2. Distribution of *E coli O157:H7* according to age grouping and sex.

Age-groups	**Sex**					
	Male			Female		
	O157:H7	Non O157:H7	O157:H7	Non O157:H7	Total	
1-20	0	0	5(11.7%)	1(33.4%)	6(8.5%)	P value = 0.002 Significant
21-40	3 (13.6%)	1(33.3%)	19(44.2%)	1(33.3%)	24(33.8%)	
41-60	9(41%)	2(66.6%)	15(34.8%)	1(33.3%)	27(38%)	
61-80	10(45.4%)	0	4(9.3%)	0	14(19.7%)	
Total	22(100%)	3(100%)	43(100%)	3(100%)	71(100%)	

According to the CDC categorization, the distribution of *E. coli* collected from the urine samples was as follows: 28% were sensitive, 45.8% of the isolates were MDR, and 24.6% of the isolates were XDR. A percentage of 1.6% of the PDR that was isolated showed resistance to colistin and all antibiotic classes. Whereas, for the swab samples, 10% of the isolates were sensitive, (50%) were MDR and (30%) were XDR, and a percentage of (10%) of PDR that was isolated showed resistance to colistin and all types of antibiotic **(Table3).**

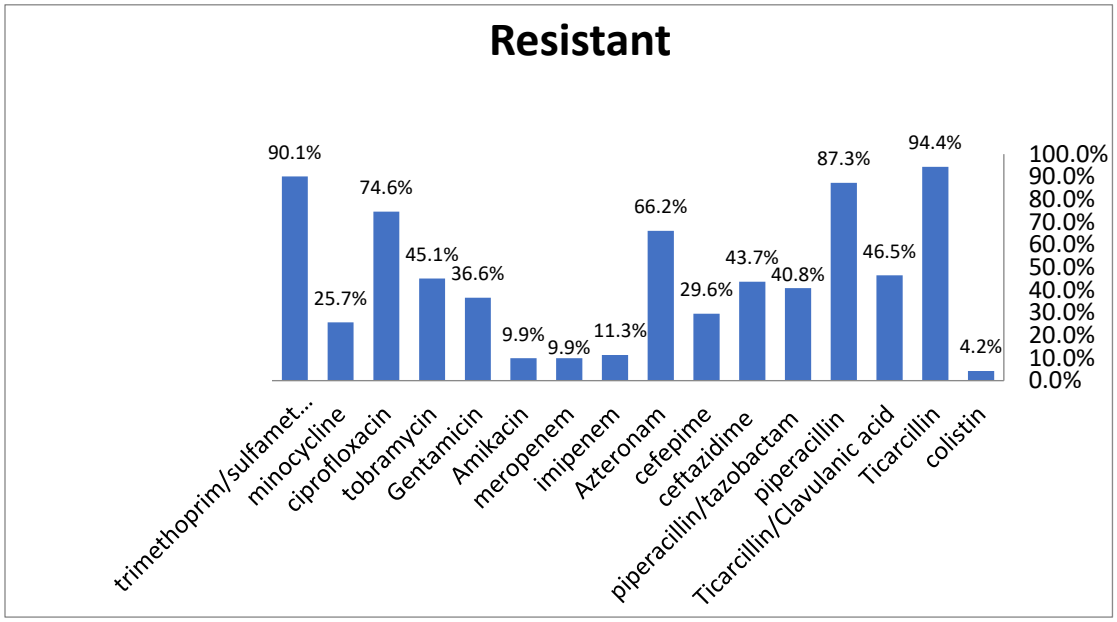
Table 3 *E coli* categorization according to the CDC definition.

Type of sample	NOT MDR		MDR		XDR		PDR		Total
	O157:H7	NON O157:H7	O157:H7	NON O157:H7	O157:H7	NON O157:H7	O157:H7	NON O157:H7	
Urine	14(23%)	3(5%)	26(42.6%)	2(3.2%)	14(23%)	1(1.6%)	1(1.6%)	0	61(100%)
Swab	1(10%)	0	5(50%)	0	3(30%)	0	1(10%)	0	10(100%)
Total	18(25.4%)		33(46.4%)		18(25.4%)		2(2.8%)		71(100%)

3.1 Phenotypic detection of antibiotic resistance

The results of susceptibility testing conducted using the automated VITEK2 system, PDR isolates showed colistin resistance (4.2%), Meropenem resistance (9.9%), and imipenem resistance (11.3%). Moderate resistance was recorded to Piperacillin/ Tazobactam (36.6%), Gentamicin (29.6%), Cefepime (25.4%) and Minocycline (23.9%). MDR show high resistance to Ticarcillin (94.4%), Trimethoprim /Sulfamethoxazole (90.1%), Piperacillin (87.3%), Ciprofloxacin (74.6%) and Azteronam (66.2%) **(Figure 2).**

Figure 2. Antibiotic resistance profile of E.coli



4. Discussion

E. coli is a part of the normal flora found in the intestines; therefore, it can readily pass from person to person in situations where personal hygiene is lacking in both men and women. *E. coli* is the main organism responsible for nosocomial UTIs (Nagarjuna et al., 2022). *E. coli* may seriously injure both humans and animals. On the other hand, if duct barriers rupture or host immunological abnormalities occur, infections may result. Fecal *E.coli* colonizes the urethra, travels through the urinary system, bladder, and sometimes the kidneys, and causes pyelonephritis in instances of UTI (Shrestha *et al*., 2022). *Escherichia coli O157:H7* is a serotype of one of the Shiga like toxin producing types; it is a significant pathogen transmitted by food and water, leading to diarrhoea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) in humans. It affects the digestive system and causes abdominal pains along with bloody diarrhea. *E. coli O157: H7* is transmitted via the fecal oral route when infected, undercooked beverages and meals are consumed, and may also spread via person to person contact by faecal shedding as well as is responsible for around (11%) of illnesses (Lim *et al.*, 2010). Additionally, they were isolated from people with burns since the *E.coli O157:H7* that was identified in those patients showed significant resistance. The research demonstrated a significant prevalence of *E.coli* in urine samples (85.9%) compared with swab samples (14.1%). Prevalence of *E. coli* (65 % in females and 35% in males) according to the results recorded by Taqi, 2023. Of the total population, 63.72% were females, and 36.28% were men, which is inconsistent with the result recorded by Sabri (Sabri and Kareem, 2020). The frequency is 90% in females and 10% in men. Females are more exposed to illness and infection due to their unique body physiology and lifestyle. Additional aspects include the physiological characteristics of the female reproductive system, such as a relatively shorter urinary tract and a reduced distance between the anus and the urethral opening. Menopause, pregnancy, decreased estrogen levels, and sexual intercourse might facilitate the migration of germs from close to the vagina to the urethra. Women, especially those between the ages of 21 and 60, have a much

greater likelihood of experiencing UTIs compared to males. However, the risk of infection is even higher among women aged 41 to 80 years. This is because, after the age of 50, the frequency of UTIs steadily rises. Urinary tract infections (UTIs) that affect adult males include prostatitis, epididymitis, orchitis, pyelonephritis, cystitis, urethritis, and infections associated with urinary catheters. UTIs in males are often linked to anatomical anomalies, which sometimes need surgical intervention due to the many protective mechanisms of the male urinary system. The results of our research are comparable to the recorded results by Magliano et al. 2012. Results confirm that the age and sex of patients play a crucial role in identifying the causes of UTIs. They may enhance accuracy in identifying the specific microorganisms responsible for the infection and provide useful data for initial therapy. As to the ECDC and CDC, acquired resistance profiles may be classified into three types. Based on the study's results, MDR *E. coli* constituted 46.4% of all isolates, showing resistance to three or more antibiotic groups. Additionally, 25.4% of isolates were identified as XDR, displaying resistance to all antibiotic groups, with at least one antibiotic in each group, except for two groups or less. The incidence of pan-colistin-resistant *E. coli* is 2.8%. These strains exhibit resistance to all antibiotic classes, including colistin, which is the last antibiotic used for treating infections caused by Gram-negative bacteria. The incidence might be caused by insufficient infection control protocols and the incorrect administration of bactericidal drugs. In addition, colistin is used in the poultry food production industry, while it is often used in our nations to enhance the development of animals used for food production (Falagas and Kasiakou, 2005). On the other hand, our results were observed to be lower than those reported from Al Hilla City, which found that 11.3% of *E. coli* bacteria were resistant to colistin (Hasan and Alwan, 2019). The probable reasons that may lead to the appearance of colistin resistance among clinical isolates in Karbala City might be the excessive use of antibiotics during the COVID-19 pandemic and the transfer of plasmid mediated resistance from animal sources through contaminated foods and recurrent urinary tract infections treated with empirical therapy (Bodro et al., 2015).The development of pan colistin resistant *E. coli* is very concerning and needs to be closely monitored and controlled, especially in Karbala City, where millions of foreign visitors come every year to visit the holy shrine of Imam Al Hussein, particularly during the Arba'in pilgrimage the world's largest annual public gathering (Nikishina and Majlesi, 2022). Further research is required to check colistin resistance in strains rather than *E. coli*.

5. Conclusion

Understanding the prevalence of colistin pan-resistance within MDR and XDR E. coli O157:H7 is crucial because it helps assess the risk of infections becoming untreatable with available antibiotics. This information is important for developing strategies to combat the spread of antibiotic resistance and for improving treatment options for infections caused by resistant strains. The study results revealed an alarming pattern of antibiotic-resistant E. coli O157:H7 isolated from UTIs samples. The prevalence of MDR was 46.4%, indicating resistance to a minimum of three antibiotic classes. The percentage of XDR cases was 25.4%, indicating resistance to nearly all classes. A pan-colistin-resistant strain accounts for 2.8% of cases. The study posits that several elements, such as the overuse of antibiotics in the context of the COVID-19 pandemic, food-mediated transmission of resistance from animals, and recurrent urinary tract infections, are the main factors leading to the emergence of colistin resistant strains. The research emphasizes the necessity for surveillance and management of antibiotic resistance. Enhanced strategies for mitigating the dissemination of antibiotic-resistant microorganisms. The current study investigated the incidence of colistin resistance in E. coli and in Karbala city only; it is necessary to investigate colistin pan resistance in other bacterial strains and in all Iraqi cities.

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Ethical Declarations

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Ethics Approval and Consent to Participate: The Ethical Approval Committee of the Faculty of Medicine, Jabir Ibn Hayyan for Medical and Pharmaceutical Sciences.

Competing Interests: The authors declare that there is no conflict of interest.

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