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Original Article

Phenotyping and Genotyping Evaluation of *E. coli* Produces Carbapenemase Isolated from Cancer Patients in Al-Basrah, Iraq

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Abstract

One of the most important nosocomial organisms that cause urinary tract infections (UTIs) in cancer patients is *Escherichia coli*. A significant cause of concern in managing UTIs is the development of carbapenem-resistant bacteria. *Escherichia coli* with carbapenem resistance has become a more serious problem, particularly in Iraq. In this regard, the present study aimed to estimate the prevalence of carbapenem-resistant *E. coli* in Al-Basrah, Iraq. Conventional tests and the Vitek®2 system were used to identify the isolates and determine the susceptibility of *E.coli* isolates to antimicrobials. In addition, *E.coli* isolates were tested by mCIM and eCIM methods. Moreover, the major carbapenemase genes, including bla_{SPM}, bla_{IMP}, $bla_{VIM,}$ and bla_{KPC} were detected by polymerase chain reaction. In total, 120 urine samples were collected from cancer patients who were suspected of having urinary tract infections at Basrah Center of Oncology Al-Sader Teaching Hospital, Basrah, Iraq. Identification of bacterial growth by using biochemical tests revealed different bacterial species. The most frequent bacteria were *E. coli* (n=22, 53.65%) isolates. The results showed that 13 (59.09%) and 11 (50%) out of 22 *E. coli* isolates were positive for the production of carbapenemase, based on the eCIM and sCIM, respectively. The majority of *E.coli* in this study possessed the bla_{VIM} gene (n=13, 59.1%), followed by the bla_{KPC} gene (n=5, 22.73%), bla_{IMP} gene (n=5, 22.73%), and bla_{SPM} gene (n=4, 18.18%). There is a spread of more than one type of carbapenemase among the *E. coli* isolates collected from UTI cancer patients in Basrah Hospital. The *E. coli* identified in the current study had a strong capacity to produce carbapenemase enzymes against the four generations of antibiotics, including imipenem and meropenem antibiotics.

Keywords: Cancer, Carbapenemase, *E.coli*, Urinary tract infections

1. Introduction

The immune system of cancer patients is compromised due to underlying malignancy, such as leukemia, as well as the harmful side effects of cancer treatment, such as chemotherapy, radiation, and bone marrow transplantation. This could result in prolonged immunosuppression, increasing the risk of infection and possibly worsening the prognosis (1).

Urinary tract infection (UTI) is one of the most prevalent infections in cancer patients (2). The UTI is identified as one of the most common microbial infections in humans, and in the community and healthcare organizations, its control and prevention is a significant health issue (3). Enterobacteriaceae is the most prevalent UTI, with uropathogenic *Escherichia coli* accounting for around 80-90% of all infections (4, 5).

The β-lactam class of antimicrobials, which includes carbapenems, is a broad-spectrum group of substances with a four-membered β-lactam ring. Carbapenems are commonly used and play a significant role in treating mixed aerobic and anaerobic infections, infections in immune-compromised patients, and infections caused by multiple drug-resistant bacterial strains (6, 7). The development of carbapenemases results in resistance to penicillins, cephalosporins, and carbapenems, which are only a few of the many hydrolyzable β-lactams that the flexible carbapenemase family can break down (8).

Carbapenem resistance is accompanied by a decrease in outer membrane permeability with increased expression of carbapenemase enzymes, simultaneous production of AmpC beta-lactamases, and extended-spectrum beta-lactamases (8). Due to the suppression of the immune system in cancer patients and the high prevalence of bacterial infections, antibiotic resistance is prevalent among them. In this research, the genotypes and phenotypic of isolated bacterial strains of *E. coli* were evaluated in cancer patients with urinary tract infections treated with carbapenems in Basrah Center of Oncology, Al-Sader Teaching Hospital, Basrah, Iraq.

2. Materials and Methods

2.1. Collection of Specimens

From October 2021 to January 2022, 120 urine samples were collected from cancer patients in Basrah Center of Oncology, Al-Sader Teaching Hospital, from patients within the age range of 18-84 years old who were suspected of having urinary tract infections. It is noteworthy that 50 (41.7%) samples belonged to males and 70 (58.3%) samples belonged to females. After explaining the purpose of the study, written informed consent was obtained from the patients.

2.2. Isolation and Identification

Conventional tests identified all the *E. coli* isolates according to Forbes, Sahm (9) and Prescott and Harley (10). The first and the second steps, including the confirmed identification, were performed by the Vitek ®2 system (bioMérieux, France).

2.3. Antibiotic Sensitivity Test

Carbapenem resistance was detected using Imipenem (10 μg; Liofilchem Company) disc according to the method of Clinical and Laboratory Standards Institute CLSI (11) and Sfeir, Hayden (12). The Vitek®2system was used in the current study to detect and confirm the antibiotic susceptibility tests of *E. coli* isolates to detect the ability to produce carbapenemase.

2.4. Molecular Detection

2.4.1. DNA Extraction

DNA was extracted from a pure culture of *E. coli* bacteria using a DNA extraction bacteria kit (Geneaid). Afterward, DNA bands were detected using agarose gel electrophoresis (1%). The presence of the carbapenemases genes was determined using a gene-specific primer targeting bla_{IMP}, bla_{VIM}, bla_{SPM}, and bla_{KPC}. The primers were obtained from the Macrogen Company, Korea (Table 1).

Table 1. Specific primers of antibiotic-resistant genesof the carbapenemases

Primers		DNA Sequences (5'-3')	product Size bp	Reference
bla_{SPM}	F	AAAATCTGGGTACGCAAACG	271	Shoja, Moosavian (13)
	R	ACATTATCCGCTGGAACA		
bla_{VIM}	F	GGTGTTTGGTCGCATATCGCAA	502	Shoja, Moosavian (13)
	R	ATTCAGCCAGATCGGCATCGGC		
bla_{IMP}	F	TCGTTTGAAGAAGTTAACG	568	Shoja, Moosavian (13)
	R	ATGTAAGTTTCAAGAGTGATGC		
bla_{KPC}	F	CATTCAAGGGCTTTCTTGCTGC	498	Mushi, Mshana (14)
	R	ACGACGGCATAGTCATTTGC		

2.4.2. Polymerase Chain Reaction Assay for the Detection of Carbapenemase Genes

polymerase chain reaction (PCR) was carried out in a total reaction volume of 25 µl consisting of 1 µl forward and 1 µl the reverse primer for each gene. Moreover, 2 µl of DNA template and 8.5 µl of nuclease-free water were added to 12.5 µl of PCR master mix (Promega, USA), and the thermal cycling machine (Bioneer, Korea) was programmed for DNA amplification. The program included an initial denaturation step at 95 °C for 5 min, denaturation and annealing at 94 °C and 60 °C, respectively for 45 seconds, and elongation for 1 min at 72 °C (35 cycles). The same program was used for bla_{KPC}, except the annealing temperature was adjusted to 55 °C for 45 s, and all primer sets had a final extension of 72 °C for 7 min (15).

2.5. Statistical Analysis

The statistical analysis was carried out in SPSS software (version 20.0). The collected data regarding group significance were evaluated using the Student's t-test. In the analysis, a *P* value of less than 0.05 was considered statistically significant.

3. Results

In total, 120 samples were collected from cancer patients in Basrah Center of Oncology Al-Sader Teaching Hospital, from patients within the age range of 18-84 years old who were suspected of having urinary tract infections. In terms of gender, 50 (41.7%) samples belonged to males and 70 (58.3%) samples belonged to females. Based on the results, 41 (34.2%) out of 120 urine samples were positive urine cultures, while 79 (65.8%) samples were negative urine cultures.

Identification of bacterial growth by using biochemical tests revealed different bacterial species, the most frequent of which was *E. coli* (n=22, 53.65%). It should be mentioned that the other species were found in 19 isolates (46.34%). The results showed that 13 (59.09%) out of 22 *E. coli* isolates were positive for produced carbapenemase based on the eCIM. Moreover, 7 (31.82%) out of 22 *E. coli* isolates were positive for produced carbapenemase types class A+D while 6 (27.27%) isolates were positive for produced carbapenemase types class A+D and class B. However, 9 (40.91%) isolates were found to be negative for produced carbapenemase based on the EDTA-modified carbapenem inactivation method (eCIM) (Figure 1).

In total, 11 (50%) isolates were positive for produced carbapenemase enzymes according to the sCIM. In addition, 5 (22.73%) isolates yielded intermediate results for the produced carbapenemase enzymes. In contrast, 6 (27.27%) isolates were negative for the produced carbapenemase based on a simplified carbapenem inactivation method (sCIM) (Table 2).

The majority of *E. coli* isolates in the current study possessed the bla_{Vim} gene (n=13, 59.1%) followed by the bla_{KPC} gene (n=5, 22.73%), bla_{IMP} gene (n=5, 22.73%), and bla_{SPM} gene (n=4, 18.18%) (Figures 2-5).

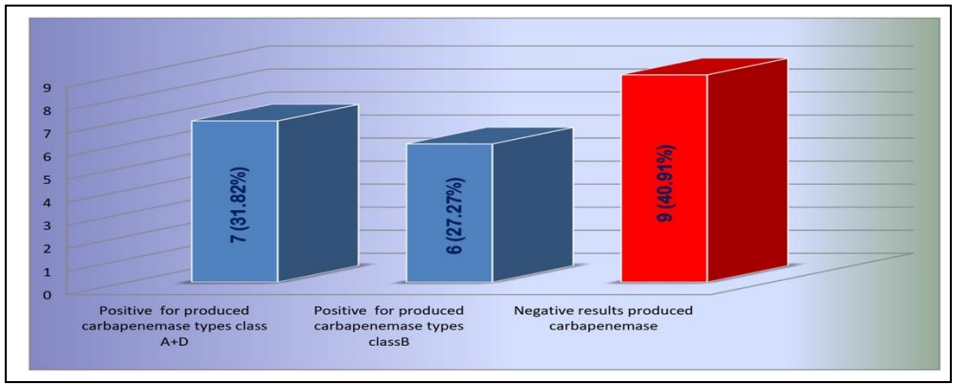

Figure 1. The *E.coli* isolates positive and negative results for produced carbapenemase types class A+D and B

Table 2. The results of produced carbapenemase by using the simplified carbapenem inactivation method (sCIM)

Isolates no.	Isolates	sCIM inhibition zone (no growth) positive results	Scim inhibition zone (6-20) positive results	sCIM inhibition zone (22-23) intermediate results	sCIM inhibition zone≤ (25-26) negative results
2,4,5,12 & 20	*E.coli*	-	-	5	-
3,6,11,15,9 & 29	*E.coli*	-	6	-	-
8,17,24,26,28 & 30	*E.coli*	0	0	0	6
10,14,19,22 & 23	*E.coli*	5	-	-	-
Total	22	5(22.73%)	6(27.27%)	5(22.73%)	6(27.27%)

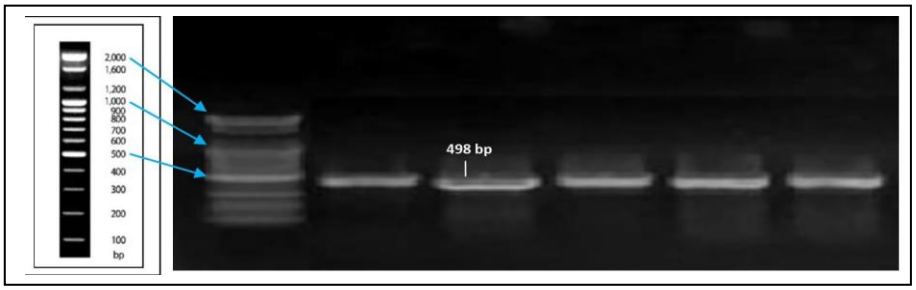

Figure 2. Agarose electrophoresis patterns show PCR amplified products of the *KPC* gene. Lane L: (2000 bp DNA ladder), Lane:(no. 1-5)

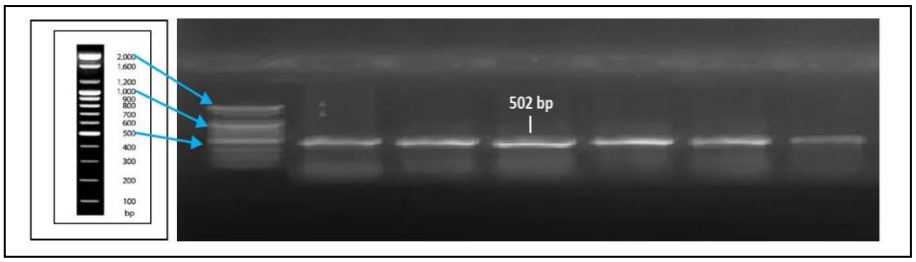

Figure 3. Agarose electrophoresis patterns show PCR amplified products of the *Vim*gene. Lane L: (2000 bp DNA ladder), Lane: (no. 1-6) *Vim* gene bands of *E.coli isolates*

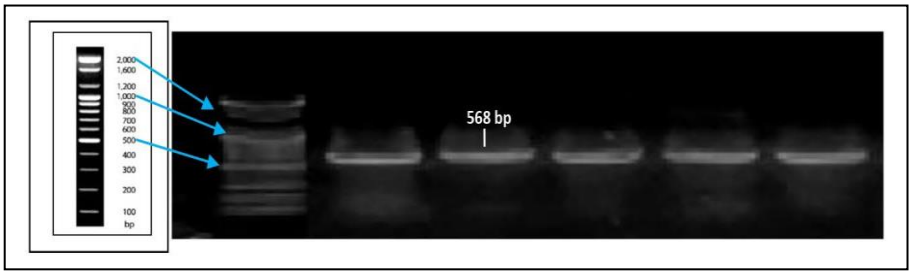

Figure 4. Agarose electrophoresis patterns show PCR amplified products of *IMP*gene. Lane L: (2000 bp DNA ladder), Lane: (no. 1-5) *IMP* gene bands of *E.coli isolates*

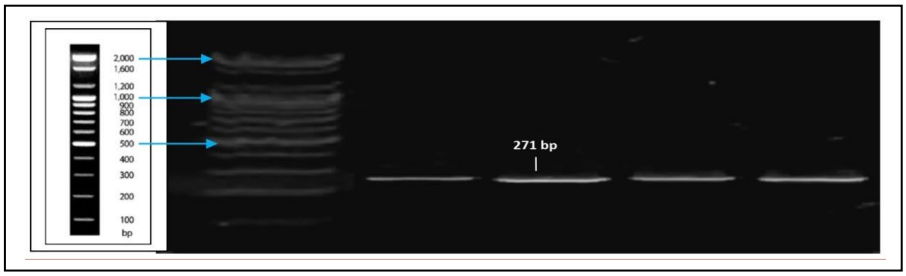

Figure 5. Agarose electrophoresis patterns show PCR amplified products of the *SPM*gene. Lane L: (2000 bp DNA ladder), Lane: (no. 1-4) *SPM* gene bands of *E.coli isolates*

4. Discussion

Cancer patients frequently have impaired immune systems due to chemotherapy and other treatments, which makes them vulnerable to opportunistic infections. The most frequent causes of morbidity and death in cancer patients include UTIs (16). The UTIs caused by *E. coli* strains cause morbidity in hospitalized cancer patients due to host defense impairments (1). Based on the findings of the current study, *E. coli* was the most common bacteria isolated from urinary tract infections with a prevalence rate of 53.65%.

A study performed by Munyi, Macharia (17) included samples of patients suffering from leukemic (31.2%) causative agents due to *E. coli.* Al-naimi and Abbas (18) in their study found *E. coli* in 21 (31.82%) isolates and Shrestha, Wei (19) reported that the most common organism isolated in their research was *E. coli* (58%). In addition, Islam, Akhter (20) declared that the rate of *E. coli* infection in their study was 53%. They described the design and performance of the eCIM, as a phenotypic method for distinguishing serine and metallo-β-lactamase (MBL) carbapenemases encoded by Enterobacteriaceae.

Notably, the eCIM format was created to supplement the mCIM format and requires low-cost "off-the-shelf" materials available to most clinical laboratories, even those in remote locations. The assay is also simple to perform and interpret. It is essential to distinguish between serine and metal-dependent carbapenemases for infection control, prevention, and therapeutic purposes (12). Results of the present study were consistent with those of a study performed by Sfeir, Hayden (12) which showed that 48.7% out of 75 isolates were positive for produced carbapenemase types class A+D. Moreover, 48.7% of the isolates were positive for produced carbapenemase types class A+D and class B.

In comparison, in this research, 45.3% of the isolates were negative for produced carbapenemase. The detection principles of the sCIM and the mCIM are similar since carbapenemases can hydrolyze carbapenem (21). Nevertheless, the sCIM experiment has fewer steps and is more convenient than the mCIM (22). TheVitek®2system was used in the current study to confirm antibiotic susceptibility tests of *E. coli* isolates to detect their ability to produce carbapenemase.

Healthcare-associated infections are frequently caused by Enterobacteriaceae, particularly in cancer patients. These infections respond well to the medicines known as carbapenems, frequently used to treat them. Recently, there has been an increase in resistance to antibiotics of the carbapenem class (23). The isolates that are resistant to carbapenems are linked to high morbidity and death rates, particularly those that generate the enzyme *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) (24). The bla_{VIM}-type MBLs also have larger substrate specificities and a stronger affinity for carbapenems, compared to other MBLs (25).

Isolates expressing bla_{VIM}-type MBLs have been found all over the world. Numerous clinical, animal, environmental, and Enterobacteriaceae samples have been discovered to contain bla_{VIM}-type MBLs. Since then, 71 *VIM* variations have been found in various nations (26). bla_{IMP}-type carbapenemases are capable of hydrolyzing nearly all β-lactams. The popular bla_{IMP}-type carbapenemases have been found in a wide range of Enterobacteriaceae family. The bla_{IMP} genes are typically found on large plasmids with various replicon types or incompatibility types (27). The $bla_{IMP\text{-}1}$ gene was found on a transmissible plasmid, and the enzyme was most similar to $bla_{IMP\text{-}1}$ and featured the typical MBL zinc-binding motif (35.5 %) (28, 29).

The findings indicated the presence and spread of carbapenemase-producing bacteria among bacterial isolates from clinical samples in Basrah, Iraq. This is most likely due to the widespread use of antimicrobials, a selective factor in hastening the emergence of resistant strains containing these enzymes (30, 31). Moreover, resistance genes mediated by transmissible

elements, such as plasmids, transposons, and integrons, play a significant role in increasing the frequency of the spread of these enzymes via horizontal gene transfer between bacterial species (32, 33). As a result of the selective pressure imposed by these drugs, resistant strains will flourish and dominate in these establishments and enter the environment via effluent water and wastewater. The environment will serve as an emergency source for the spread of these strains, which will be added to the existing ones (32, 34).

There is a spread of more than one type of carbapenemase among the *E. coli* isolates from UTI cancer patients in Basrah Hospital. The *E. coli* that was identified in the current study had a strong capacity to produce carbapenemase enzymes against the four generations of antibiotics, including imipenem and meropenem antibiotics.

Authors' Contribution

Study concept and design: A. A. A.
Acquisition of data: S. S. M. A.
Analysis and interpretation of data: A. A. A.
Drafting of the manuscript: A. A. A. and A. A. A.
Critical revision of the manuscript for important intellectual content: S. S. M. A.
Statistical analysis: A. A. A.
Administrative, technical, and material support: A. A. A.

Ethics

The Ethics Committee of Al-Sader Teaching Hospital approved this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

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