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Article

Genotypic Detection of Carbapenems Resistance Genes in Acinetobacter baumannii Isolated from Urinary Tract Infection Patients

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ABSTRACT

Acinetobacter baumannii is a Gram-negative bacterium characterized by its short, round, rod-shaped morphology. It is an opportunistic pathogen that poses a significant threat, particularly to immunocompromised patients, often those with hospital stays lasting less than 90 days. Between June 2022 and July 2023, 214 urine samples were collected from individuals suspected of having urinary tract infections (UTIs). These samples were subjected to antibiotic resistance testing, focusing on detecting specific genes related to carbapenem resistance, namely blaNDM, blaKPC, and blaVIM.The study's results revealed a notable trend in antibiotic resistance among the bacterial isolates. Ceftazidime, cefotaxime, and ceftriaxone, commonly used antibiotics for UTIs, showed a high resistance rate among the tested isolates. This resistance highlights the challenges healthcare professionals face when treating UTIs caused by Acinetobacter baumannii. On the other hand, the isolates displayed a comparatively lower resistance rate to imipenem and meropenem, two necessary carbapenem antibiotics. This lower resistance to carbapenems is encouraging as these drugs are often considered the last line of defense against multidrug-resistant bacterial infections. The presence of carbapenem resistance genes, such as blaNDM, blaKPC, and blaVIM, in the Acinetobacter baumannii isolates is of particular concern. These genes confer resistance to carbapenem antibiotics, crucial for treating severe infections caused by multidrug-resistant bacteria. In conclusion, the study aims to study the growth of antibiotic resistance in Acinetobacter baumannii, especially in urinary tract infections in immunocompromised patients with more extended hospital stays. It also highlights the need for Surveys and periodic examinations to detect the spread of bacteria and their resistance.

Keywords: Carbapenems, UTI, genes, blaNDM, blaKPC, and blaVIM.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative bacterium that is small, spherical, and rod-shaped. An opportunistic bacterium [1]. Regularly, it has been shown to colonize the skin, oropharynx, and respiratory tract. Recently, it has been shown that *A. baumannii* determines a “red alert” in hospital settings due to the emergence of antibiotic resistance [2]. The phenomenon of multi-drug resistance (MDR) organism’s phenomenon has progressively become a cause of severe nosocomial and community-acquired infections [3]. Over time, antibiotics became less effective due to the emergence of many resistance mechanisms, such as secretion of beta-lactamase enzymes, receptor modulation, and others [4]. Carbapenem antibiotics are considered one of the most effective antibiotics today and the least affected by bacterial resistance; the resistant-isotype testing showed that the isolates were effective against a wide range of bacterial illnesses [5]. Carbapenems are the most potent

beta-lactam antibiotics, exhibiting broad-spectrum antibacterial action against Gram-positive and Gram-negative bacteria. Combining a carbapenem and beta-lactam rings gives this compound its peculiar molecular structure[6]. Most beta-lactamases are immune to this mix (ESBLs) of antibiotics, including ampicillin, carbenicillin, and the extended-spectrum beta-lactamases [7]. Gram-negative bacteria resistance to carbapenem is a global public health issue. Such resistance, especially when mediated by genes expressing carbapenemase, rapidly develops and generates significant outbreaks, significantly reducing the treatment options available [8]. Resistance to β-lactams, notable carbapenems, is mainly caused by enzymatic breakdown by -lactamases in the Enterobacteriaceae. The NDM-1, KPC, was first identified in 2008, although NDM-1 was found in water samples from New Delhi since 2006. [9]. The majority of NDM-1 infections are from the Indian subcontinent [10]. Horizontal gene transfer across bacteria promotes the transmission of antibiotic-resistance genes [11]. These plasmids have populations with identical structures but distinct antibiotic drug-resistance cassette compositions [12]. This mechanism has allowed antibiotic resistance genes to spread quickly among Enterobacteriaceae and other pathogens like *Acinetobacter baumanii* [13]. Antibiotic resistance genes [14], such as the extended-spectrum -lactamase CTX-M-15 in *Escherichia coli* ST131 and KPC in *Klebsiella pneumoniae* sequence type (ST) 258, may clonally propagate in successfully pathogenic strains [15,16]. Due to HGT and clonal growth [17], KPC and NDM-1 quickly spread after their first appearance. Comparable mobile elements will likely make KPC and NDM-1 accessible in comparable pathogen populations [18]. This is because KPC and NDM-1 have similar distributions and resistance spectra (both give resistance to practically all B-lactam antimicrobials). Clinical Enterobacteriaceae isolates from Pakistan and the United States [18] tested negative for carbapenemase, NDM-1, and KPC. This research aimed to phenotypically and genetically evaluate the antibacterial efficacy of imipenem and meropenem. The study aimed to investigate antibiotic resistance in Acinetobacter baumannii isolated from urinary tract infections (UTIs). Specific objectives included assessing resistance to common UTI antibiotics, detecting carbapenem resistance genes, and evaluating resistance to carbapenem drugs. The research focused on immunocompromised patients with shorter hospital stays. The findings provide insights into the antibiotic resistance landscape of Acinetobacter baumannii, informing strategies for UTI management.

MATERIALS AND METHODS

Isolation and identification of *Acinetobacter baumanii*

Urine samples were randomly collected from 214 outpatients with suspected UTIs from June 2022 to July 2023. The urine inoculates onto blood agar plates and MacConkey agar. The colony-formed unit method grows a singular, refined bacterial colony. The urine specimens that contain lower than 10^5 CFU / ml are eliminated. All bacteria secluded are resembled according to colony morphologic and criterion microbiological experience as colony morphologic, gram lines, oxidase experience, catalase, IMVIC tests, coagulation trial, and outgrowth in Maconkey agar (OxoidTM). The *Acinetobacter baumanii* suspected colonies were diagnosed depending on the vitec2 system.

Antimicrobial sensitivity test

Disk diffusion method on Muller-Hinton agar medium (Oxoid, UK) was carried out to determine the susceptibility of Acinetobacter baumannii isolates against five standard antibiotic disks including Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Imipenem (10 µg), and Meropenem (10µg). MacFarland microbial suspension was used as an inoculum of approximately 1×10^8 CFU/ml. The cultures were incubated at 37 degrees Celsius for 18h under aerobic conditions, according to Kirby-Baur[19]. Zones of inhibition were calculated and interpreted as recommended by the National Committee for Clinical Laboratories Standard guidelines (Clinical and Laboratory Standards Institute,2023).

PCR for detecting genes

The DNA extraction by extraction DNA kit. The primers used in this study and the PCR thermocycling conditions are described in Tables 1 and 2 for detecting three resistance genes associated with carbapenems.

gene	sequence	bp	Reference
blaNDM	F- GGTTT-GGCGATCTGGTTTTC	699	20
	R- CGGAATGGCTCATCAC-GATC		
blaKPC	F- CGTCTAG-TTCTGCTGTCTTG	798	21
	R- CTTGTCATCCTT-GTTAGGCG		
blaVIM	F-AGTGGTGAG-TATCCGACAG	261	22
	R- ATGAAAGTGCGTGGA-GAC		

Table 1: The primer sequence used in PCR for *Acinetobacter baumannii.*

Gene		Temperature (°C) / Time				Cycles Number
	premier Denature	Denature	Anneal	Extension	last Extension	
blaNDM	95°C -5m	94°C - 1min	55°C-1min	72°C-2min	72°C-5min	30
blaKPC	95°C -5m	95°C - 1min	55°C-1min	72°C-1min	72°C-5min	30
blaVIM	95°C -5m	94°C - 1min	58°C-40sec	72°C-50sec	72°C-6min	34

Table 2: PCR thermal cycling conditions for *Acinetobacter baumannii* resistance gene.

RESULTS AND DISCUSSION

Total bacterial isolates

Out of (214) collected urine samples, the result showed that 184 samples (85.98%) were G- bacterial isolates and 28 samples (13.08%) were G+ bacterial isolates. In contrast, the results showed 2 samples (0.93 %) with no growth, as shown in Figure 1.

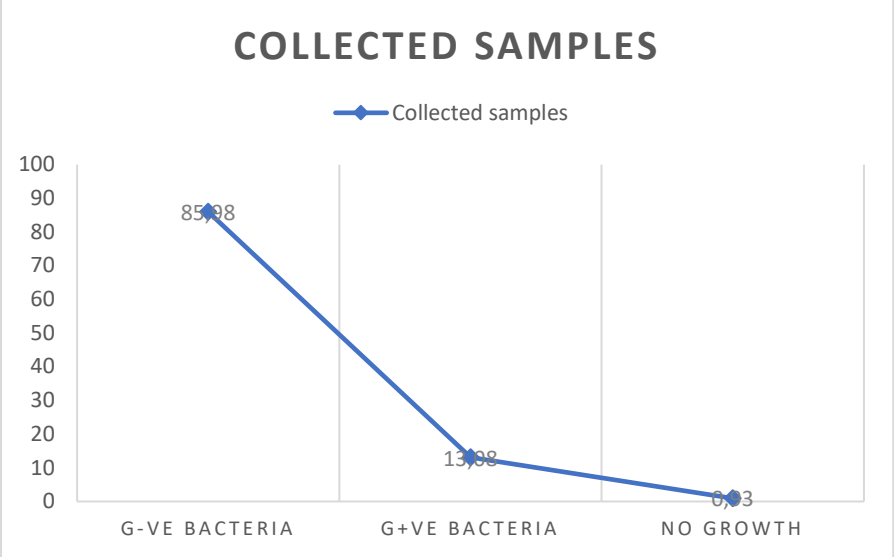

Figure 1: Percentage of total samples isolated from unhealthy with UTI.

The study results proved that the gram-negative bacterial isolates were 89 isolates (48.36%) for *E. coli*, 62 isolates (33.69%) for *K. pneumonia*, 19 isolates (10.32%) for *A. bumanni*, 8 isolates (0.43%) for *Pseudomonas aeruginosa*, and 6 isolates (0.32%) for *Proteus spp*.). Gram-positive bacterial isolates were 21 isolates (75%) for *S. aureus* and 7 isolates (25%) for *E. faecalis*, as shown in figure-2.

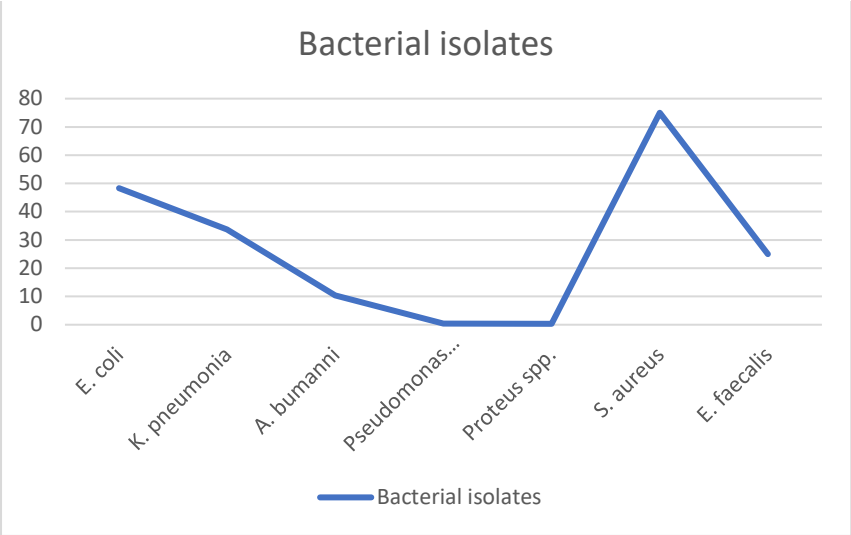

Figure 2: Bacterial isolates according to collected samples.

Antimicrobial sensitivity testing

In this study, there are 5 different antimicrobials were used. The results showed that 17 (89.47%) isolates exhibited a high resistance rate to ceftazidime, followed by cefotaxime and ceftriaxone in 15 (78.94%) isolates. On the other hand, the *A. Baumann* showed notably lowest resistance to imipenem and meropenem 13(68.42%) isolates and 12(63.15%), respectively, as shown in Figure 3.

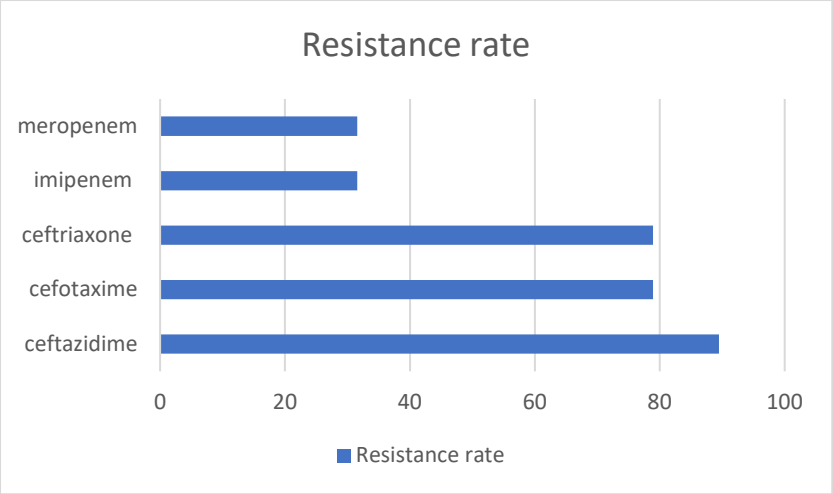

Figure 3: Antimicrobial susceptibility patterns for *A. baumannii.*

Carbapenem's resistance-associated genes:

Carbapenemase-producing bacteria due to the acquisition of various antimicrobial resistance. *A. baumannii* causes a variety of infections, such as UTI, bacteremia, septicemia, catheter-associated infections, and wound infections. In the present study, most of the *A. baumannii* were recovered from urine (n=19). Similarly, a study conducted in Hong Kong also reported that 20.4% and 13% of *A. baumannii* were recovered from urine culture in Iran. Antimicrobial resistance has been seen from the initial use of these drugs and is a growing global concern. *Acinetobacter baumannii* treatment of these infections is often tricky, and carbapenems are currently the antibiotics of choice. In this study, there is an emergence of remarkable resistance to carbapenem antibiotics (imipenem and meropenem 31.58% and 36.85%, respectively); in Iran, it has been proved that the *A. baumannii* was isolated from UTI and showed resistance to imipenem and meropenem (78% and 44%) respectively. Also, another study in Iran by Alavi-Moghadam et al. in 2014 showed that 100% of isolates were resistant to imipenem. Carbapenemases belong to the B-lactamases classes (A, B, and D), enzymes that play the main role in B-lactam resistance. In *A. baumannii* bla (KPC, VIM, and NDM), genes are encoded for carbapenemases the class A (i.e., blaKPC) exhibiting a minor role in phenotypic resistance. The results indicated that 5 isolates (26.31%) were carrying the *blaNDM* gene, as shown in (Figure-4); this result agrees with [1, 9], and [10.] Twenty percent of *A. baumannii* PCR results employing gene-specific primers included the blaNDM-1 gene. Furthermore, forty percent of *A. baumannii* isolates were found to be blaNDM-2 gene-positive when their DNA was amplified using blaNDM-2 primers. Carbapenems are a kind of -lactam antibiotic that has strong antibacterial action against *Acinetobacter baumannii*. However, the emergence and dissemination of acquired carbapenem resistance in these species have questioned the efficacy of treatment and control measures. In bla (NDM-1) genes, they are responsible for carbapenem resistance by encoding enzymes that partly hydrolyze carbapenems and other B-lactams [2.] Only two bactericidal drugs, colistin and fosfomycin, and one bacteriostatic drug are effective against bla NDM-1 producers (tigecycline). According to the study, one of the most distinguishing features of NDM producers is that they are not only nosocomial pathogens but also Gram-negative community members, such as *Acinetobacter baumannii*, which can operate as a natural reservoir for bla (NDM) genes in Enterobacteriaceae [3.] Thus, screening and detecting NDM-1 *A. baumannii* producers is necessary to aid inappropriate treatment. On the other hand, three isolates (18.75%) were carrying the *blaVIM* gene. The discovery of MBL-producing organisms has been difficult using phenotypic approaches

such as the double-disc synergy test, MBL E-test, and combination disk test; however, molecular technologies, particularly next-generation sequencing, will throw some light on their detection [7]. Phenotypic techniques only find some MBL-producing strains since they are not sensitive enough. As a result of the discovery of blaVIM-1 using PCR in 14.3% of *A. baumannii* isolates that had been determined to be MBL negative by E-test, it is clear how important it is to use molecular methods in everyday practice to find these concealed MBLs. Due to the lack of fitness cost and NDM lactamases being favored over other MBLs, they do not hinder bacterial development and have spread worldwide among Gram-negative bacteria. This finding conflicts with [4]. Carbapenemase genes from class A, blaKPC, even though the bacterial isolates had not possessed the blaKPC gene. According to reports, Kuwait and America 5 had a 75% and 18% prevalence of bla GES, respectively. The prevalence of blaKPC in *A. baumannii* is rarely observed. *A.baumannii* clinical As this gene is found on the transportable elements, which may be passed from one bacterium to another and across the community, isolates containing the blakpc gene are disseminated in Iran. The blakpc gene's presence in These mechanisms is necessary for the formation of *A.baumannii* strains that are resistant to a variety of innate and acquired antimicrobials (carbapenems are the selective antibiotic for treating the infections caused by multiple-drug resistant *A.baumannii* strains). Therefore, the judicious use of antibiotics for infection control in hospitals, particularly in ICU departments, can significantly delay the emergence of these resistant strains and their associated diseases. This amplifies the significance of prudent antibiotic use in hospitals for managing infections, especially in the intensive care unit, and lowers the risk of nosocomial transmission and potential outbreaks [6].

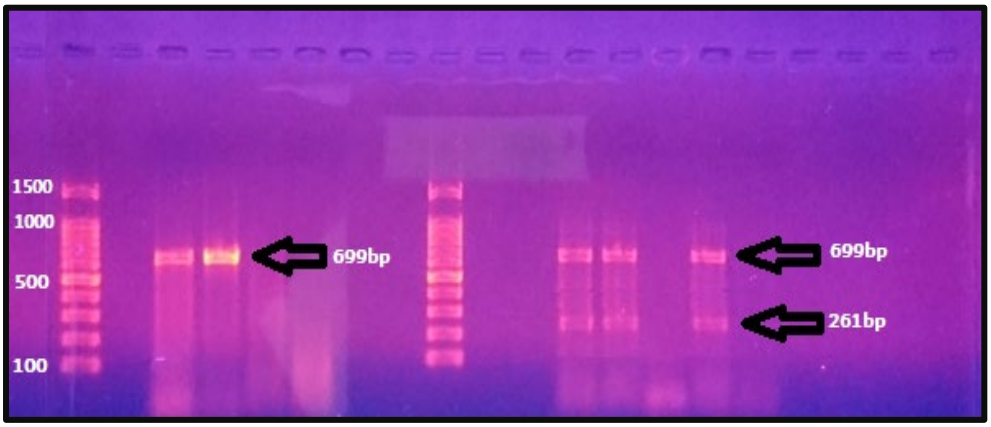

Figure 4: Gel electrophoresis of PCR products of *blaNDM* (699bp) and *blaVIM* (261bp).

CONCLUSIONS

Numerous Acinetobacter baumannii isolates were found to harbor the carbapenem resistance genes, namely blaNDM and blaVIM, indicating their genetic predisposition for carbapenem resistance. Notably, the blaKPC gene was conspicuously absent in these isolates. These findings indicate a multifaceted resistance profile, as these isolates also displayed phenotypic resistance to a broad spectrum of commonly prescribed antibiotics. This highlights the complexity of antibiotic resistance mechanisms employed by Acinetobacter baumannii and raises concerns about the efficacy of conventional treatment approaches. The presence of carbapenem resistance genes underscores the clinical challenge posed by this pathogen, necessitating ongoing vigilance and Non-payment of antibiotics without laboratory tests in order to limit the spreading of bacteria resistance, especially in hospitals and create units or specialized teams in each hospital or medical center interested in developing high-definition programs for the use of antibiotics.

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