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GENETIC DETERMINATION OF CARBAPENEMASES AMONG CARBAPENEM - RESISTANT *PROTEUS MIRABILIS* ISOLATED FROM CLINICAL SAMPLES, IRAQ

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Abstract

***Background and aims*:** *Proteus mirabilis* is an enterobacterial species that naturally colonizes the gastrointestinal lumen. It is an opportunistic pathogen responsible for serious infections of the human urinary tract, respiratory tract, wounds, otitis media, and blood. It poses a potential threat to patients via the production of β-lactamases, which decrease the efficacy of antimicrobial treatment and impair the management of its pathogenicity. This study was established to determine the prevalence of carbapenemases of *P. mirabilis* isolated from various clinical specimens.

***Materials and methods*:** In this study clinical samples were collected from (urine, wound and ear swab), and among these isolates, *Proteus mirabilis* was identified by phenotype and genotype ***Results*:** The study includes the collection of 250 samples from different clinical sources, including urine (40.4%), wounds (43.6%) and ear swabs (16%) from patients attending Al- Kut Hospitals and private clinics, The prevalence of *P. mirabilis* was 10.4% among all collected samples, female infection rate was 30.7%, male infection rate was 69.2%. The age group ≥50 years was commonly infected with *P. mirabilis*. All *Proteus mirabilis* isolates were examined for their resistance against 17 antibiotics belonging to different classes, the highest rates of resistance were against class Tetracyclines antibiotic (doxycyclin) was 100%, and maximal sensitivity has been to norfloxacin, meropenem,doprapemen was 92,3%. In a phenotypic analysis production carbapenemase, it was discovered that 11% of clinical isolates was *Proteus mirabilis.* Genotypically isolates were carbapemase producers, 26.9% of isolates were carbapemase producers KPC-type carbapemase, while other gens (blaIMP, blaVIM, blaOXA, blaSIM and NDM-1) were absent among all isolates.

***Conclusion*:** There is an urgent need to monitor hospitalized patients and improve healthcare in order to reduce the incidence of infection and outbreaks of infection with antibiotic-resistant *Proteus*.

Keywords: Proteus mirabilis, Antibiotic susceptibility, Phenotypes and genotype of Carbapenemase, Proteus mirabilis

Introduction

Proteus mirabilis belongs to family *Enterobacteriacea* and can cause different clinical diseases including urinary tract infection, wound infection, meningitis in infant, rheumatoid, endocarditis,

septicemia, cystic fibrosis because it produces many virulence factors include (adhesion, toxins, flagella, enzymes production like urease, biofilm and highly resistance phenotype to antibiotics) [1-3]. *P. mirabilis* is one of the most common causative agents of urinary tract infections (UTIs), particularly in catheterized patients [4]. *Proteus mirabilis* is an opportunistic pathogen, causing a variety of community-acquired and nosocomial illnesses. *Proteus mirabilis* biofilm production is a significant resistance mechanism because it enhances resistance gene transfer, renders bacterial colonies antibiotic-resistant, increases antibiotic metabolism [5]. Additionally, the rise of *Proteus mirabilis* infections that form biofilms and are multi-drug resistant is a significant worldwide public health concern that calls for non-antibiotic therapy [6]. *Proteus mirabilis* is poses a potential threat to patients via the production of β-lactamases, which decrease the efficacy of antimicrobial treatment and impair the management of its pathogenicity β-lactam resistance, mediated by the synthesis of β-lactamases, is being more and more frequently reported among P. mirabilis. Extended-spectrum β-lactamases (ESBLs), AmpC, and carbapenemases are the most common β-lactamase enzymes [7]. Carbapenems are the remaining treatment option against serious ESBL- and AmpC-related infections [8]. Unfortunately, treatment failure is observed due to the rapid propagation of carbapenem-resistant isolates coexpression of AmpC, ESBL, or carbapenemase enzymes are the most common mechanisms of carbapenem resistance in *Enterobacteriaceae* [9]. The extensive resistance of Gram-negative bacteria is associated with the transfer of resistance genes via transferable genetic elements such as plasmids, which can readily pass through mutant clones and spread rapidly between countries. Most of this spread is therefore undetected as the normal human flora acquires those resistance genes and becomes a silent source of endogenous infections[10].In view of the increasing prevalence of Proteus resistance to various antimicrobials, especially β-lactam antibiotics, the objective of this study is to detect mechanisms of resistance to β-lactams (i.e., ESBLs, AmpC, and carbapenemases) among *P. mirabilis* isolates collected from healthcare facilities using phenotypic and molecular testing, to support the potential therapeutic options for treating these complicated clinical infections. Then, we determined the genetic diversity of different β-lactamase producing *P. mirabilis* isolates.

Materials and Methods

Samples

A total 250 specimens (urine, wound and otitis media) were collected from patients with different ages admitted to Al-Zahraa teaching hospital, Al-Karama teaching hospital, Al-Kut hospital and from private clinics in Waist province from both sex, male and female. Each patient's details has been recorded, ie name, age, gender, underlying clinical condition, and the date the sample was collected. *Proteus mirabilis* was provisionally identified based on characteristic growth on blood agar, non-lactose-fermenting colonies on MacConkey's agar media, and various biochemical reactions [11-12], and were confirmed using the automated Vitek 2 system. The purified isolates were preserved at -20°C in glycerol (25% v/v). A routine antimicrobial susceptibility test was performed by the Kirby–Bauer disk diffusion method against all *P. mirabilis* isolates on Mueller Hinton agar and the results were interpreted in accordance with the Clinical and Laboratory Standards Institute [13] criteria. The antibiotics used were piperacillin amoxicillin/clavulanic acid,

aztreonam, imipenem, doprapemen, meropenem, doxycyclin cefoxitin, ceftazidime, cefotaxime, ciprofloxacin, gentamicin, and amikacin.

Detection of carbapenemase enzyme A modified Hodge test to confirm the release of carbapenemases from *P. mirabilis* isolates in accordance with CLSI guidelines [14] DNA of 26 *P. mirabilis* isolates was extracted depending on extraction protocol used by following the manufacturer's protocol. The extracted genomic DNA was then confirmed by gel electrophoresis in a 1% agarose gel after staining with safe dye and visualized under a UV transilluminator documentation system. Suspensions containing DNA were stored at -20°C until their use as a template for PCR. Phenotypically confirmed carbapenemase positive Proteus isolates were subjected to PCR using specific primers forcarbapenemase genes (blaKPC, blaIMP, blaNDM, blaVIM and blaSIM) [15], (Table 1)

Table 1 Primers' sequence of gram-negative bacteria

MultiplexPCR pool	Primers	Sequences (5'-3')	Size (bp)	References
Multiplex-IMP VIM SIM	F	TTGACACTCCATTTACDG	180bp	[16]
	R	GATYGAGAATTAAGCCACTCT		
	F	GATGGTGTTTGGTCGCAT	390bp	
	R	CGAATGCGCAGCACCAGA		
	F	TACAAGGGATTCGGCATCG	570bp	
	R	TAATGGCCTGTTCCCATGTG		
Monoplex NDM-1	F	ACCGCCTGGACCGATGACCA	264bp	
	R	GCCAAAGTTGGGCGCGGTTG		
Monoplex KPC	F	CATTCAAGGGCTTTCTTGCTGC	538bp	
	R	ACGACGGCATAGTCATTTGC		
OXA-48	F	GCGTGGTTAAGGATGAACAC	438 bp	[17]
	R	CATCAAGTTCAACCCAACCG		

Statistical analysis

All collected data were documented and analyzed using the GraphPad Prism Software [18].

Results

Identification of clinical isolates

This study was conducted on a total of 250 clinical specimens, including (urine, wound and ear swab), urine 101 (40.4%), wounds 109 (43.6%) and ear swabs 40 (16%) which were collected from patients with different ages admitted in hospital Table (1). Out of 250 samples proportion of bacterial growth was isolated: 200 (80%) positive culture, whereas no growth specimens are 50 (20%). The prevalence of *P. mirabilis* was 26 (10.4%) among all collected samples, Isolates were

identified by traditional phenotypic and biochemical tests as well as molecular methods, where the confirmation rate was 100%. Female infection rate was 8 (30.7%) male infection rate was 18 (69.2%). The age group ≥ 50 years was most commonly infected with P. mirabilis. The results showed that wounds infected 18 (69%) with *P. mirabilis* were more common from urin and ear sample. Diabetic 13 (50%) wounds were more affected by *P. mirabilis* than surgical wounds 5 (19.2%).

Antimicrobial resistance pattern

All *Proteus mirabilis* isolates were examined for their resistance against 17 antibiotics belonging to different classes, the highest rates of resistance were against class Tetracyclines antibiotic (doxycyclin) with (100%), and maximal sensitivity has been to (ciprofloxacin, levofloxacin) (80.7%) and (norfloxacin, meropenem,doprapemen) (92.3%), **(**Figure 1).

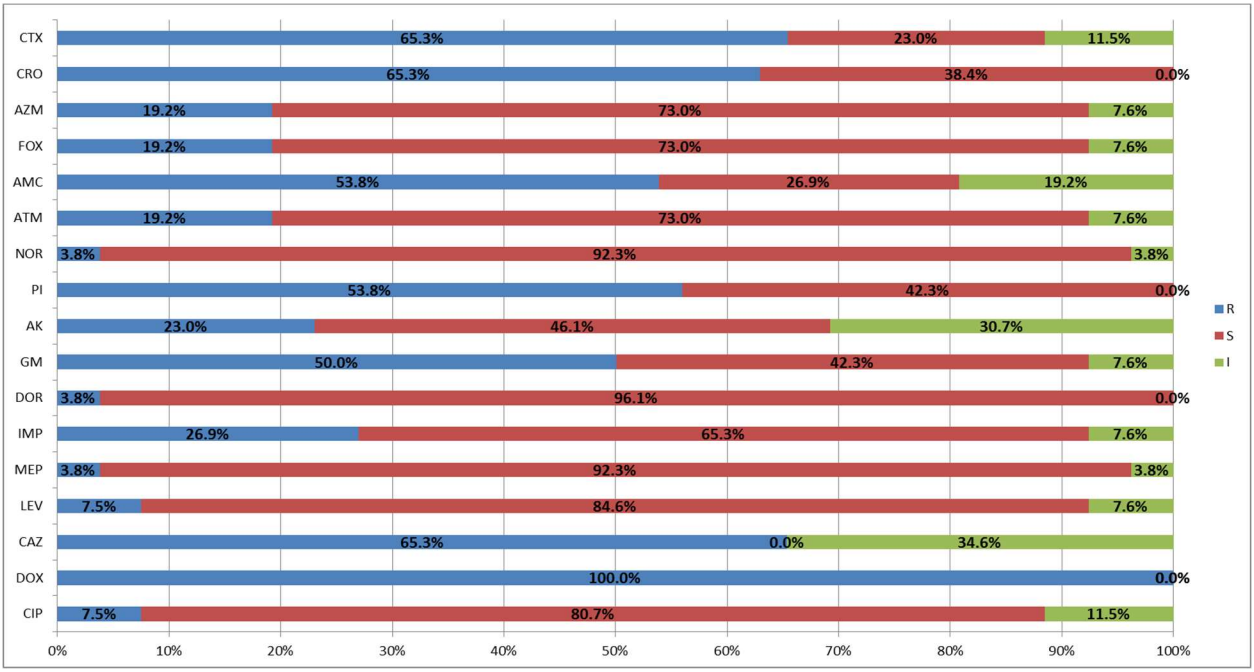

Figure (1): Frequency of antibiotics resistance profiles of *Proteus mirabilis*

Phenotypic detection of carbapenemases

In a phenotypic analysis carbapenemase production, it was discovered that 3 (11 %) of the clinical isolates of the *Proteus mirabilis*

Molecular detection

Tests were conducted to confirm the identification using 16S rRNA after diagnosis of bacteria by biochemical test Vitek2. DNA of 26 isolated was extracted and conventional PCR was performed to amplify gene and results revealed that all isolates were *Proteus mirabilis.* Figure (2)

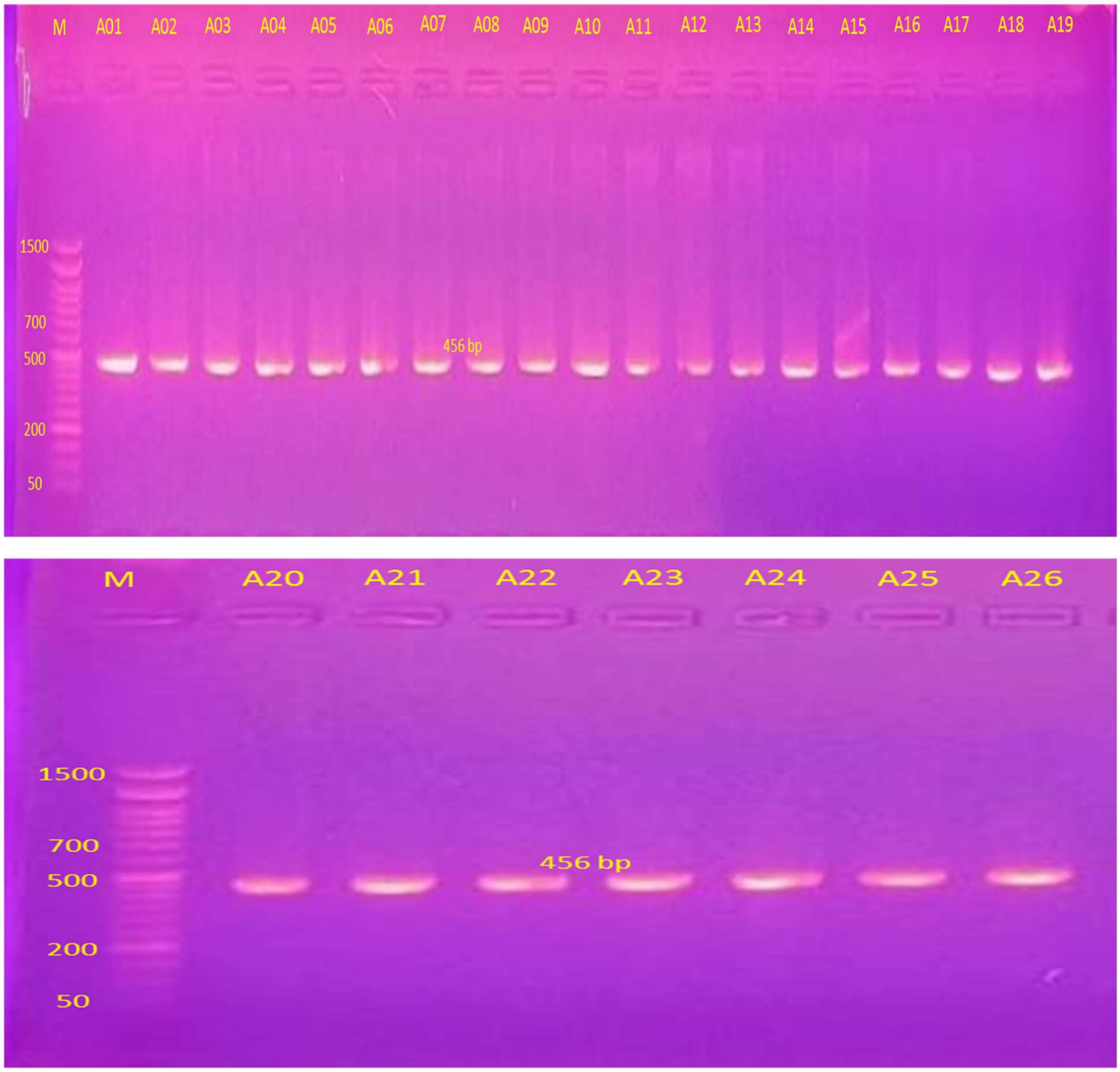

Figure (2): Electrophoretic analysis (1.5% agarose) showing ureR-amplification products of potential of *Proteus mirabilis*. Primers ureR -F&R were employed. M, 1 kb GeneRuler (Fermentas); ureR amplification product from *Proteus mirabilis* (456 bp)

Genotypically isolates were carbapemase producers 7(26.9%) of the isolates were carbapemase producers KPC-type carbapemase,while other gens (blaIMP, blaVIM, blaOXA, blaSIM and NDM-1) were absent among all isolates (Figure 3).

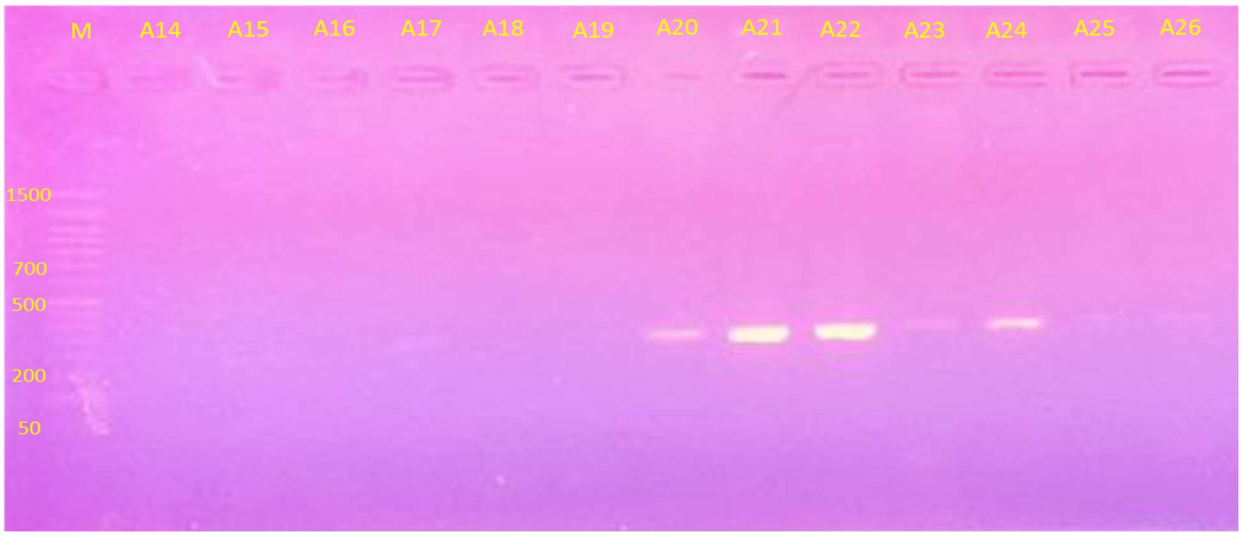

Figure (3): Electrophoretic analysis (1.5 % agarose) showing blaKPC-amplification products of potential of *Proteus mirabilis*. Primers blaKPC -F&R were employed. M, 1 kb GeneRuler (Fermentas); blaKPC amplification product from *Proteus mirabilis* (538 bp)

Discussion

In the present study, 26 (13%) samples were positive and 174 (87%) samples were negative *P. mirabilis* isolates were isolated from various clinical samples: urine, wound, ear swab. The results showed that wounds infected 18 (69%) with *P. mirabilis* were more common from urine and ear sample. Diabetic 13 (50%) wounds were more affected by *P. mirabilis* than surgical wounds 5 (19.2%). these percentage differences may be due to the difference in the geographical location of the source of the samples. The incidence of wound infection may be attributed to the high sensitivity of the exposed area of the wound to microbial invasion, whether the wound was spontaneous or resulting from a surgical operation. Just as the wounds become more susceptible to infections by not following the correct methods in terms of hygiene, and by not paying attention to sterilization rules by hospital staff, as well as other factors related to the personal hygiene of the patient himself and the medical staff [19]. Concerning urine samples, the percentage of *Proteus mirabilis* isolates that appeared in this study is 8 (30.7%) of the total 26 isolates of *Proteus mirabilis* and this result is close to the results of[20], as the *Proteus mirabilis* were isolated from urine samples with percentages (40%). In the present study (100%) of *Proteus mirabilis* isolates were high resistant to doxycycline, this observation was in accordance with results of other studies conducted by[21] who demonstrated that 90,6% of isolates were resistant to doxycycline. In addition it was in accordance with results of other studies conducted by [22] demonstrated that 95 % of isolates were resistant to doxycycline, but disagree with [23] recorded that the resistant to doxycycline antibiotic was (39.7%). likewise, high resistance rate (65.3) was reported against Cephalosporin antibiotic (ceftazidim, ceftiaxon and cefatoxim), the resistance rate was comparable with the study results with [24], The majority of isolates (70%) exhibited resistance to Cephalosporins. Therefore, to decrease the chance of microorganisms attaining resistance to this drug should be carefully and wisely used. Also, resistance was shown against class Penicillin

antibiotic (amoxilin clavulanic acid, piperacillin) with (53.8%), resistance another study recorded 64% [25] Gentamycin is an aminoglycoside antibiotic which is broad-spectrum and inhibitor for protein synthesis. In this study, *P. mirabilis* isolates show resistance to gentamycin (50%). This result agrees with the researcher [26] recorded 46.32%. In contrast another study recorded lower resistance 50% [27] while disagrees with [28] were the isolates show low resistance (7.5%). The prevalence of carbapenemase producers 3 (11%) by phenotype, which was comparable to the results of other studies conducted in Baghdad City (33.3%) by [29]. Carbapenemase enzymes confer resistance to broad-spectrum β-lactam antibiotic and it is a one of the important carbapenem resistant mechanisms in gram-negative bacteria. The differences in prevalence may be due to strains and time variations but overall indicate high incidence of MBLs among bacteria in our area [30].

In this study among 26 isolates was found that only gene for carbapenemase enzyme in among *Proteus mirabilis* is KPC by the detection of genes encoding these enzymes, using most common carbapenemases specific primers for blaIMP, blaVIM, blaKPC, blaSIM, blaGIM, blaSPM and NDM-1. Whereas, no PCR-amplification products with blaIMP, blaVIM, blaOXA, blaSIM and NDM-1carbapenemase genes. The percentage of presence of the gen 7(26.9%) of the total isolates of *Proteus mirabilis* bacteria Figure (2), this a result agree with these studies [31] which showed that 45% of the isolates of *Proteus mirabilis* bacterium had this gene. but disagree with this study in Wasit [32] which showed that 0% of the isolates of *Proteus mirabilis* bacterium had this gene. The rapid emergence of carbapenem resistance in Gram-negative bacteria is a major public health problem. *Klebsiella pneumoniae* carbapenemase (KPC) enzyme hydrolyzes most β-lactam antimicrobial agents, including carbapenems, and the global spread of blaKPC genes has been well documented. KPC-producing bacteria, usually belonging to the order Enterobacterales, have been detected across the planet, and several KPC variants have been described. [33]. Seven KPC variants have been described (KPC-1=2 to KPC-8). These enzymes hydrolyze carbapenems, penicillins, and monobactams more efficiently than extendedspectrum cephalosporins and are inhibited by clavulanate [34]. Recently, it was shown that KPC-encoding genes were housed in a transposon element that may be transported by plasmids, facilitating mobilization. There have also been reports of clonal spread and two carbapenemase-producing plasmids from *Proteus mirabilis* , pT18 and pT211 (both carrying blaKPC-2), were characterized through whole genome sequencing. [35]. other gens (blaIMP, blaVIM, blaOXA, blaSIM and NDM-1) noticed that isolates don't carry this genes Figure (3). The lack of amplification of genes can be explained by the existence of a potential new enzyme due to the high rate of mutations in the bla genes [36]. These results agree with the researcher [37]. Where it was found that no PCR-amplification products with bla IMP, bla VIM, bla NDM and bla SIM genes among Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, *Proteus mirabilis* and Klebsiella oxytoca isolates. But disagree with this study [38] and [39].

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