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INVESTIGATION OF THE PREVALENCE OF CARBAPENEM RESISTANT PSEUDOMONAS AERUGINOSA ISOLATES ISOLATED FROM DIFFERENT CLINICAL SAMPLES IN AL-DIWANIYAH GOVERNORATE

[1]*Farah Awid Abed, [2]Fatima Raheef and [3]Dhuha M. Jabir* and [4]Salam Jasim Mohammed

[1]Ministry of High Education, Department of Biology, College of Science, University of Al- Qadisiyah, Iraq.
[2,3]Department of Biology, College of Science, University of Al-Qadisiyah, Al Diwaniyah, Iraq.
[4]Department of Family and Community Medicine, Medical College, University of Kufa, Iraq.

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***Corresponding Author: Farah Awid Abed**
Ministry of High Education, Department of Biology, College of Science, University of Al- Qadisiyah, Iraq.

ABSTRACT

Back ground: Carbapenem is the last choice treatment for multi-antibiotic resistant *Pseudomonas aeruginosa* infections and the spread of resistant strains to this antibiotic is a serious problem. **Methods:** In the current study, 250 clinical samples were collected from different pathological cases in Al-Diwaniyah governorate, distributed as follows: 50 smears for burn injuries, 50 samples from the intensive care unit, 50 samples for wounds, 50 sputum samples for patients with acute pneumonia, and 50 urine samples for patients suffering from urinary tract infection. all collected samples were cultured on the blood and MacConkey agar medium. the bacteria were diagnosed by biochemical methods and staining. *Pseudomonas aeruginosa* were isolated, then an antimicrobial sensitivity test was performed using Vitek -2 compact system (BioMérieux Company \France). At last the ability of carbapenem-resistant bacteria to produce the carbapenemase enzyme was investigated by the NitroSpeed-Carba NP test. **Results:** the results showed that there were 70 carbapenem-resistant isolates(28%), and most of the isolates were from infection and wound patients(n= 40, 16%), while the fewest isolates were from burn injuries(n=10). The study also found that the most produced carbapenemase was the class D. **Conclusion:** There is a significant proportion of isolates that are resistant to carbapenems, because they produce carbapenase enzyme class D.

KEYWORDS: *Pseudomonas aeruginosa*, hospital acquired infection, carbapenem resistant, carbapenemase enzyme, OXA-48.

INTRODUCTION

The important multi-drug resistant organism *Pseudomonas aeruginosa* primarily affects immunocompromised patients and causes serious infections especially with burns patients, wound infections, and other conditions. Beside many other disorders like cystic fibrosis, keratitis and it is the most isolated species at Intensive Care Units (ICU).[1] The widespread spread of bacteria is due to its ability to grow under harsh conditions such as high temperatures, extreme pH, and lack of nutrients.[2] It also has many virulence factors that increased resistance of bacteria to many antibiotics represented by LPS which act in tissue damage, biofilm formation and antibiotic tolerance[3], and outer membrane proteins which play crucial role in antibiotic resistance, in addition to many other pathogenic factors.[4] Carbapenem is the most recommended choice treatment for multi-antibiotic resistant *P. aeruginosa* infections.[5] The spread of resistant strains to this antibiotic is a serious problem. Carbapenem-resistant *P. aeruginosa* (CRPA) is an important cause of hospital-acquired infections worldwide.[6] Particularly in patients with compromised immune systems or those undergoing invasive medical procedures.[7] This Gram-negative bacterium is notorious for its ability to develop resistance to multiple antibiotics, making it challenging to treat and control.[8] The emergence of carbapenem resistance in *P. aeruginosa* is a significant public health concern, given the limited treatment options available.[9-11] Carbapenems are broad-spectrum antibiotics that have traditionally been used as a last resort for treating multidrug-resistant infections.[12] However, the overuse and misuse of these antibiotics have led to the development of resistance mechanisms.[13] The resistance of bacteria to carbapenem has been attributed genetically to" The loss of porin Opr D and acquiring resistant genes encoding carbapenem-hydrolyzing (carbapenemase) accounted mainstays of

resistance".[14] Which can inactivate the drug.[15] Investigating the prevalence of CRPA isolates in different clinical settings is essential for understanding the scope of the problem and implementing effective control measures.[16] Surveillance studies have shown that CRPA infections are most commonly associated with intensive care units (ICUs), where patients are often exposed to invasive devices and are at higher risk of developing infections.[17] However, CRPA has also been reported in other clinical settings, including surgical wards, burn units, and outpatient clinics. Several risk factors have been identified for the acquisition of CRPA, including prior antibiotic exposure, prolonged hospital stay[18], mechanical ventilation, and immunosuppression. In addition, the spread of CRPA is facilitated by the ability of *P. aeruginosa* to survive on environmental surfaces and form biofilms, which can protect the bacteria from disinfectants and antibiotics[19], carbapenem resistance has been reported more widely distributed.[20] Production of carbapenem-hydrolyzing enzymes(so-called carbapenemases) is one of several methods used by bacteria to resist carbapenem,[21] According to the amino acid identities of the main clinically significant carbapenemases found in clinical isolates, they can be divided into three different classes: molecular classes A (such as carbapenemase [KPC] enzymes), B (known as New- Delhi *metallo-lactamase* [NDM], VIM [Verona integron-encoded metallo-lactamase], and D (such OXA-48) and its derivatives).The basic aim of the presented study was investigation of CRPA prevalence in clinical samples in Al- Diwanyia province for identifying the patterns of resistance and developing appropriate infection control measures.

METHODS

Sample collection and cultivation: A total of 250 clinical samples distributed as follows[50] burn swabs,[50] wounds swabs (pus or discharge),[50] urine samples,[50] septum samples and[50] samples from intensive care unit were aseptically collected. The samples were transported to laboratory for bacteriological processing. Swabs were streaked on Blood agar and MacConkey agar and incubated at 37°C for 24 h and or 48 h if required. Purified colonies were first identified through bacteriological reactions and cultural traits.[22,23] All isolates were then subjected to Vitek2 automated bacterial species identification system[24] for identification of bacterial species. Confirmed isolates of *P. aeruginosa* were stored at -20°C in nutrient broth supplemented with 20% glycerol until further processing.[25]

Antibiotic Sensitivity Testing: A sensitivity test of isolated bacteria to antibiotics was conducted using Vitek-2 compact test (BioMérieux Company\ France), after preparing a pure culture of the growing bacteria and then transferred to the brain heart infusion medium.[26]

Investigation about carbapenemase-producing *P aeruginosa* isolates: For the purpose of investigating the ability of bacteria to produce carbapenmase enzyme and to determine the class of produced enzyme (A,B and D) "The same protocol of the Nitro Carba-Carba NP test as the one used for Enterobacterales was followed (Nordmann *et al*., 2020)[27]" For each bacterial isolate, 1 to 2 calibrated 1-µl loopfulls of tested colonies, grown overnight at 37°C on Mueller-Hinton agar (Hi media \India), specimen plating (e.g., blood agar plates) were selected. To extract the enzymes, the sample was re-suspended in one of the following:

(i) 100 µl of a Tris-HCl 20 mmol/liter lysis buffer (B-PER II bacterial protein extraction reagent; Thermo Scientific Pierce, Rockford, IL, USA) containing 0.1 mM ZnSO4 (CarlRoth GmbH; reference no. K301.1), pH 7.5, in tubes 1 and 2;

(ii) 100 µl of a Tris-HCl 20 mmol/liter lysis buffer containing 0.1 mM ZnSO4 and 25 mM dipicolinic acid (Sigma Aldrich; reference no. P63808), pH 7.5, in tube 3;

(iii) 100 µl of a Tris-HCl 20 mmol/liter lysis buffer containing 0.1 mM ZnSO4 and avibactam sodium hydrate (0.04 mM [12.2 µg/ml]; MedChemExpress; reference no. HY-14879B) in tube 4; or

(iv) 100 µl of a Tris-HCl 20 mmol/liter lysis buffer containing 0.1 mM ZnSO4 and vaborbactam (50 µg/ml; MedChemExpress; reference no. HY-19930) in tube 5Tubes were then vortexed vigorously for 30 to 60 s until complete resuspension. Then, 50 µl of distilled water was added to tube 1 (control) and 50 µl of ertapenem sodium (ETP) (80 µg/ml; Sigma-Aldrich; reference no. SML1238) to tubes 2 to 5. Following incubation at room temperature for 5 min, 50 µl of 1 g/liter nitrocefin (Toronto Research Chemicals, Canada; reference no. N493815) was added into each tube 1 to 5. The final concentrations of ETP were 20 µg/ml"

RESULTS

Results of isolation and diagnosis of bacteria from clinical samples

In this study, 250 samples were collected for different clinical cases, as shown above in the collection of samples, and the number of bacteria was as shown in Table 1

Table 1: Number of collected clinical samples.

Sample	Number	Ratio
Burn	43	17.2%
Wound	45	18%
ICU	48	19.2%
Urine	35	14%
Septum	40	16%
Total	211	84.4%

Table Number 2: The proportions of bacterial species isolated from clinical samples according to Vitek test results.

Bacteria	Wounds	Burns	ICU	urine	Septum
E.coli	30	40	35	45	12
Pseudomonas aeuroginosa	41	45	20	25	46
Acinatobacter bummanii	42	37	34	32	35
Klebssilla pneumonia	40	24	40	44	39
Other	30	25	38	40	30

These results came after investigating the diagnosis of bacteria by biochemical methods, in addition to the Vitek -2compact system test, where ID-GN (gram negative bacillus distinguishing proof) used. The above table shows the wide spread of *P aeruginosa*, as it constituted the highest percentages for most of the clinical samples under study. The reason for this can be attributed to the ability of the mentioned bacteria to grow in various conditions and environments, as well as its high ability to resist sterilization, high temperature conditions and change in pH (28).

Antibiotic sensitivity results

Beside the identity detection of bacteria Vitek -2compact system was used to investigate the sensitivity of bacteria to antimicrobial agents. As shown in Table 3A,B below.

Table 3A: The results of antibiotic sensitivity testing, B the number and ratio of MDR and CAPA bacteria.

Antibiotic	No. of isolates
Ceftizdime	60
Cefipime	65
Cefotaxime	59
Impnime	58
Meropnime	12
Gentamicin	60
Ciprofloxacin	50
Amikacin	53
Norfloxacin	48
MDR *P. aeruginosa*	CAPA
100 40%	70 28%

Carbapenimase production results

The results of carbapenemase production by carbapenem-resistant isolates were read and interpreted according to the change in the color of the tubes as a result of nitrocefin hydrolysis as follows:

1. A color shift from yellow to red (nitrocefin hydrolysis) in tubes 1, 2, and 3, and no color change (nitrocefin hydrolysis) in tubes 4 and 5, which contain ertapenem and avibactam. contains both ertapenem and vaborbactam, indicating the synthesis of class A carbapenemase (e.g., KPC).
2. When tube color shift from yellow to red (nitrocefin hydrolysis) in tubes refers to The presence of a class B carbapenemase, and the absence of any color change (no nitrocefin hydrolysis) in tube 3which containing ertapenem and dipicolinic acid.
3. The creation of a class D carbapenemase, such as OXA-48 and its variants, is indicated by a color shift from yellow to red (nitrocefin hydrolysis) in tubes 1, 2, 3, and 5, and the absence of any color change (no nitrocefin hydrolysis) in tube 4 containing ertapenem and avibactam.
4. The absence of any change in color (i.e no hydrolysis) in tubes 2 to 5 and a color change from to red in tube 1 solely containing water suggest lack in activity of the enzyme but synthesis of a noncarbapenemase -lactamase, such as TEM-1 or CTX-M-type enzymes.
5. The absence of any color change (i.e., no nitrocefin hydrolysis) in any of the tubes (1–5) suggests that there is no -lactamase activity.

DISCUSSION

The results show that 211(84.4%) clinical samples gave a positive result for the presence of bacterial infections, while 39 of them did not give a positive result, despite the passage of 24 hours since the patient was in the hospital. The reason may be due to the patient's genetic and immune readiness to resist infection. The table number 2 which demonstrate the Vitek results that associated with bacterial identity part. These results came after investigating the diagnosis of bacteria by biochemical methods, in addition to the Vitek -2compact system test, where ID-GN (gram negative bacillus distinguishing proof) used. The above table shows the wide spread of *P aeruginosa*, as it constituted the highest percentages for most of the clinical samples under study. The reason for this can be attributed to the ability of the mentioned bacteria to grow in various conditions and environments, as well as its high ability to resist sterilization, high temperature conditions and change in pH.[28]

As regarding the investigation about the isolated bacteria to produce carbapenimase enzyme. the results showed that there are 70 isolates resistant to carbapenem antibiotics, 18 are carbapenemase A producers, and 24 are class D producers. It is clear from the above results that the percentage of isolates producing carbapenemase class D (OXA-48) is the most common among the isolated bacteria. While the rest 28 may be resistant to carbapenems for many other reasons than the ability to form carbapenemase, such as the overproduction of AmpC beta-lactamase and inactivation of the OprD outer membrane protein or the efflux of drug[17], Carbapenemase production is the least common cause of carbapenem resistance in bacteria.[29] The ability of bacteria to produce the carbapenimase class D which also known as OXA-48enzyme is due to the presence of

genes coded by plasmid, the OXA-48 was firstly discovered at 2011 in many countries such Egypt, Sudan, Algeria, Tunisia, and France by Nordmann and Naas 2011, Poirel, 2011; Jayol *et al.*, 2016; Mohamed et al., 2018) and firstly diagnosed molecularly in Iraq in P. aeruginosa by Al-abedi & Al-Mayahi 2019. Who proofed that carbabenemase Class D can be identified molecularly in P. aeruginosa isolates by determination of blaOXA-48 gene.

CONCLUSION

There is a significant proportion of isolates that are resistant to carbapenems, because they produce carbapenase enzyme class D, Although phenotypic methods for the investigation of carbapenemase production are considered to be of little sensitivity, they can be used primarily in the investigation of the enzyme.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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