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Research Article

Carbapenem resistant strains of pseudomonas species at Minia university hospitals

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Abstract

Objectives: The prevalence of Beta-lactamase producing strains of Pseudomonas aeruginosa (P.aeruginosa), which is an important etiological agent of nosocomial infections, has been reported widely. Therefore, proper and rapid detection of carbapenemase producing isolates is essential. Aim of the work: The aim of this study was to detect the prevalence of bla-IMP gene in carbapenem resistant P.aeruginosa strains isolated from Minia university hospitals. Patients and Methods: This study was conducted through the period of June 2018 to September 2018, on 40 P.aeruginosa strains isolated from different specimens, carbapenem resistant strains were collected and evaluated for bla-IMP gene expression using PCR. **Results:** Out of 40 P.aeruginosa strains, 26 isolates were carbapenem resistant using (Vitek-2, bioMérieux, France) expressing bla-IMP gene was in 46%. **Conclusion:** Resistance to Carbapenems in our hospitals have started to constitute a significant problem, which require proper diagnosis and effective treatment. The most effective antibiotic against carbapenem resistant isolates in this study was colistin. Genetic investigations for most common genes responsible for carbapenem resistance are recommended to identify the major cause of resistance and should be applied on a large number of clinical specimens. However, this could be limited due to its high cost.

Keywords: Carbapenem, bla-IMP, metallo-β-lactamases, P.aeruginosa.

Introduction

P. aeruginosa is a Gram negative aerobic, bacteria distributed widely in nature and can survive on a variety of surfaces and in hospitals [1]. It is an opportunistic pathogen causing about 10-20% of nosocomial infections in the form of bacteremia and sepsis in ICU, cystic fibrosis, urinary tract infections, pneumonia, burn infection and wound infection [2].

Multidrug-resistant (MDR) P. aeruginosa is resistant to one antibiotic in three or more anti-pseudomonal antibiotics (penicillin/ cephalosporin, Carbapenems, quinolones and aminoglycosides) [3].

MDR P. aeruginosa becoming a major health concern because the organism is inherently resistant to many antibiotic classes and is able to acquire resistance to all effective antimicrobial drugs [4].

P. aeruginosa acquires many mechanisms of resistance towards Carbapenems through production of metallo-β-lactamases (MβLs) enzymes, especially IMP (imipenem active metallo-β-lactamase) and VIM (Verona integron-encoded metallo β-lactamase) [5, 6].

Carbapenemase resistance refers to Ambler classes A, B, and D [7, 8], but MβL VIM and IMP belong to Ambler B. MβLs are inhibited by ethylene-diamine-tetra-acetic acid (EDTA) and sodium mercapto-acetic acid (SMA), but not affected by β-lactase inhibitors such as clavulanic acid, sulbactam and tazobactam [9].

The aim of this study was to detect the prevalence of bla-IMP gene in P.aeruginosa strains isolated from patients at Minia university hospitals.

Material and methods

Isolation and identification P. aeruginosa

This work was conducted on 40 pseudomonas species, which isolated from clinical specimens submitted to microbiology unit at Minia University hospitals for culture and sensitivity through the period of June 2018 to September 2018. All isolates were subjected to the following:

-Routine culture on blood and MacConkey agar media for 24-48 hours incubation at 37 °C. Then identification and AST was done using (VITEK-2, bioMérieux - France).

-All Carbapenems (Imipenem and Meropenem) resistant pseudomonas isolates were preserved for evaluation of bla-IMP gene expression using PCR.

-DNA extraction was done using (Promega Co., USA) kits following the manufacturer's protocol.

-PCR detection: specific primer designed from (bla-IMP): forward primer: (5'-CTA CCG CAG CAG AGT CTT TG-3'), Reverse primer: (5'-AAC CAG TTT TGC CTT ACC AT-3') (Operon Co., Germany) [10].

As a control for our work, the primer pair (5'-ATGGAAATGCTGAAATTCGGC-3') and (5'-CTTCTTCAGCTCGACGCGACG-3') was selected in order to amplify conserved regions of a target gene in P. aeruginosa and thus generate a PCR amplicon with a certain molecular weight (500 bp) that can be identified by gel electrophoresis.

Statistical analysis

Statistical Package for Social Science (Inc., Chicago, version 21). Statistical significance was recognized when $p < 0.05$. The numerical data were expressed as means and standard deviations. The qualitative data were expressed in frequencies and percentages. Student's t-test was applied in order to compare between two independent groups. Frequencies of categorical variables were compared using the x^2 test and Fishers' exact test when appropriate. For quantitative data, the comparison between the three groups was performed by an analysis of variance (ANOVA) followed by post-hoc Newman-Keuls multiple comparison tests. A two tailed $p < 0.05$ was considered significant.

Results

In this study, 40 Pseudomonas isolates were collected from clinical specimens admitted to microbiology unit at Minia university hospitals, through the period of June 2018 to September 2018, and distributed according to the type of specimens as illustrated in table (1).

Table (1): Distribution of Pseudomonas according to the type of specimens:

Types of specimens	**No. (%)**
Wound swab	6(15%)
Sputum	2(5%)
Urine Culture	24(60%)
Blood culture	8(420%)
Total	**40**

Table (2): Antibiotic resistance pattern of isolated Pseudomonas spp.:

Antibiotic	Resistant	Sensitive
	No. (%)	No. (%)
Meropenem	26 (65%)	14 (35%)
Imipenem	٢٥(62.5%)	١٥ (37.5%)
Cefazolin	30 (75%)	10 (25%)
Cefepime	24 (60%)	16 (40%)
Amikacin	24 (60%)	16 (40%)
Gentamicin	22 (55%)	18 (45%)
Ciprofloxacin	24 (60%)	16 (40%)
Levofloxacin	22 (55%)	18 (45%)
Ceftazidime	28 (70%)	12 (30%)
Colistin	0 (100%)	٤٠ (0%)

Table (3): Distribution of carbapenem resistant Pseudomonas isolates according to the type of specimens:

Types of specimens	No. (%)
Wound swab	4 (15.4%)
Urine	22 (84.6%)
Total	**26**

Table (4): Antibiotic resistance pattern of the carbapenem resistant isolates:

Antibiotic	Resistant	Sensitive
	No. (%)	No. (%)
Cefepime	**20 (76.9%)**	**6 (23.1%)**
Amikacin	**18 (69.2%)**	**8 (30.7%)**
Ciprofloxacin	**20 (76.9%)**	**6 (23.1%)**
Levofloxacin	**11 (42.3%)**	**15 (57.7%)**
Cefotaxime	**٢٤ (92.3%)**	**٢ (٧.٦%)**
Ceftazidime	**26 (100%)**	**0 (0%)**
Colistin	**0 (0%)**	**26 (100%)**

In this study, the resistance pattern of bla-IMP gene was expressed in 30% of all isolates representing about 46% of carbapenem resistant strains.

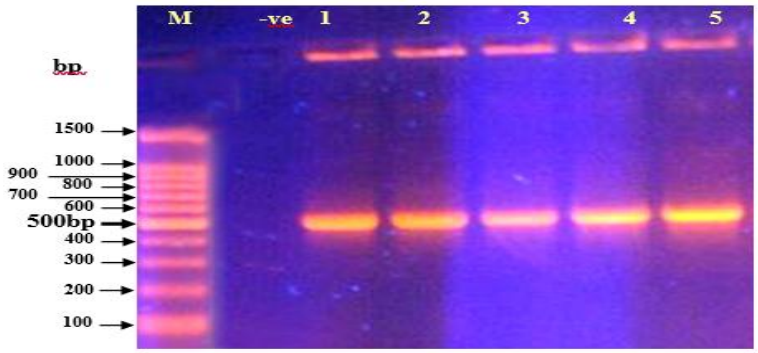

Figure (1): PCR detection of bla-IMP gene on agarose gel electrophoresis. Lanes (1, 2, 3, 4, and 5) were positive samples for bla-IMP gene

Discussion

MDR P. aeruginosa is considered a major problem ؛different antibiotics are commonly used for its treatment such as aminoglycosides, beta-lactamases؛ and quinolones [11]. The antibiotic resistance pattern in pseudomonas hesitates rapidly, especially in the case of imipenem [12]. Carbapenems (e.g. imipenem and meropenem) are often the last resort antibiotics used to treat MDR P.aeruginosa [13]. Production or acquisition of genes of MβLs (e.g. IMP and VIM) is considered the increasing cause of antibiotic resistance to Carbapenems [14, 15].

The MβLs producing P.aeruginosa in Egypt vary according to infection control policies and procedures utilized in hospitals [16]. The present study showed that 65% of clinical isolates of P. aeruginosa were resistant to Carbapenems (imipenem and meropenem). These results were near to that of Rehab Mousa et al. [17] who reported 69% of his isolates. In our study, the resistance rate of P. aeruginosa to imipenem was 62.5% and to meropenem was 65%. Carbapenem resistance rates can vary according to local antibiotic regulations, strain origin, and geographic location.

The bla-IMP gene was expressed in 30% of all pseudomonas isolates representing about 46% of carbapenem resistant strains. In Saudi Arabia, Al-Agamy et al. found that the prevalence of resistance to carbapenem was 34% and about 22% of them produced MBLs [18]. On the other hand, our results disagree with a study from Iran, which reported that only 8 (9.75%) of MBL-producing P. aeruginosa isolates were positive for bla-IMP [19]. In a study done in Iran, 100 isolates were resistant to imipenem, about 70 (70%) were found to be MBLs producers [20]. Therefore, detection of the MBL-producing strains was necessary for both; proper specific treatment and to decrease the nosocomial spread of resistance strains [21].

In the present study, it was found that P.aeruginosa isolates exhibited different rates of resistance towards aminoglycosides, which include Amikacin and Gentamicin with a percentage (60 % and 55%) respectively. Previous studies from Iran have approved the high resistance rates of the P. aeruginosa to aminoglycosides (gentamycin, amikacin) which was similar to current results [22].

The current work revealed that the resistance towards ciprofloxacin and levofloxacin were (60% and 55%) respectively. these results disagreed with Al.Fahadawi et al who found in his study on pseudomonas isolates which gave good effectiveness towards ciprofloxacin and norfloxacin, where the percentage of sensitivity was (85.3%, 76.5%) and the percentage of resistance was (11.8%, 17.6%) respectively [23]. Whereas other researchers reported similar rates of resistance to quinolones [24]. While, Corona-Nakamura et al. [25] showed that P. aeruginosa isolates were susceptible to antibiotic ciprofloxacin. This difference may be due to the continuous development of MDR strains of P. aeruginosa worldwide.

Colistin is one of polymyxin class of antibiotics, which has a wide range of activity against most Gram-negative bacteria. Colistin nowadays is used widely for clinical application especially MDR P. aeruginosa. Based on the breakpoint reported by CLSI [26] for colistin against P. aeruginosa, a susceptible breakpoint of $\leq$ 2mg/L and a resistant breakpoint of $\geq$ 4mg/L. Interestingly, colistin was the most effective antibiotic in our study since all of our tested carbapenem resistant isolates (n=26) were found to be sensitive to colistin.

Conclusion

Carbapenem resistance among pseudomonas strains require further surveillance, strong preventive measures and application of infection-control policies. In addition, to routine phenotypic methods for detecting carbapenemases production molecular confirmation by PCR of different carbapenemases producers is required for all isolates in order to identify the hidden genes. Also, periodic surveillance program is very crucial for each area to determine the most effective treatment polices.

Conflict of interest

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

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