Evaluation of Three Carbapenemase-Phenotypic Detection Methods and Emergence of Diverse VIM and GES Variants among Pseudomonas aeruginosa Isolates in Tunisia

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Abstract: Background: Since 2012, few reports on the molecular epidemiology of Pseudomonas aeruginosa were reported in Tunisia. Objectives: This study aimed to evaluate carbapenem-resistance determinants and molecular epidemiology and to compare the carbapenemase-phenotypic detection methods of multidrug-resistant P. aeruginosa isolates. Methods: During a period of four years (2014 to 2017), all imipenem-ceftazidime-resistant P. aeruginosa isolates were retrospectively selected at the microbial laboratory of Charles Nicolle hospital of Tunis. These isolates were examined by the modified Hodge test, modified carbapenem inactivation method (mCIM), and another mCIM, called CIMTris, and their performance was evaluated using PCR analysis as the gold standard. Results: A total of 35 isolates were recovered among patients hospitalized in different units. All strains were colistin-susceptible. All carbapenem-resistant isolates showed a high-level resistance to carbapenems. CIMTris and mCIM showed 96.15% and 46.15% sensitivity and 44.44% and 100% specificity, respectively, for detecting carbapenemase production. Conclusions: CIMTris is a promising approach for detecting carbapenemase activity in P. aeruginosa and merits further testing. Moreover, this study described the first detection of GES-5- and GES-9-producing P. aeruginosa in Tunisia as well as the co-occurrence of the blaGES-5 and blaVIM-11 carbapenemase genes in one isolate. These findings are of great concern because the rapid dissemination of MDR strains represents a major therapeutic and epidemiological threat.

Keywords: Pseudomonas; GES; VIM; ICU

1. Introduction

Pseudomonas aeruginosa is an important pathogen that causes various opportunistic and acute nosocomial-acquired infections, especially in immunocompromised patients. Its high intrinsic resistance and ability to develop multidrug resistance produce serious therapeutic problems. Carbapenems have been considered first-line agents to treat severe cases of P. aeruginosa infections [1]. Resistance to carbapenems can stem from the production of carbapenemases or other mechanisms such as mutation in the oprD gene, the overproduction of cephalosporinases, the over-expression of efflux pumps, or combinations of these mechanisms [2].

The most commonly reported carbapenemases among P. aeruginosa are metallo-β-lactamases (MBLs) (e.g., the Verona Imipenemase (VIM), Imipenemase (IMP), Sao Paulo metallo enzyme (SPM), German imipenemase (GIM), and New Delhi metallo-β-lactamase (NDM) types) and, to a lesser extent, Ambler class A carbapenemases (e.g., Klebsiella pneumoniae carbapenemase (KPC) and some Guyana extended-spectrum (GES)-type enzymes [3]. MBL enzymes are also able to hydrolyze penicillin and cephalosporins [4,5]. Moreover, the
MBL VIM-2, initially reported in France [4], has emerged and has been reported to be the main MBL determinant in *P. aeruginosa* isolates in Tunisia and worldwide during the past two decades [6–9]. Their increase in recent years, which is associated with high mortality, morbidity, long hospital stays, and increased costs, emphasizes the need for the detection of these isolates to avoid therapeutic failures and nosocomial outbreaks [8,9].

It should be noted that detecting carbapenemases is more difficult in *P. aeruginosa* compared to *Enterobacteriaceae* [10]. Currently, several phenotypic methods are available, but most of them are unsuitable for clinical laboratories to perform on a routine basis [10,11]. Thus, standardized carbapenemase detection methods using routine phenotypic screening tests are still controversial.

Since 2012, few reports on the molecular epidemiology of *P. aeruginosa* were reported in Tunisia [6]. The aim of our study was, therefore, to evaluate changes in carbapenem-resistance determinants and molecular epidemiology and to compare carbapenemase-phenotypic detection methods of multidrug-resistant *P. aeruginosa* isolates recovered at Charles Nicolle Hospital of Tunis, Tunisia, during a 4-year period.

2. Materials and Methods

2.1. Bacterial Isolates

A total of 80 imipenem-ceftazidime-resistant *P. aeruginosa* (ICRPA) isolates were retrospectively selected from the frozen stocks kept in brain heart infusion with 20% glycerol at −80 °C at the microbial laboratory of Charles Nicolle hospital of Tunis during a period of four years (2014 to 2017).

From frozen stock, each isolate was subcultured twice on tryptic soy agar (Biorad, Marnesta la Coquette, France), incubating each subculture in ambient air at 35 °C ± 2 °C for 18 to 24 h to ensure purity and viability. Thus, only 35 non-duplicated clinical ICRPA isolates were successfully subcultured and included in this study.

Bacterial identification was performed using the API 20NE system (bioMérieux, Marcy l’Etoile, France).

2.2. Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing to 16 antibiotics (ticarcillin, ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, aztreonam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, tobramycin, amikacin, netilmicin, ciprofloxacin, levofloxacin, and fosfomycin) was performed by the agar disk diffusion method on Mueller–Hinton (MH) agar plates (Bio-Rad, Marnesta la Coquette, France), according to the CA-SFM guidelines (http://www.sfm-microbiologie.org/, accessed on 1 January 2017).

The minimum inhibitory concentrations (MICs) of imipenem and meropenem were determined by the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [M100-S25]. Colistin MICs were determined by the broth microdilution method using a commercialized kit (UMIC, Biocentric, Bandol-France).

*Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains in antimicrobial susceptibility testing and MICs.

2.3. Phenotypic Detection of Carbapenemase Production

The phenotypic detection of carbapenemases was performed by a modified Hodge test, modified carbapenem inactivation method (mCIM), and another mCIM, called CIMTris, which uses 0.5 M Tris-HCl buffer rather than water for extraction, as previously reported [10]. All carbapenemase phenotypic methods were assessed twice by different raters.

The performance of the carbapenemase phenotypic tests was evaluated using PCR analysis for *bla*<sub>VIM</sub> and *bla*<sub>GES</sub> as the gold standard (Table 1) [12]. Phenotypic method sensitivities and specificities were calculated according to Ilstrup [12,13].
Table 1. Oligonucleotides used in this study.

| Gene | Primer a | Sequence (5′–3′) c | Product Size (bp) b | Reference |
|------|----------|-------------------|--------------------|------------|
| blaGES | MultiGES-F | AGTCGGCTAGACCGGAAG | 399 | [14] |
| blaGES | MultiGES-R | TTTGCCCTGTAGCAGGAT | | |
| MultiOXA-48 F | | GCTTGCTCGTGGCAGGAA | 281 | [14] |
| MultiOXA-48 R | | TGGACACCTCAATTCGAG | 232 | [14] |
| blaimp | MultiIMP-F | GATYGAGAATTAAGCCACCT | 390 | [15] |
| blaimp | MultiIMP-R | CGAATGCCAGCACGAC | 537 | [15] |
| blavim | MultiVIM-F | GATGGTGTTTGGTCGATA | 798 | [15] |
| blavim | MultiVIM-R | CGAATGCCAGCACGA | 579 | [15] |
| blaKPC | MultiKPC-F | CATTCAAGGCCTGTTTGT | 621 | [15] |
| blaKPC | MultiKPC-R | CGAATGCCAGCACGAC | 579 | [15] |
| blabic | MultiBIC-F | TCATTCCGCGGCTGACCTCAC | 621 | [15] |
| blabic | MultiBIC-R | CGAATGCCAGCACGAC | 579 | [15] |
| blaNDM | MultiNDM-F | GCTTGCTCGTGGCAGGAA | 232 | [15] |
| blaNDM | MultiNDM-R | CGAATGCCAGCACGAC | 579 | [15] |
| blaAIM | MultiAIM-F | TGCAGACCCACCTCGGAT | 322 | [15] |
| blaAIM | MultiAIM-R | GGTTTGGCGATCTGGTTTTC | 699 | [15] |
| blagim | MultiGIM-F | TCGACACACCTCTGTGCTGAA | 477 | [15] |
| blagim | MultiGIM-R | AACTCCCGACTCTGGA CGAC | 579 | [15] |
| blasim | MultiSIM-F | TACAGAGGATTCGGCAGAC | 477 | [15] |
| blasim | MultiSIM-R | AACTCCCGACTCTGGA CGAC | 579 | [15] |
| bladm | MultiDIM-F | GCTTGCTCGTGGCAGGAA | 232 | [15] |
| bladm | MultiDIM-R | GGTTTGGCGATCTGGTTTTC | 699 | [15] |
| blasp | SPM-F | AAAATCTGGGTACGCAAACG | 271 | [15] |
| blasp | SPM-R | ACATTATCCGCTGGAACAGG | | |

a F, sense primer; R, antisense primer. b Nucleotide numbering begins at the initiation codons of genes. c D = A or G or T; Y = C or T.

2.4. Detection and Characterization of Beta-Lactamase Genes

The molecular detection of carbapenemase-encoding genes (blaGES, blaKPC, blaOXA-48, blaimp, blavim, blanDM, blasp, blabic, blaAIM, blagim, and bladm) was performed by PCR with previously reported conditions (Table 1) [14,15]. All PCR products were sequenced using a DNA sequencer (ABI PRISM 3130; Applied Biosystems, Foster City, CA, USA) [16].

3. Results

The 35 isolates had been obtained among patients hospitalized in different units (intensive care units, 63%; surgery ward, 26%; urology, 6%; and external consultation, 6%) (Figure 1) and from different types of samples (Figure 2) (low respiratory samples, 46%; pus, 14%; urine culture, 11%; blood culture, 11%; rectal samples, 9%; and material, 6%).

All strains were colistin-susceptible (MIC range 1–4 µg/mL) and were resistant to gentamicin (91%), tobramycin (91%), netilmicin (100%), amikacin (83%), ciprofloxacin (94%), and fosfomycin (100%) (Figure 3). All carbapenem-resistant isolates showed a high-level resistance to carbapenems. The MIC ranges of imipenem and meropenem were 4–512 µg/mL and 4–256 µg/mL, respectively.

Carbapenemase-encoding genes were detected in 26 strains (74%) and were identified as: blagES (n = 14), blagES (n = 2), blavim (n = 1), blavim (n = 9), and blavim (n = 1). The association between blagES and blavim was found in two strains (S15) (Table 4). None of the strains harbored the genes blakPC, blaimp, blaoXA-48, blasp, blanDM, blabic, blaim, blagim, blasim, and bladm.
Figure 1. Distribution of *P. aeruginosa* isolates according to wards.

Figure 2. Distribution of *Paeruginosa* isolates according to sample types.

Figure 3. Antibiotic resistance rates among *Paeruginosa* isolates.
Table 2. Characteristics of imipenem- and ceftazidime-resistant *P. aeruginosa* isolates (*n* = 35).

| Strains | Specimen | Ward      | Date of Isolation (Day/Month/Year) | Minimal Inhibitory Concentration (µg/mL) | Resistance to Non β-lactams | Phenotypic Detection of Carbapenemases | bla Genes |
|---------|----------|-----------|------------------------------------|------------------------------------------|----------------------------|--------------------------------------|-----------|
|         |          |           |                                    |                                          |                            | mCIM | CIMTris | mHodge |                      |           |
| S₁      | Pus      | Urology   | 21 August 2014                     | 512 128 4                                | GEN, AMN, NET, TOB, CIP, FOS | -   | -      | -      |                      | -         |
| S₂      | Urine    | ICU       | 21 August 2014                     | 16 4 4                                  | GEN, AMN, NET, TOB, CIP, FOS | -   | +      | -      | **blaVIM-2**         |           |
| S₃      | Pulmonary | ICU       | 21 August 2014                     | 32 16 4                                 | GEN, AMN, NET, TOB, CIP, FOS | -   | +      | -      | **blaVIM-2**         |           |
| S₄      | Pulmonary | ICU       | 30 August 2014                     | 512 128 4                               | GEN, AMN, NET, TOB, CIP, FOS | +   | +      | +      | **blaVIM-1**         |           |
| S₅      | Pulmonary | ICU       | 8 September 2014                   | 32 16 1                                 | GEN, AMN, NET, TOB, CIP, FOS | -   | +      | -      | **blaGES-9**         |           |
| S₆      | Material  | Surgery   | 10 September 2014                  | 16 8 2                                  | GEN, AMN, NET, TOB, CIP, FOS | -   | +      | -      | **blaGES-9**         |           |
| S₇      | Pulmonary | ICU       | 23 September 2014                  | 8 4 4                                   | GEN, AMN, NET, TOB, CIP, FOS | -   | +      | -      | **blaGES-5**         |           |
| S₈      | Pulmonary | Surgery   | 30 October 2014                    | 16 8 2                                  | GEN, AMN, NET, TOB, CIP, FOS | -   | +      | -      | **blaGES-5**         |           |
| S₉      | Pulmonary | ICU       | 24 December 2014                   | 512 256 1                               | GEN, AMN, NET, TOB, CIP, FOS | +   | +      | -      | **blaVIM-2**         |           |
| S₁₀     | Pulmonary | ICU       | 20 December 2014                   | 8 4 1                                   | GEN, NET, TOB, CIP, FOS     | -   | +      | -      | **blaGES-5**         |           |
| S₁₁     | Urine    | EC        | 06 February 2015                   | 8 8 4                                   | GEN, AMN, NET, TOB, CIP, FOS | -   | -      | -      |                      | -         |
| S₁₂     | Pulmonary | Surgery   | 27 June 2015                       | 16 16 4                                 | GEN, AMN, NET, TOB, CIP, FOS | -   | +      | -      |                      | -         |
| S₁₃     | Urine    | EC        | 30 June 2015                       | 16 8 4                                  | GEN, NET, TOB, CIP, FOS     | -   | +      | -      |                      | -         |
| S₁₄     | Pulmonary | Surgery   | 19 September 2015                  | 8 8 2                                   | GEN, AMN, NET, TOB, CIP, FOS | -   | +      | -      | **blaGES-5**         |           |
Table 3. Characteristics of imipenem- and ceftazidime-resistant *P. aeruginosa* isolates (*n* = 35).

| Strains | Specimen | Ward      | Date of Isolation (Day/Month/Year) | Minimal Inhibitory Concentration (µg/mL) | Resistance to Non β-lactams | Phenotypic Detection of Carbapenemases | bla Genes |
|---------|----------|-----------|-----------------------------------|------------------------------------------|-----------------------------|---------------------------------------|-----------|
|         |          |           |                                   | Imipenem (4–8) * | Meropenem (2–8) * | Colistin (4) * | mCIM | CIMTris | mHodge |          |
| S15     | Pus      | Surgery   | 30 September 2015                | 128           | 128           | 4          | GEN, AMN, NET, TOB, CIP, FOS | -       | +       | -       | *blaGES-5, blaVIM-11* |
| S16     | Pus      | ICU       | 18 March 2016                    | 16            | 8             | 2          | GEN, NET, TOB, CIP, FOS | +       | +       | +       | *blaGES-5* |
| S17     | Pulmonary | ICU       | 06 April 2016                    | 16            | 8             | 2          | AMN, NET, TOB, CIP, FOS | -       | +       | -       | *blaGES-5* |
| S18     | Pus      | ICU       | 26 May 2016                      | 32            | 32            | 16         | GEN, AMN, NET, TOB, CIP, FOS | -       | +       | -       | *blaGES-5* |
| S19     | Blood    | ICU       | 13 June 2016                     | 4             | 4             | 2          | GEN, AMN, NET, TOB, FOS | +       | +       | -       | *blaVIM-2* |
| S20     | Blood    | ICU       | 12 July 2016                     | 32            | 8             | 2          | GEN, AMN, NET, TOB, CIP, FOS | -       | +       | -       | *blaGES-5, blaVIM-2* |
| S21     | Blood    | Surgery   | 18 August 2016                   | 32            | 16            | 2          | AMN, NET, TOB, CIP, FOS | -       | +       | -       | *blaGES-5* |
| S22     | Blood    | ICU       | 16 August 2016                   | 32            | 8             | 4          | GEN, AMN, NET, TOB, CIP, FOS | -       | +       | -       | *blaGES-5* |
| S23     | Pulmonary | Surgery   | 21 August 2016                   | 16            | 8             | 4          | AMN, NET, TOB, CIP, FOS | +       | +       | -       | *blaGES-5* |
| S24     | Puncture | Surgery   | 13 October 2016                  | 16            | 8             | 1          | GEN, AMN, NET, TOB, CIP, FOS | -       | +       | -       | *blaGES-5* |
| S25     | Pulmonary | Surgery   | 31 October 2016                  | 16            | 8             | 2          | GEN, AMN, NET, TOB, CIP, FOS | +       | +       | -       | *blaGES-5* |
| S26     | Material | ICU       | 25 October 2016                  | 16            | 8             | 2          | GEN, AMN, NET, TOB, CIP, FOS | -       | -       | -       | -       |
| S27     | Pulmonary | ICU       | 15 February 2017                 | 128           | 64            | 1          | AMN, NET, TOB, CIP, FOS | +       | +       | -       | *blaVIM-2* |
| S28     | Pus      | ICU       | 17 February 2017                 | 16            | 8             | 1          | GEN, AMN, NET, TOB, CIP, FOS | -       | +       | -       | -       |
Table 4. Characteristics of imipenem- and ceftazidime-resistant *P. aeruginosa* isolates (*n* = 35).

| Strains | Specimen | Ward       | Date of Isolation (Day/Month/Year) | Minimal Inhibitory Concentration (µg/mL) | Resistance to Non β-lactams | Phenotypic Detection of Carbapenemases | bla Genes |
|---------|----------|------------|------------------------------------|----------------------------------------|-----------------------------|--------------------------------------|-----------|
|         |          |            |                                    |                                        |                             |                                      |           |
|         |          |            |                                    |                                        |                             | mCIM       | CIMTris | mHodge |
|         |          |            |                                    |                                        |                             |                                       |           |
| S\textsubscript{29} | Pulmonary | ICU        | 24 June 2017                       | 16                                     | GEN, AMN, NET, TOB, CIP, FOS | -          | -       | -      |
| S\textsubscript{30} | Urine     | Urology    | 28 August 2017                    | 16                                     | GEN, AMN, NET, TOB, CIP, FOS | -          | +       | -      |
| S\textsubscript{31} | Rectal    | ICU        | 16 September 2017                 | 16                                     | GEN, AMN, NET, TOB, CIP, FOS | +          | +       | -      | bla\textsubscript{GES-5} |
| S\textsubscript{32} | Pulmonary | ICU        | 09 October 2017                   | 8                                      | GEN, AMN, NET, TOB, CIP, FOS | +          | +       | -      | bla\textsubscript{VIM-2} |
| S\textsubscript{33} | Rectal    | ICU        | 09 October 2017                   | 32                                     | GEN, AMN, NET, TOB, CIP, FOS | +          | +       | +      | bla\textsubscript{VIM-2} |
| S\textsubscript{34} | Rectal    | ICU        | 22 November 2017                  | 64                                     | GEN, AMN, NET, TOB, CIP, FOS | +          | +       | -      | bla\textsubscript{GES-5} |
| S\textsubscript{35} | Pulmonary | ICU        | 30 November 2017                  | 32                                     | GEN, AMN, NET, TOB, CIP, FOS | +          | +       | -      | bla\textsubscript{GES-5} |

EC: External consultation; ICU: Intensive care unit; *: MICs interpretive standard; mCIM: modified carbapenem inactivation method; CIMTris: carbapenem inactivation method Tris; mHodge test: modified Hodge test; +: Positive test; -: Negative; GEN: gentamicin; TOB: tobramycin; AMN: amikacin; NET: netilmicin; CIP: ciprofloxacin; FOS: Fosfomycin.
Of the 35 ICRPA strains tested, 26 (74.2%) harbored acquired carbapenemase-encoding genes, and 24 of these were positive on CIMTris. Four of the nine isolates not harboring acquired carbapenemase genes were negative on CIMTris, whereas for the remaining five were positive. Thus, CIMTris showed 96.15% sensitivity and 44.44% specificity for detecting carbapenemase production.

The testing of mCIM in ICRPA isolates showed that 12 of 26 (46.15%) isolates harboring carbapenemase genes were positive on mCIM. All the 14 mCIM-negative isolates harbored acquired carbapenemase genes. Nine of the nine (100%) isolates not harboring acquired carbapenemase genes were negative on mCIM. Thus, mCIM showed a sensitivity of 46.15% and a specificity of 100% for detecting carbapenemase activities (Tables 5 and 6).

Table 5. Comparison of three phenotypic methods for carbapenemase detection in *Pseudomonas aeruginosa* strains.

| Phenotypic Tests | mCIM | CIMTris | mHodge Test |
|-----------------|------|---------|-------------|
| True positive   | 12   | 28      | 3           |
| False positive  | 0    | 3       | 0           |
| Specificity (%) | 34.8 | 100     | 25          |

mCIM: modified carbapenem inactivation method; CIMTris: carbapenem inactivation method Tris; mHodge test: modified Hodge test; %: percentage.

Table 6. Comparison of three phenotypic methods for carbapenemase detection in *Pseudomonas aeruginosa* strains according to carbapenemase encoding genes.

| Phenotypic Tests | PCR Results |
|-----------------|-------------|
|                 | Carbapenemase Coding Genes | *bla* 
VIM | *bla* 
GES |
| mHodge test     | Positive (n) | 3 | 1 |
|                 | Negative (n) | 24 | 15 |
|                 | Sensitivity (%) | 12.5 | 30 | 5 |
|                 | Specificity (%) | 100 | 100 | 86.66 |
| mCIM            | Positive (n) | 7 | 5 |
|                 | Negative (n) | 20 | 10 |
|                 | Sensitivity (%) | 46.15 | 63.7 | 26.31 |
|                 | Specificity (%) | 100 | 83.33 | 62.5 |
| CIMTris         | Positive (n) | 9 | 15 |
|                 | Negative (n) | 7 | 5 |
|                 | Sensitivity (%) | 96.15 | 81.81 | 74 |
|                 | Specificity (%) | 44.44 | 29 | 21.42 |

mCIM: modified carbapenem inactivation method; mHodge test: modified Hodge test; CIMTris: carbapenem inactivation method Tris.

4. Discussion

In our study, most of the collected ICRPA strains were isolated from low respiratory samples in ICU patients, confirming the data of a previous study [17]. It has been reported that most of the nosocomial infections caused by carbapenemase-producing *P. aeruginosa* (CPPA) most frequently affect patients with pneumonia associated with mechanical
ventilation, and this is the main cause of chronic respiratory infection in immunocompromised patients.

Our study revealed that all isolated ICRPA remained susceptible only to colistin, indicating the dissemination of multidrug-resistant (MDR) strains and an emerging problem in our hospital. The problem of bacterial resistance to commonly used antibiotics is worldwide [2,5,8,17]. The management of ICRPA infections represents a difficult therapeutic challenge due to the increasing resistance levels of these organisms to most classes of antimicrobial agents.

The acquisition of carbapenemase genes by P. aeruginosa is an important cause of MDR, and therefore, the rapid and correct detection of carbapenemases is crucial [2]. Molecular methods are the gold standard in the identification of carbapenemase-producing strains, but phenotypic methods have been developed due to the high cost of molecular methods and their inability to detect new carbapenemase genes [2]. However, some phenotypic assays are still not accepted for non-fermentative Gram-negative bacilli [10]. In this study, we evaluate the performance of three phenotypic methods in the detection of carbapenemase-producing P. aeruginosa, including the modified Hodge test, mCIM, and CIMTris. The CIMTris differed from the mCIM by the Tris-HCl buffer that was used instead of water during the MEM inactivation step. The CIMTris showed markedly higher sensitivity than the mCIM and modified Hodge test (96.1% vs. 46.1 and 12.5%). The Tris-HCl buffer used in the CIMTris seemed to effectively extract carbapenemases of class A and B. However, the specificity of the CIMTris was lower than the other two methods. This could probably be explained by the degradation of meropenem by other carbapenemases that were not detected in this study. Our results show that CIMTris is useful, simple, and accessible to clinical laboratories for detecting carbapenemase production in ICRPA, but PCR is needed to confirm the presence of carbapenemase-encoding genes, as previously reported [10].

Of the 35 ICRPA strains, 26 were harboring carbapenemase-encoding genes, and 16 strains were carrying class A beta-lactamases. The coexistence of blaGES-5 and blaVIM-11 was found in one strain. Thus, our results show a predominance of blaGES-5, which is not in agreement with earlier studies carried out in Tunisia [6,8] and worldwide [2–4] that showed a dissemination of blaVIM-2. Interestingly, we report here for the first time in North Africa the emergence of blaGES-5 and blaGES-9 harboring CPPA isolates that had been reported in European [18,19], Asian [20], South African [21], and South American [22] studies.

The high levels of resistance among isolated P. aeruginosa, especially in ICUs where there are critically ill patients who underwent invasive procedures using multiple devices and broader spectrum antibiotics, emphasizes the need for measures to prevent the clinical dissemination of these isolates. A similar scenario was described by Koutsogiannou et al., who also reported the clonal dissemination of MDR P. aeruginosa in a university hospital [23].

5. Conclusions

Carbapenemase enzymes among P. aeruginosa isolates in Tunisia remain poorly investigated. This study reveals new information about carbapenemase enzymes among P. aeruginosa isolates in Tunisia by demonstrating the first detection of GES-5 and GES-9 carbapenemases as well as the co-occurrence of blaGES-5 and blaVIM-11 in one isolate. Moreover, the CIMTris is a promising approach for detecting carbapenemase activity in P. aeruginosa and merits further testing. These findings are of great concern because the rapid dissemination of MDR strains represents a major therapeutic and epidemiological threat and requires the implementation of strict hygiene procedures and regular surveillance studies.

Author Contributions: Conceptualization, S.F. and I.B.B.B.; methodology, S.F. and E.M.; software, S.F. and E.M.; validation, S.F., E.M., A.F., L.K. and I.B.B.B.; formal analysis, S.F. and E.M.; investigation, S.F. and E.M.; resources, A.F. and L.K.; data curation, S.F and E.M.; writing—original draft, S.F., E.M. and I.B.B.B. All authors have read and agreed to the published version of the manuscript.
Funding: This research was funded by the Ministry of Higher Education and Scientific Research of Tunisia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Patient consent was waived, only specimen from routine diagnostic were used.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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