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Detection of Metallo-beta-lactamase Producing *Pseudomonas aeruginosa* in an Abidjan Hospital, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KMKG, NKG and NPNDM participated in the design of the study, in the collection and analysis of the data. Authors EJT, FK and KMKG analyzed and interpreted the data. Authors AAT and SPAN coordinated the study. The manuscript was designed by author KMKG. All authors contributed to the revision and approved the final version.

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ABSTRACT

Metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* has emerged as a threat to hospital infection control, due to its ability of multi-drug resistance. This study was carried out to determine the prevalence of MBL producing *P. aeruginosa* in an Abidjan Hospital, Côte d'Ivoire.
Methods: A prospective study was undertaken to detect MBLs in *P. aeruginosa* isolates obtained from various clinical samples. A total of 88 strains were screened for imipenem resistance by the disc diffusion method. Detection of MBLs were further done by phenotypic methods, using imipenem-EDTA disk synergy (DST) test and combined disk test (CDT).

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Results: Out of 88 isolates, 14 isolates (15.9%) were imipenem resistant. Out 14 imipenem resistant *P. aeruginosa* strains, 11 were positive for MBL production by CDT and by DST.
Conclusion: Majority (78.6%) of imipenem resistant *P. aeruginosa* were MBL positive. The prevalence of MBL producing-*P. aeruginosa* increased from 10.4% in 2011 to 12.5% in 2015 in Abidjan, Côte d'Ivoire. Early detection of carbapenemases and MBL can be performed in hospitals and laboratories from the following rapid and less expensive tests.

Keywords: Metallo-beta-lactamases; Pseudomonas aeruginosa; multidrug resistance.

1. INTRODUCTION

Pseudomonas aeruginosa is one of the most important nosocomial pathogens and is responsible for infections with a high mortality rate [1] Owing to its persistence in the hospital environment, as a survival strategy an array of multidrug resistance mechanisms are often seen in such hospital isolates [2] Consequently, treatment options are narrowed down to only a few antibiotics. Carbapenems are the antibiotics of choice for severe *Pseudomonas* infections. However, resistance to this novel antibiotic is increasing worldwide [3].

Carbapenem resistance in *P. aeruginosa* is most common due to the production of Metallo-β-lactamases (MBLs) [4]. MBLs, which require divalent cations (usually zinc ions) as metal cofactors for enzymatic activity, can hydrolyze all β-lactams including carbapenems (except aztreonam) [2]. On the whole, eleven genes of MBLs have been detected, namely *blah* (IMP, VIM, NDM, SPM, GIM, AIM, SIM, DIM, KHM, FIM and TMB) [5]. Nosocomial outbreaks caused by MBL producing *P. aeruginosa* have been reported in several countries worldwide [6–10]. In Africa, previous studies have highlighted MBL-producing *P. aeruginosa* as being implicated in clinical infections in Egypt [11], Libya [12] and Tanzania [13]. In Abidjan Côte d'Ivoire, Katy *et al* described strains of *P. aeruginosa* carrying bla_{VIM-2} in 2013 [14]. The aim of this study was to determine the prevalence of MBL producing *P. aeruginosa* in the university hospital centre of Abidjan-cocody during the year 2015.

2. MATERIALS AND METHODS

2.1 Bacterial Strains and Identification

Eighty-eight *P. aeruginosa* strains isolated from various clinical specimens were collected from January to December 2015. The identification of the strains was carried out by the API® 20NE gallery. An inoculum of 0.5 Mc Farland is inoculated on API® 20NE (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The principle is based on microtubule inoculum with a suspension that rehydrates the media. Incubation was done at 37℃ for 24 hours. After incubation, the reading of the gallery was done by referring to the Table of reading. The identification of *P. aeruginosa* strains is obtained using the API® 20NE identification table.

2.2 Disc Susceptibility Test

Sensitivity to antibiotics was determined by the disc diffusion method using Mueller-Hinton agar according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [15]. The antibiotic discs tested were ticarcillin (75 µg) ticarcillin-clavulanic acid (75/10 µg), piperacillin (100 µg), piperacillin/ tazobactam (100 µg/ 10 µg), cefepime (30 µg), ceftazidime (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), gentamicin (10 µg) tobramycin (10 µg) and colistin (10 µg) (Oxoid Ltd, Basingstoke Hampshire England).

2.3 Imipenem-EDTA Combined Disk Method (CDT)

Imipenem-EDTA combined disk method (CDT) was performed as described by Yong et al. [16]. A lawn culture of test isolates was prepared. After allowing it to dry for five minutes, two imipenem discs, one with a 4 µl of 0.5 M EDTA andthe other a plain imipenem disc, were placed on the surface of agar plates approximately 30 mm apart. The plates were incubated overnight at 37℃. An increase in zone diameter of ≥ 7 mm around the imipenem+EDTA disk in comparison to imipenem disk alone indicated production of MBL (Fig. 1).

2.4 Imipenem-EDTA Double-Disk Synergy Test (DDST)

Imipenem-EDTA double-disk synergy test (DDST) was performed as described by Lee et al. [17] Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the Antibiogram Committee of the French Society of Microbiology [15]. An imipenem (10 µg) disk was placed 20 mm centre to centre from a blank disk containing 10 µL of 0.5 M EDTA (750 µg). Enhancement of the zone of inhibition in the area between imipenem and EDTA disk in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result for MBL production (Fig. 2).

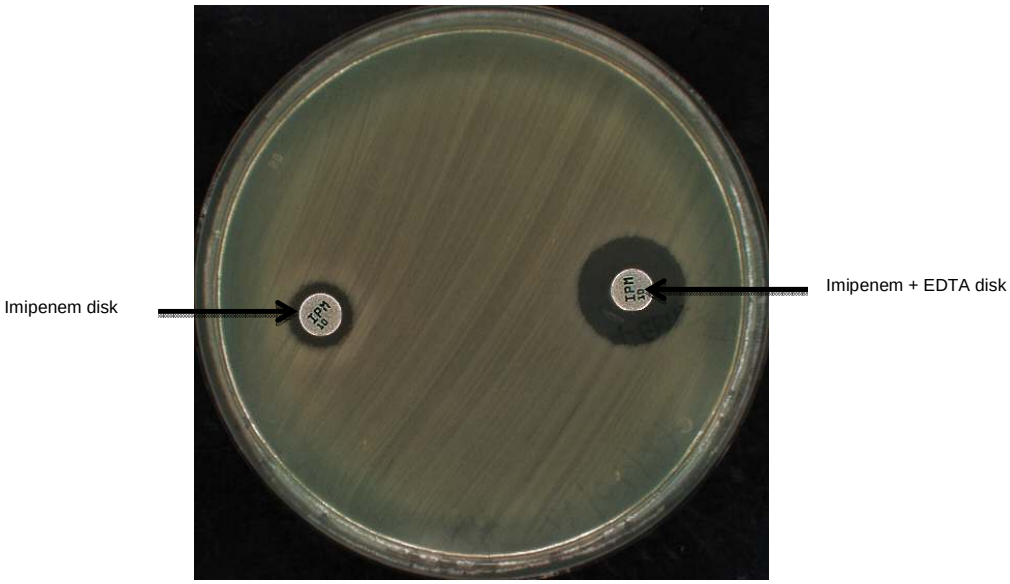

Fig. 1. Combined disk test using imipenem and imipenem-EDTA (CDT). Imipenem + EDTA disk (on the right) produced ≥ 7 mm larger zone of inhibition than the imipenem disk (on the left)

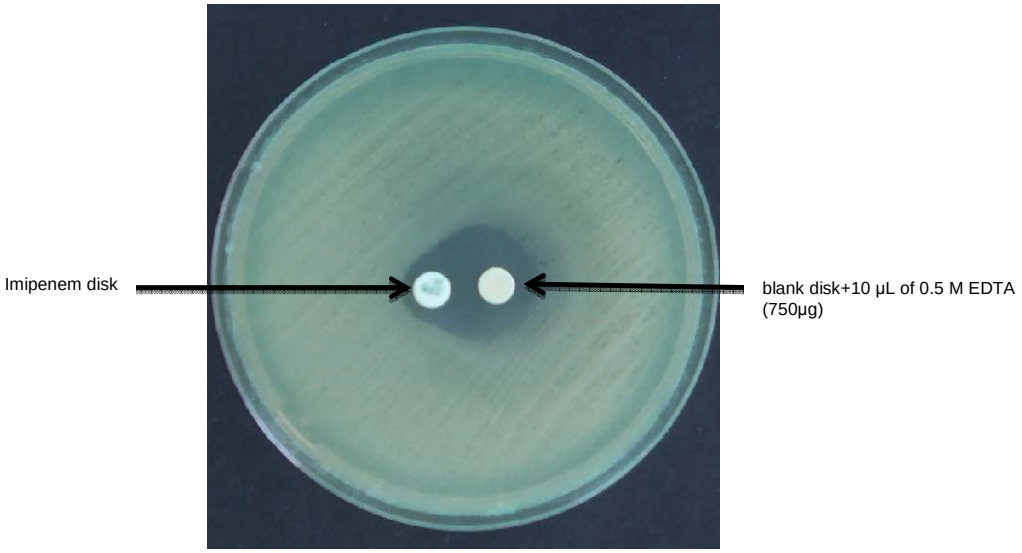

Fig. 2. Imipenem-EDTA double-disk synergy test (DST). Imipenem disk (on the left) produced a large synergistic zone of inhibition towards the imipenem + EDTA disk (on the right)

3. RESULTS

The 88 strains were recovered from 10 different departments of the university hospital centre of Abidjan-cocody. The results revealed a high prevalence of *P. aeruginosa* infections in the medical department (51.1%), followed by pneumology (15.9%), neurology (5.6%), ICU (3, 4%), endocrinology and gynaecology-obstetrics (2.2%). In the urology, pediatric, rheumatology and ENT departments the infection rate due to *P. aeruginosa* was 1.1%. The information relating to the remaining thirteen (14.8%) strains of *P. aeruginosa* has not been reported. The distribution of *P. aeruginosa* in clinical samples is summarized in Table 1.

Out of the 88 isolates of *P. aeruginosa* screened for imipenem resistance by disk diffusion test, 14 isolates (15.9%) were imipenem resistant. High rates of resistance to ticarcillin and ticarcillin + clavulanic acid were observed (42.05%), while those of colistin and amikacin were of 1.14% and 5.68% respectively. The antibiotic resistance profile of these 88 strains is summarized in Table 2.

All 14 *P. aeruginosa* strains showing resistance to imipenem 10 µg disk in the screening test were further tested by imipenem-EDTA disk synergy test and combined disk test. 11 out of 14 strains were MBL producers by combined disk test and imipenem-EDTA disk synergy test. All 11 MBL producing - *P. aeruginosa* were resistant to more than three other antibiotics. The susceptibility pattern of MBL producing - *P. aeruginosa* strains are shown in Table 3. Of the 11 MBLs positive, 5 (45.45%) were isolated from abscess, 2 (18.18%) from drain, 1 (9.1%) from urine, 1 (9.1%) from ascites, 1 (9.1%) from pleural fluid and 1 (9.1%) from swabs. 5 (45.45%) of these strains came from the Department of Medicine, 3 (27.3%) from pneumology, 2 (18.18%) from the Endocrinology Department and 1 (9.1%) from the Maternity department.

Table 1. Distribution of *P. aeruginosa* in clinical samples

Source of *P. aeruginosa* strains	Number of isolated (N=88)	Percentage (%)
Pus	36	40.9
Sputum	16	18.18
Pleural liquid	7	7.95
Blood	4	4.54
Bronchial aspiration	5	5.68
Urine	3	3.4
Drain	3	3.4
Tracheal aspiration	2	2.27
Cerebrospinal fluid	2	2.27
Ascitic fluid	1	1.13
Wound	1	1.13
Stools	1	1.13
Undetermined	7	7.95

Table 2. Susceptibility profile of the 88 strains of *P. aeruginosa* in the antibiotics used in this study

Antibiotics	Total resistant (%)	Total sensitive (%)
Amikacin	9 (10.23)	79 (89.78)
Cefepime	26 (29.54)	62 (70.46)
Ceftazidime	19 (21.59)	69 (78.41)
Ciprofloxacin	15 (19.32)	71 (80.69)
Colistin	1 (1.14)	87 (98.86)
Gentamicin	22 (25)	66 (75)
Imipenem	14 (15.91)	74 (84.09)
Levofloxacin	19 (21.59)	69 (78.41)
Piperacillin	20 (22.73)	68 (77.27)
Ticarcillin	37 (42.05)	51 (57.95)
ticarcillin clavulanic acid	37 (42.05)	51 (57.95)
Tobramycin	16 (18.18)	72 (81.82)

Table 3. Susceptibility pattern of MBL producing-*P. aeruginosa* strains to commonly used anti-pseudomonal antibiotics

Antibiotics	Resistant (%)	Sensitive (%)
Amikacin	4 (36.36)	7 (63.64)
Cefepime	11 (100)	0
Ceftazidime	11 (100)	0
Ciprofloxacin	6 (54.55)	5 (45.45)
Colistin	0	11 (100)
Gentamicin	11 (100)	0
Piperacillin-tazobactam	5 (45.45)	6 (54.55)
Tobramycin	11 (100)	0

4. DISCUSSION

The resistance of *P. aeruginosa* strains in this study was higher for beta-lactams than for other families of antibiotics. Indeed, a resistance rate of 42.05% to ticarcillin and ticarcillin + clavulanic acid and 22.73% for piperacillin was obtained. This result is different from a study in Iraq [18] where the resistance rates were 100 and 16.6% respectively for ticarcillin and ticarcillin/clavulanic acid. In Cameroon, however, 35.5 and 17.6% respectively were reported [19]. The resistance rate to piperacillin was close to 23.5% of Kamga et al. [19]. The cefepime and ceftazidime resistance rates were 29.54 and 21.59% respectively. These resistance rates are higher than previous studies in Morocco [20] and in Cameroon [19], but lower than reported in Iraq [18]. The findings in these studies could be attributed to the hyper production of beta-lactamases through resistance genes and mutational processes [21,22]. Of the 88 strains of *P. aeruginosa,* 15.91% were resistant to imipenem. According to the latest data from the AMICI (Observatoire de la Résistance des Micro-organisms aux anti-infectieux en Côte d'Ivoire), this rate was 10.4% in 2011 [23]. Moreover, this rate is higher than those obtained in Iraq (5.5%) [18] and in Morocco (11%) [20] but lower than that obtained in Mexico in 2010 [24].

Among the aminoglycosides, amikacin was the most active molecule on these strains studied with 10.23%. This result is different from those reported in Côte d'Ivoire with a resistance rate of 5% [25] and 6% in Morocco [20]. Concerning the fluoroquinolones, rates of resistance of 19.32, 21.59% in ciprofloxacin and levofloxacin were observed. Colistin was the most active molecule on these *P. aeruginosa* with a resistance level of 1.14%. Several studies have shown that *P. aeruginosa* strains are less resistant to colistin [7,18–20], this could be due to their low use in the treatment of *P. aeruginosa* infections.

Several studies have described the different methods for detecting MBL in *P. aeruginosa* [26–28]. Although MIC detection is the standard technique of MBL detection, DST and CDT are comparable to the first one. These are at the same time simple, reliable, less cumbersome and cheap, as indicated in previous reports [28,29]. Using these two methods (DST and CDT) in this study, the prevalence of MBL in *P. aeruginosa* was 12.5%. This result is similar to the results obtained by Lee et al. [30] who reported 100% sensitivity and specificity with CDT. However, with CDT, Kaly et al. [7] obtained 72.73% specificity. Therefore, these tests can be set up in small laboratories. The prevalence of MBL in *Pseudomonas* in this study is lower than in India (22.4%) [7] and Greece (50%) [31]. However, it is higher than a report obtained in 2011 in India [6]. In Africa, precisely in Egypt 11, Tunisia 3 and Uganda [32], studies have reported a prevalence of MBL-producing *P. aeruginosa* at 68.7%, 68% and 14.29%, respectively.

In this study, all MBL positive strains were sensitive to colistin and 100% resistant to ceftazidime, cefepime, gentamicin and tobramycin. These results are in agreement with those reported in the literature [6,7]. Marked levels of resistance were also observed for MBL-producing *P. aeruginosa* to amikacin, piperacillin/tazobactam and ciprofloxacin. This is largely due to the fact that the MBL genes may be located on plasmids or mobile genetic elements carrying other resistance genes to other antibiotics family. These MBL positive strains are usually resistant to beta-lactams, aminoglycosides and fluoroquinolones. However, they remain sensitive to colistin and polymyxin B [2]. In this study, the production of MBL was not the only resistance mechanism to imipenem. Three strains of *P. aeruginosa* resistant to imipenem did not produce MBL. Studies have shown that carbapenem resistance may be non-

enzymatic with loss of porin D (OprD) and the presence of inducible or derepressed AmpC [33,34].

At the level of hospital services, half of the MBL positive strains were from the Department of General Medicine, (45.45%). In addition, the predominance of MBL positive strains was higher in case of pus with a rate of 45.45% when compared to the other 5 biological products. Kali et al. [7] had previously reported a high prevalence of strains *P aeruginosa* in pus (67.3%).

5. CONCLUSION

The majority (78.6%) of imipenem resistant *P. aeruginosa* were MBL positive. These strains showed multi-resistance to other families of antibiotics but whether all were sensitive to colistin or not is yet to be found. The prevalence of *P. aeruginosa* increased from 10.4% in 2011 to 15.91% in 2015 in Côte d'Ivoire. The prevalence of MBLs in *P. aeruginosa* causing clinical infection was 12.5% in 2015. This increase must appeal to all actors in the health sector to take measures for early detection of carbapenem- and MBL-positive strains in hospitals using rapid and less expensive tests.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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