Phenotypical Characterization of *Pseudomonas aeruginosa* Clinical Strains Isolated in Abidjan (Côte d'Ivoire)

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Authors' contributions

This work was done in collaboration between all authors. Authors NPNDM and NKG wrote the protocol. Author NPNDM wrote the first draft of the manuscript. Authors KMKG and VG managed the analyzes of the study. Authors AAT, JET and BKT managed the documentary research. Authors SPAN and MD made the corrections. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: Objective of this study was to determine the prevalence of clinical *Pseudomonas aeruginosa* strains expressing antibiotic resistance mechanisms of the family of β-lactams, aminoglycosides and fluoroquinolones in Abidjan.
Study Design: It is a retrospective study.

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Place of Study: Laboratories of Bacteriology and virology of Pasteur Institut, Abidjan, Côte d'Ivoire.
Methodology: From January 2012 to December 2015 of *P. aeruginosa* strains from various hospital departments and pathological products of patients have been identified using conventional methods of Bacteriology and Maldi-Tof. Susceptibility strain to antibiotics was tested using the standard Mueller-Hinton agar diffusion susceptibility test method according to CA-SFM 2015.
Results: Two hundred and eight *Pseudomonas aeruginosa* strains were isolated which 35.83% coming from service of medicine. Among these strains 46.63% were isolated from abscess and 9.62% from urine. Antibiotics resistance profile showed a resistance of preferably β-lactams especially to ticarcillin (30.77%). Colistin was the most active antibiotic. Different types of phenotypes have been identified. Regarding β-lactams, the most observed phenotype were the wild ones with 61.06% of all the strains and 12.02% for the efflux phenotype. For aminoglycosides 69.23% were wild-type and 75.96% were wild type for fluoroquinolones.

1. INTRODUCTION

Pseudomonas aeruginosa belongs to the family Pseudomonadaceae. This family includes many species. *P. aeruginosa* is far the most frequently isolated species from pathological products, it is a ubiquitous non-fermentative Gram-negative bacillus [1]. In the hospital, *P aeruginosa* behaves essentially as an opportunistic pathogenic germ in that it infects patients who have been weakened by surgery, resuscitation or even medicine (invasive diagnostic techniques, broad-spectrum antibiotics) [1,2,3]. *P. aeruginosa* has a poor membrane permeability which gives it natural resistance to many antibiotics. The severity of nosocomial infections with *P. aeruginosa* is conditioned by specific virulence to the species. It also depends on the ability of the pathogen to accumulate antibiotic resistance mechanisms and resulting therapeutic difficulties [4]. *Pseudomonas aeruginosa* has natural resistance to Penicillin G, A and M, cephalosporin of 1st and 2nd generation and certain C3Gs such as cefotaxime. The mechanism is the production of an inducible chromosomal Cephalosporinase (Amp-C) not inhibited by clavulanic acid [1,4,5]. In addition to this enzymatic resistance, there may be an efflux system (MexA-MexB-OprM) which causes the antibiotic to be expelled from the bacterial cell. This mechanism is opposed to beta-lactams, tetracyclines, phenicolates and fluoroquinolones thus exhibiting cross-resistance. It may be isolated or associated with a decreased permeability of the bacterial wall. The latter may be selective with respect to the imipenem by reduction or loss of the D2 porin (OprD) or extended to the other beta-lactams. Against aminoglycosides, *Pseudomonas aeruginosa* has mostly enzymatic mechanisms but also non-enzymatic mechanisms such as impermeability of the bacterial wall and the MexXY-OprM efflux system [6]. Facing fluoroquinolones, it involves target mutations (DNA gyrase) and impermeability phenomena [7].

The abuse of antibiotics, non-compliance and the anarchic consumption of antibiotics have contributed to a change in the susceptibility profile of microbial species and to the emergence of multi-resistant germs. Studies by Cholley in French and Sefraoui in Algeria [5,8] mentioned the increasing frequency of *Pseudomonas aeruginosa* strains multi-resistant to almost all therapeutic antibiotics except colistin. Thus, the dramatic evolution of antibiotic resistance is hampered by the absence of new antipseudomonal therapies and requires rigorous monitoring of local epidemiology and the state of current resistance to adapt the use of antibiotics to our context. Therefore, detection of resistance would prevent or slow down the spread of multi-resistant strains. In Côte d'Ivoire, although some studies have reported resistance phenotypes in strains of *Pseudomonas aeruginosa* [9], there is very little information on the mechanisms of resistance to antibiotics. The aim of this study was to determine the proportion of strains of *Pseudomonas aeruginosa* expressing antibiotic resistance mechanisms in the β-lactam, aminoglycoside and fluoroquinolone family in Abidjan (Côte d'Ivoire).

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Bacteria

A descriptive study has been carried out in the laboratories of medical bacteriology, the Antibiotics and Natural Substances Unit and the resistance monitoring of Microorganisms to the anti-Infectives (ASSURMI) of the Institut

Pasteur of Côte d'Ivoire and some health institutions (University Hospital and University and private clinics) of Abidjan city. *Pseudomonas aeruginosa* isolates were clinical origin and stored at the Biological Resource Center (CeReB) of the Institut Pasteur of Côte d'Ivoire from January 2012 to 31 December 2015 were used. The strains were isolated of pathological products from patients of different medical departments. The genus and the species of strains were verified and confirmed by conventional methods of classical microbiology, after subculturing on agar cetrimide medium and basis of cultural criteria (the appearance of the green coloration which indicates the pyoverdine secretion or a pigment pyocyanin) and biochemical (use of the media Urea-indole, disks oxidase). Biochemical identification was also performed using MALDI TOF (BioMérieux SA, Lyon, French).

2.2 Activity of Antibiotics Tested

Activity of thirteen antibiotics was determined by the standard antibiogram method on Muller Hinton medium (MH). Antibiogram used during the period covered by this study is the classical Kirby-Bauer method by the diffusion of antibiotics from disks impregnated on ordinary MH agar. After 18 to 24 hours incubation in an oven at 37℃., the diffusion diameters of the various antibiotics were measured and compared with the reference diameter of the Antibiogram Committee of the French Society of Microbiology [10]. The choice of discs takes into account both natural resistances and therapeutic possibilities (ceftazidime, imipenem, amikacin, ciprofloxacin). However, some antibiotics have been tested for the sole purpose of consolidating the identification. This is the case of kanamycin (still inactive) and colistin (still active in vitro). A quality control of the antibiotics tested and of the culture media used was carried out using the *P. aeruginosa* strain ATCC 27853. The interpretation was made according to the criteria recommended by the Antibiogram Committee of the Society French of Microbiology [10] and the interpretative reading of the antibiogram revealed the different phenotypes expressed by *P. aeruginosa* in β-lactams: cefepime (FEP, 30 µg), ceftazidime (CAZ, 30 µg), imipenem (IMP, 10 µg) amikacin (AKN, 30 µg), gentamycin (GMN, 15 µg, tobramycin (TMN, 10 µg), piperacillin (PIP, 75 µg), ticarcillin (TIC, 75 µg), ticarcillin / clavulanic acid) and fluoroquinolones (ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg). Detection of broad spectrum. β-lactamases was facilitated on antibiogram by bringing ticarcillin / clavulanic acid, ceftazidime, cefepime [11]. In this study, other resistance phenotypes were determined for all strains according to the method described by Mesaros et al. [7]. All resistant bacteria corresponded to strains classified as resistant (R) or intermediate (I). The calculated resistance levels were the sum of the levels of intermediate strains (I) and resistant strains (R). The data was entered and analyzed on the Excel software.

3. RESULTS

3.1 Prevalence of *Pseudomonas aeruginosa* Strains by Sampling Sources and Services

A total of 208 *Pseudomonas aeruginosa* strains were isolated from the different sampling sources. of Specimens sources consisted of urine, blood for blood culture, abscess area, sputum and other products (tracheal aspirations, drain, gum, bronchial aspirations, ascites, pleural fluid, wounds, stools, catheter and cerebrospinal fluid). *P. aeruginosa* strains isolated from the abscess area were the most representative. With a prevalence of 46.63% from all strains isolated. This prevalence was 9.62% with strains isolated from urine and at unspecified sites. The prevalence was less than 5% from *Pseudomonas aeruginosa* strains isolated from the pleural fluid, blood, probes, wounds, catheter, tips of drains and cerebrospinal fluid. Very low prevalence of less than 1% were observed with *P. aeruginosa* strains isolated from stool, ascites fluid, tracheal aspiration and gums Table 1.

Medicine service and Undetermineted service were the most represented as shown in Fig. 1 with respective isolation frequencies of 35.58% and 23.07%. Follow-up of the pneumology services (9.61%), pediatrics (6.73%) and resuscitation (5.77%). The proportions of *P. aeruginosa* strains present were less than 5% in neurology, ENT, gastroenterology, urology, surgery, stomatology (Fig. 1). The lowest prevalence were observed in endocrinology, gynecology-obstetrics, traumatology, nephrology and rheumatology. The prevalence of strains isolated in these services was less than 1% (Fig. 1).
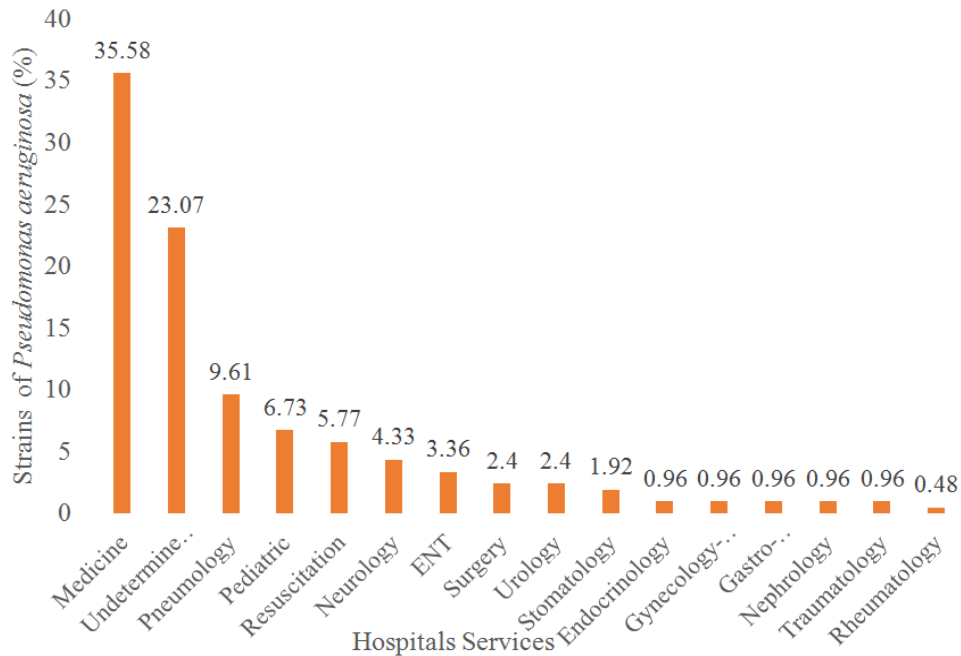
Fig. 1. Frequency of *Pseudomonas aeruginosa* strains isolated by hospital service

3.2 Evaluation of the Resistance Level of *Pseudomonas aeruginosa* Strains to Antibiotics

Pseudomonas aeruginosa strains showed different levels of resistance to antibiotics (Fig. 2). Beta-lactam resistance levels were as follows: ceftazidime (17.78%), cefepime (22.60%), imipenem (13.46%), meropenem (13, 46%), ticarcillin (30.77%), ticarcillin + clavulanic acid (29.32%) and piperacillin (19.71%). For aminoglycosides, the highest rate was observed for gentamycin (20.19%) followed by tobramycin (18.75%), while amikacin was the most active antibiotic in this family (10, 57%). For fluoroquinolone family, the resistance rate was 15.48% form ciprofloxacin and 21.15% for levofloxacin. Finally, *Pseudomonas aeruginosa* strains showed very low resistance to colistin (2.88%) (Fig. 2).

3.3 Occurrence of *Pseudomonas aeruginosa* Strains According to Resistance Phenotypes to Beta-lactams, Aminoglycosides and Fluoroquinolones

The interpretive reading of the antibiogram revealed various resistance phenotypes. The wild-type phenotype grouping the strains having acquired no resistance in each family of antibiotics tested. These strains represent the majority phenotype with a frequency of 61.06% for β-lactams. The phenotypes of acquired resistance expressed for β-lactams were the hyper production of cephalosporinases "CASE" with 9.13% of the strains expressing this phenotype. The CASE phenotype was expressed in the same proportion of 9.13% of *P. aeruginosa* strains, alone or associated with the phenotype "OprD deficient". The "efflux" phenotype was expressed with 12.02% of the isolated *P. aeruginosa* strains. In all strains, 13.46% expressed a resistance phenotype to imipenem. This phenotype is characterized by loss of the porin "deficient OprD" alone representing 3.37% of the strains, or associated with other resistance mechanisms to β-lactams at 10.09%. With 4.33% of prevalence, the product of penicillinase « PASE » was less expressed at *Pseudomonas aeruginosa* strains (Fig. 3).

With regard to aminoglycosides, the distribution of *Pseudomonas aeruginosa* strains according to resistance phenotypes to aminoglycosides shown in Fig. 4. From this figure, the wild-type or all-aminoglycoside phenotype represented 69.23% of all the strains tested. The most resistant phenotypes were resistant gentamycin-tobramycin phenotypes (GMN / TMN) with 13.94%. The frequency of 10.10% of strains was observed with the gentamycin-tobramycin-resistant amikacin phenotype (GMN / TMN /

AKN). All strains tested, tobramycin-resistant amikacin phenotype (TMN / AKN) was the least phenotype represented with 0.48%.

For fluoroquinolones, Fig. 5 shows that the majority of *Pseudomonas aeruginosa* strains isolated in this study were wild-type, susceptible to all antibiotics of the fluoroquinolone family tested (type I phenotype). The frequency of *P. aeruginosa* strains expressing the type I phenotype (wild-type phenotype) was 75.96%.

The *P. aeruginosa* strains resistant to all the antibiotics of the family fluoroquinolones tested expressed the phenotype ciprofloxacin-levofloxacin resistant (phenotype of type IV). With type IV phenotype, a frequency of 12.50% strains tested was observed. The resistant ciprofloxacin phenotype (type II phenotype) characterized by resistance of *P. aeruginosa* strains to ciprofloxacin alone, was expressed with 3.85% of strains tested.
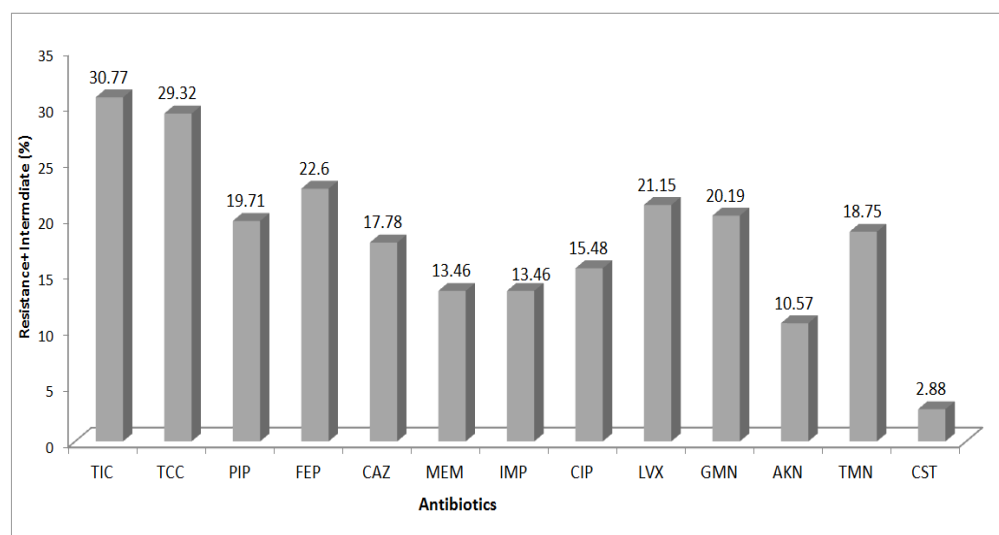

Fig. 2. Resistance level of *Pseudomonas aeruginosa* strains
Ticarcillin (TIC), Ticarcillin + clavulanic acid (TCC), Piperacillin (PIP), Cefepime (FEP), Ceftazidime (CAZ), Meropenem (MEM), Imipenem (IMP), Ciprofloxacin (CIP), Levofloxacin (LVX), Gentamycin (GMN), Amikacin (AKN), Tobramycin (TMN), Colistin (CST)

Table 1. Frequency of *Pseudomonas aeruginosa* strains by biological samples and their resistance to antibiotics

| Biological samples	Number of strains (%)	Number of susceptible strains (%)	Number of resistant strains (%)
Abscess	97 (46.63)	52 (53.6)	45 (46.4)
Undetermineted	20 (9.62)	13 (65)	7 (35)
Urine	20(9.62)	11 (55)	9 (45)
Blood	7 (3.37)	3(42.86)	4 (57.14)
Sputum	18 (8.65)	7 (38.89)	11 (61.11)
Tracheal aspirations	2 (0.96)	2 (100)	-
Bronchial aspirations	11 (5.29)	6 (54.54)	5 (45.46)
Drain	3 (1/44)	-	3 (100)
Ascite fluid	1 (0.48)	1(100)	-
Probes	6 (2/88)	2 (33.33)	4 (66.67)
Catheter	4 (1.92)	2 (50)	2 (50)
Gum	2 (0.96)	1 (50)	1 (50)
Cerebrospinal fluid	3 (1.44)	3 (100)	-
Wound	3 (1.44)	1 (33.33)	2 (66.67)
Pleural fluid	10 (4.81)	10 (100)	-
Stool	1 (0.48)	1 (100)	-
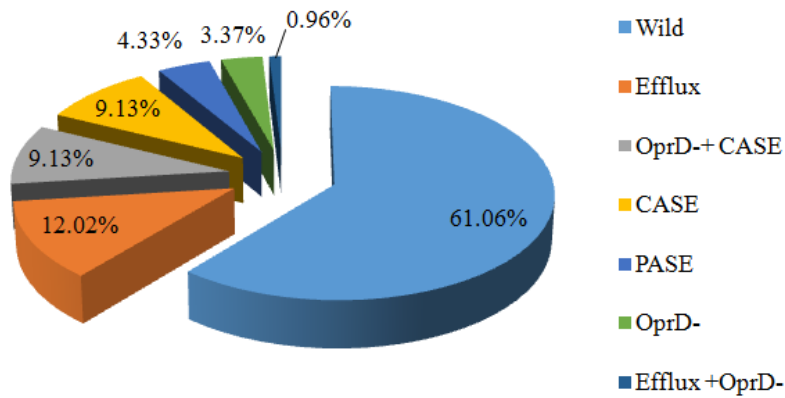
Fig. 3. Breakdown strains by beta-lactam resistance phenotypes
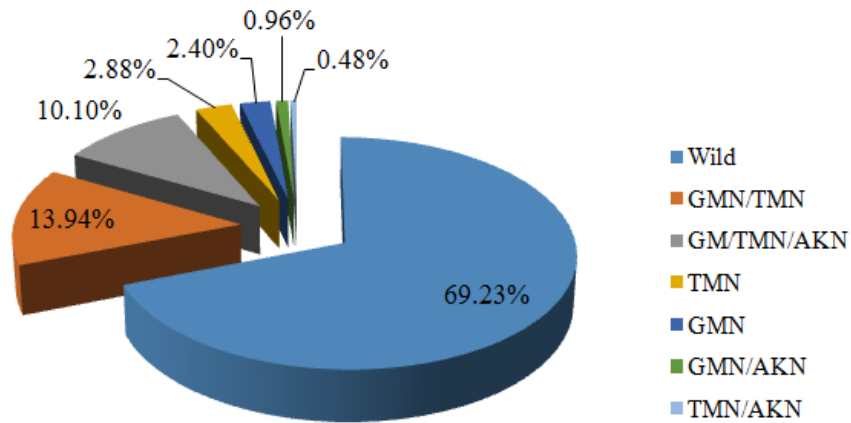*CASE: hyper-produced cepholosporinase, PASE: penicillinase, OprD-: porin OprD deficient*

Fig. 4. Distribution strains according to resistance phenotypes from aminoglycosides
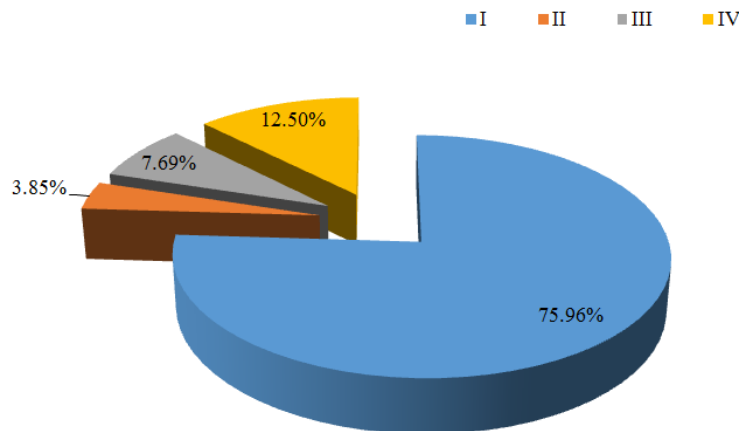*GMN: Gentamycin, TMN: Tobramycin, AKN: Amikacin*

Fig. 5. Distribution of the strains according to resistance phenotypes to fluoroquinolones
I: Wild phenotype (Sensitive to ciprofloxacin and levofloxacin), II: Phenotype of resistance to ciprofloxacin,
III: Phenotype of resistance to levofloxacin, IV: Phenotype of resistance to ciprofloxacin and levofloxacin

4. DISCUSSION

This study made it possible to demonstrate a high prevalence of the population of *P. aeruginosa* strains isolated in hospital departments and biological products. This high prevalence has been demonstrated by previous surveillance studies [12]. At the level of hospital services, *Pseudomonas aeruginosa* was found in all types of services during the four years. However, Medicine Department showed a predominance of isolation with a frequency of 35.48% of strains isolated. Studies in Morocco and Cameroon, *Pseudomonas aeruginosa* have revealed similar results in Medicine Department [3,13]. In addition, other services were concerned as revealed by previous studies [12]. In Algeria, studies have shown a higher isolation frequency of P. aeruginosa in the resuscitation department [8]. In Europe, large burns and resuscitation services are the most affected by *Pseudomonas aeruginosa* infections [14,15,16]. The congestion of the service as well as the lack of isolation of the infected patients could explain the diffusion of the bacterium. In addition, the circulation of patients between different units of the same hospital but also between hospitals, nationally or internationally, can contribute to the spread of germs. Transmission by nursing staff remains an important factor in the spread of strains, due to non-compliance with hygiene regulations due to overwork, which is the usual situation in this medical service. To reduce transmission by Hand washing, and the use of hydro-alcoholic solutions are essential as a standard hygiene measure. It is therefore necessary to insist on the importance of isolation practices and on prevention through the application of traditional hygiene rules. The therapeutic protocols used in each department also play a role in the emergence of epidemics by selection pressure. The spectrum of *P. aeruginosa* infections includes pneumonia, meningitis, skin lesions, septicemia, urinary tract infections, ENT infections [1,4]. It is capable of infecting any site of the human organism. Abscess specimens were the main isolation site for P. aeruginosa strains during the study, with a prevalence of 46.63% of the strains isolated. Similar results were obtained in Morocco [3,17]. For these authors, suppuration showed a high prevalence of strains of *P. aeruginosa* compared to other biological products. However, urine showed a high prevalence of *P. aeruginosa* strains in studies in Cameroon and Egypt [13,18]. The study of the susceptibility of *P. aeruginosa* strains carried out with 14 antibiotics belonging to different families revealed that the majority of resistant strains were given to at least three antibiotics of different families. This indicates the presence of multidrug-resistant *Pseudomonas aeruginosa* (PAMR) in Côte d'Ivoire. Colistin remained the most active antibiotic on *P. aeruginosa* with a low resistance rate of 2.88%. Work carried out in the Mediterranean and in Africa revealed no colistin resistant *P. aeruginosa* [5,8,13]. However, a 2% resistance rate was recorded with the work carried out by Goli and al on *Pseudomonas aeruginosa* strains from the hospitals of Tabriz in Iran [19]. This colistin activity is due to its lesser use in therapy in *P. aeruginosa* infections and probabilistic antibiotic therapy. In addition, 48.65% resistance levels were observed by *Pseudomonas aeruginosa* strains isolated from the wounds of patients hospitalized at the Brazzaville University Hospital in Congo [20]. Similarly, 33% intermediate sensitivity was observed in Singapore [21]. This difference in colistin activity can be explained by the isolation environment of the strains, the local habits in consumption and the prescription of colistin. In assessing previous data from the Observatory of Microorganism Resistance to Anti-Infectives in Côte d'Ivoire (ORMICI) [9], it was found that the antibiotic resistance rate of the β-lactam family was considerably lowered, except for the imipenem. With this antibiotic, resistance rate rose from 10.4% to 13.46%. This decrease would be due to an awareness in the consumption of antibiotics belonging to the β-lactam family. In aminoglycoside family, amikacin remained the most active. Resistance rate of 10.57% obtained is close to that obtained by Louzi which is 12.9% and higher than those obtained in Côte d'Ivoire with the resistance rate of 5% [22] and Morocco 6% [17]). For fluoroquinolone family, ciprofloxacin remained more active. Ciprofloxacin has been shown to be most active of quinolones in [23], which confirms the results of our study.

The study of beta-lactam resistance phenotypes revealed no strain expressing the ESBL phenotype. In addition, resistance to beta-lactam antibiotics has been observed by combining several phenotypes, noted in two cases, should encourage a doubling of vigilance in order to avoid the diffusion of multi-resistant strains. In this study, the efflux phenotype alone is represented by 12.02% of isolated strains which is consistent with the results of Sefraoui [8] with 17.5% overproduction of MexAB-OprM active efflux systems. Louzi et al. [3] with 4.8%. Efflux Phenotype: this is a non-enzymatic mechanism of resistance. This mechanism contributes to the

resistance in diffusion rate of the antibiotics by actively expelling them towards the extracellular medium [24]. Ticarcillin alone is a good indicator of the efflux phenotype of the MexAB-OprM effluent pump [7]. In this study, the efflux mechanism was the most representative of resistance mechanism for beta-lactam antibiotics family. Similar results have been reported in France by Cavallo et al. [25].

The phenotype penicillinase (PASE), it was recorded with a frequency of 4.33% of strains isolated. These results differ from the results of Kamga et al. [13] with a frequency of 26.5%. This mechanism confers inactivation on carboxypenicillins and ureidopenicillins, but not on ceftazidime and carbapenems with production of penicillinase. Hyper-produced cepholosporinase (CASE) with a frequency of 9.13%, inhibits the activity of most β-lactams except for the imipenem [13,26,27]. This result is similar to that of Louzi [3] with a frequency of 9.7. But higher than those reported by Sefraoui [8] and Kamga [13] with respective frequencies of 4.6% and 5.9%. For this mechanism, ceftazidime is always less active. The activity of cefepime was not inhibited by this enzyme [28]. The loss of porin or phenotype "OprD deficient" was expressed alone with a frequency of 3.37%. It is a non-enzymatic resistance characterized by selective resistance to imipenem. These results are consistent with those of Sefraoui [8] and *Kamga* et al. [13]. The sometimes abusive use of broad spectrum antibiotics, including carbapenems, is a risk factor for the selection of this resistance mechanism [1]. Modification of the outer membrane protein OprD remains the most frequent mechanism of resistance to imipenem.

The determination of the resistance phenotype to aminoglycosides and other antibiotics is more delicate on classical antibiogram [29]. Nevertheless, the resistance phenotype to gentamycin, tobramycin and amikacin (GMN / TMN / AKN), in this study was expressed with 10.10% strains isolated. These results are consistent with those reported by Teixeira et al [30]. This could be due to the production of N-acetyltransferase enzymes, AAC (6) -II, which catalyze the acetylation of the -NH2 function. The modification of AAC contributes of this resistance species to most aminoglycosides used in therapeutics [5]. This phenotype could also be due to the action of an active efflux mechanism. Indeed, the MexXY-OprM active efflux system is capable of exporting aminoglycosides to the external environment and causing resistance to this family of antibiotics [6,27]. The resistance

phenotype to gentamycin and tobramycin (GMN / TMN) was the most expressed resistance phenotype with a frequency of 13.94%. These results differ from results obtained by *Teixeira et al* with 2.04% strains [30]. This mechanism is determined by the enzymes ANT (2 '), AAC (3) -II and AAC (6') - II. Resistance phenotype to tobramycin and amikacin (TMN / AKN) characterized by enzyme AAC (6 '). I was the least represented in this study with 0.48% strains. While this was the most representative phenotype described by Teixeira et al. [30]. DNA gyrase is an enzyme necessary for fluoroquinolones activity. The mutations of *GyrA* genes generate resistance by alteration of the target. The type II phenotype was expressed with a frequency of 3.85% strains. This phenotype would probably be due to mutations in the regions of gyrA (DNA gyrase) genes. The type IV phenotype with an expression frequency of 12.50% would be the result of mutations at gyrA in association with ParC which affect the binding of fluoroquinolones to their targets [5,31]. Resistance phenotypes to the fluoroquinolones observed could thus be attributed to the association of the action of a gyrase encoded by a plasmid gene and an efflux pump [7,24].

5. CONCLUSION

This work reports a high rate of multidrug-resistant *Pseudomonas aeruginosa* (44.71%). In this study, the good sensitivity to amikacin suggests the use of the beta-lactam-amikacin combination as a first-line probabilistic treatment for *Pseudomonas aeruginosa* infections and the imipenem-amikacin combination for targeted treatment of multidrug-resistant *P. aeruginosa*. These strains accumulate several resistance mechanisms that may be due to mutations or gene acquisitions. The most resistant phenotypes, often unknown, are an integral part of our clinical practice bacteriology context, so it would be essential to systematically characterize the resistance expressed by strains with respect to antibiotics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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