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Emergence of Antibiotic Resistance and Correlation with the Efflux Pump in *Pseudomonas aeruginosa* Isolated from Abidjan Hospital

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Abstract: ***Emergence of antibiotic resistance of Pseudomonas aeruginosa clinical strains is increasingly becoming a public health trouble shooting. The main objectives of the present study were to determine the resistance levels of different antibiotics used in therapy and to correlate the expression of four efflux pumps genes with the phenotypic resistance to antibiotics. 208 isolates of P. aeruginosa from the various biological products, stored from January 2012 to December 2015 at the Center of Biological Resources (CeReB) of the Institut Pasteur of Côte d'Ivoire have been assessed to clearly understand sensitivity of 14 antibiotics using diffusion method. As results, this work highly showed a clear emergence as regards the resistance of P. aeruginosa strains to the different classes of selected antibiotics. Nevertheless, high resistance was preferentially observed for β-lactams, in particular for ticarcillin (30.77%). Colistin (2,4%) was the most active antibiotic in this study. This work also demonstrated the correlation between the P. aeruginosa strains resistance and the efflux pump mechanism using RT-PCR techniques. 83% of the ticarcillin-resistant strains expressed the constitutive mexB gene, 52% cefepime- resistant strains expressed regulated mexD gene, 59% of imipenem and ciprofloxacin-resistant strains expressed regulated mexF gene and finally 62% of gentamicin-resistant strains could express constitutive mexY gene.***

Keywords: Emergence, Antibiotic, resistance, *Pseudomonas aeruginosa*, efflux pump

1. Introduction

Pseudomonas aeruginosa is a non-fermentative Gram-negative bacterium, a saprophyte of the environment, notably in water, moist soils and plants. Commensal bacterium from the human to the intestinal area, *Pseudomonas aeruginosa* is able to infect any organism area [13], [15]**.** It is frequently opportunistic and detected in common human infections in intensive care units [24], [27]**.** In nosocomial diseases classification, *Pseudomonas aeruginosa* is the third one after *Escherichia coli* and *Staphylococcus aureus* [1]. In Côte d'Ivoire, although there are no national data, several studies have been demonstrated the extent of *Pseudomonas aeruginosa* in nosocomial diseases in different health facilities [9], [34]**.** It results in additional drug costs and longer hospital stays with patients. However, abuse of antibiotics, non drug-compliance and anarchic consumption of antimicrobials contributed to the changes in the susceptibility profile of microbial species and to the emergence of multi-resistant germs. *P. aeruginosa* constitutively expresses an efflux system (MexAB-OprM) and poor membrane permeability conferring natural resistance to many antibiotics used in therapy. In addition to this natural resistance, this germ is characterized by its particular ability to acquire and accumulate numerous resistance mechanisms that could be linked to the appearance of multi-resistant strains [22]**.** This accumulation of resistance mechanisms has become problematic. In extreme cases it leads to a therapeutic stalemate due to the emergence of strains known to be totally resistant to the antibiotics currently available on the market [22, 32]**.** Active efflux is an important non-enzymatic mechanism in antibiotic resistance in *P. aeruginosa*. It also contributes to the development of multidrug-resistant. *P. aeruginosa* harbours four genetically different three-component efflux family systems belonging to the resistance-nodulation-cell division (RND), MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM [16], [18], [19]. The first component of each system contains a protein located in the cytoplasmic membrane including MexB, MexD, MexF and MexY. The pump functions as an energy-dependent pump with wide substrate specificity. The second component is closed to outer membrane protein (OprM, OprJ, OprN and OprM). The third protein (MexA, MexC, MexE and MexX) is located in the periplasmic area [19]**.** Efflux mechanisms contribute predominantly to the decline of antimicrobials in *Pseudomonas aeruginosa*, and therefore contribute to the emergence of resistance [16], [21], [22].

In this work we investigated the emergence of multiresistance *of Pseudomonas aeruginosa* and correlated antibiotic resistance and gene expression of the four efflux pumps most commonly used by *Pseudomonas* strains.

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2. Materials and Methods

2.1 Strains

A total of 208 bacterial strains of *Pseudomonas aeruginosa* coming from clinical origin have been previously conserved at the Biological Resource Center (CeReB) of Côte d'Ivoire Institut Pasteur from January 2012 to December 2015. The genus and species of isolates were verified and confirmed. Verification of the strains of *Pseudomonas aeruginosa* has been carried out by conventional methods of classical microbiology, after enrichment on the brain heart broth (BCC liquid medium) and subculture on Agar containing Cetrimide, based on the cultural characteristics (pyocyanine and pyoverdine colonies). In addition, identification using MALDI TOF techniques has been also used, and molecular biology using the amplification of 16S RNA, *recA*, and *rpoB* house keeping genes.

2.2 Study of sensitivity antibiotic

Antibiotic activity has been determined by the environment Disk agar diffusion method of Kirby Bauer on agar medium Mueller-Hinton-like. Following antibiotics have been tested according to the recommendations of the Committee of the susceptibility of the French Society for Microbiology [7] including β-lactam antibiotics (aztreonam (ATM, 30µg), cefepime (FEP, 30 µg), ceftazidime (CAZ, 30 µg), imipenem (IMP, 10 µg) Meropenem (MEM 10 µg), piperacillin (PIP, 75 µg), ticarcillin (ICT 75 µg), ticarcillin / clavulanic acid (TCC, 75/10 µg)), aminoglycosides (amikacin (AKN, 30 µg) gentamicin (GEN, 10 µg), tobramycin (TMN, 10 µg)) and fluoroquinolones (ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg) and colistin (CST, 10 µg). Antibiotic discs were placed using a manual dispenser while respecting the location of the antibiotic discs for *Pseudomonas aeruginosa* recommended in the CA-SFM [7]. The plates were then stored at room temperature (25 ± 2 ° C) in bench during 15 minutes to allow a pre-release antibiotics before the incubated at 37 ° C for 18 to 24 hours. Interpretation of the results has been made by the automated method capture boxes with a reading camera incorporated into the ADAGIO (BIORAD, France). The results are transcribed into sensitive (S), intermediate (I), resistant (R) categories as recommended by CA-SFM [7]. Kanamycin disc has been assessed to control the natural resistance of *P. aeruginosa* strains. The quality control of the antibiotics tested and of the culture media used was carried out from the *P. aeruginosa* strain of reference ATCC 27853. The strains with intermediate sensitivity were not classified as resistant. Data have been selected and analyzed on Excel sofware.

2.3 Assessment of Efflux Mechanism/RT-PCR Analysis

In order to understand the efflux mechanism for the possible contribution of the MexAB-OprM and MexEF-OpN, MexCD-OprJ, MexXY-OprM efflux systems to the observed resistance, PCR techniques has been used after reverse transcription (RT-PCR) to amplify the *mexB*, *mexD*, *mexF* and *mexY* multiplexes genes was used. Briefly, bacterial colonies were suspended in 200 µL of PBS, centrifuged for 5 min at 5000 rpm, at 4 ° C. After centrifugation pellet has been used to extract the total RNAs with the ReliaPrepTM RNA Cell Miniprep kit (Promega) according to the protocol recommended by the manufacturer. The RNA extracts were stored at -20 ° C until use. The reverse transcription was carried out in a volume of 30 µL according to the manufacturer procedures. The RNA mixture are transferred into Eppendorf tubes of the "Mastercycler personal" type and are introduced into the thermocycler. The amplification reactions are carried out in a thermocycler (Thermocycler 9700 ABI / Applied Biosystem). 1.5% Agarose gel electrophoresis in the presence of TBE has been used. 5 µL of amplification products have been deposited for visualization.

Table 1: List of primers used in this work for genes expression encoding the efflux pumps [29].

Genes	*Primers*	*Sequences (5'-3')*	*Size of amplicons (pb)*
mexB	primer F	5'-ACTTCTTCAGCTTCAAGGAC-3'	155
	primer R	5'-GAGCATGAGGAACTTGTTG-3'	
mexD	primer F	5'-CTACCCTGGTGAAACAGC-3'	250
	primer R	5'-AGCAGGTACATCACCATCA-3'	
mexF	primer F	5'-CATCGAGATCTCCAACCT-3'	350
	primer R	5'-GTTCTCCACCACCACGAT-3'	
mexY	primer F	5'-GCTACAACATCCCCTATGAC-3'	445
	primer R	5'- AACTGGCGGTAGATGTTG-3'	

3. Results

3.1 Prevalence of *P. aeruginosa* strains during the year of collection

Among the 208 strains *P. aeruginosa*, 34 (16.35%) and 22 (10.58%) have been collected respectively in 2012 and 2013. In 2014, 64 (30.77%) were isolated. 88 (42.30%) strains of *P. aeruginosa* were collected in 2015 (**Figure 1**)

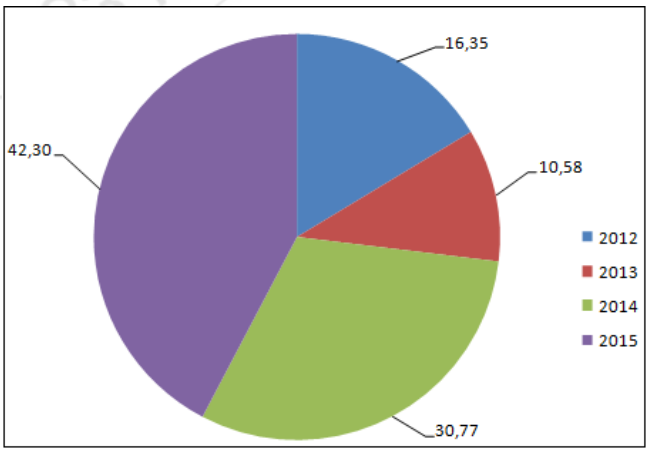

Figure 1: Distribution of *Pseudomonas aeruginosa* strains from 2012 to 2015

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3.2 Prevalence of strains of *P. aeruginosa* basing on hospital services

Strains have been isolated from 16 hospital centers. General services and Pneumology showed a greater occurrence of strains of *P. aeruginosa*. For all three services the isolation frequencies of *P. aeruginosa* were 35.58% (74) in Medicine service, 23.07% (48) in Undetermineted service and 9.61% (20) in Pneumology **(Figure 2)**. These isolation frequencies varied in the same proportions in the Pediatric, Resuscitation, Neurology, ENT, Surgery, Urology and Stomatology area. 14 (6.73%) in Pediatrics, 12 (5.77%), in Resuscitation, 9 (4.33%) in Neurology, 7 (3.36%) in ENT, 5 2.40%) in Surgery, 5 (2.40%) in Urology and 4 (1.92%) in Stomatology (**Figure 2**). Low prevalences have been observed in Endocrinology 2 (0.96%), Gynecology-Obstetrics 2 (0.96%), Gastroenterology 2 (0.96%), Nephrology 2 (0, 96%), Traumatology 2 (0.96%) and Rheumatology 1 (0.48%).

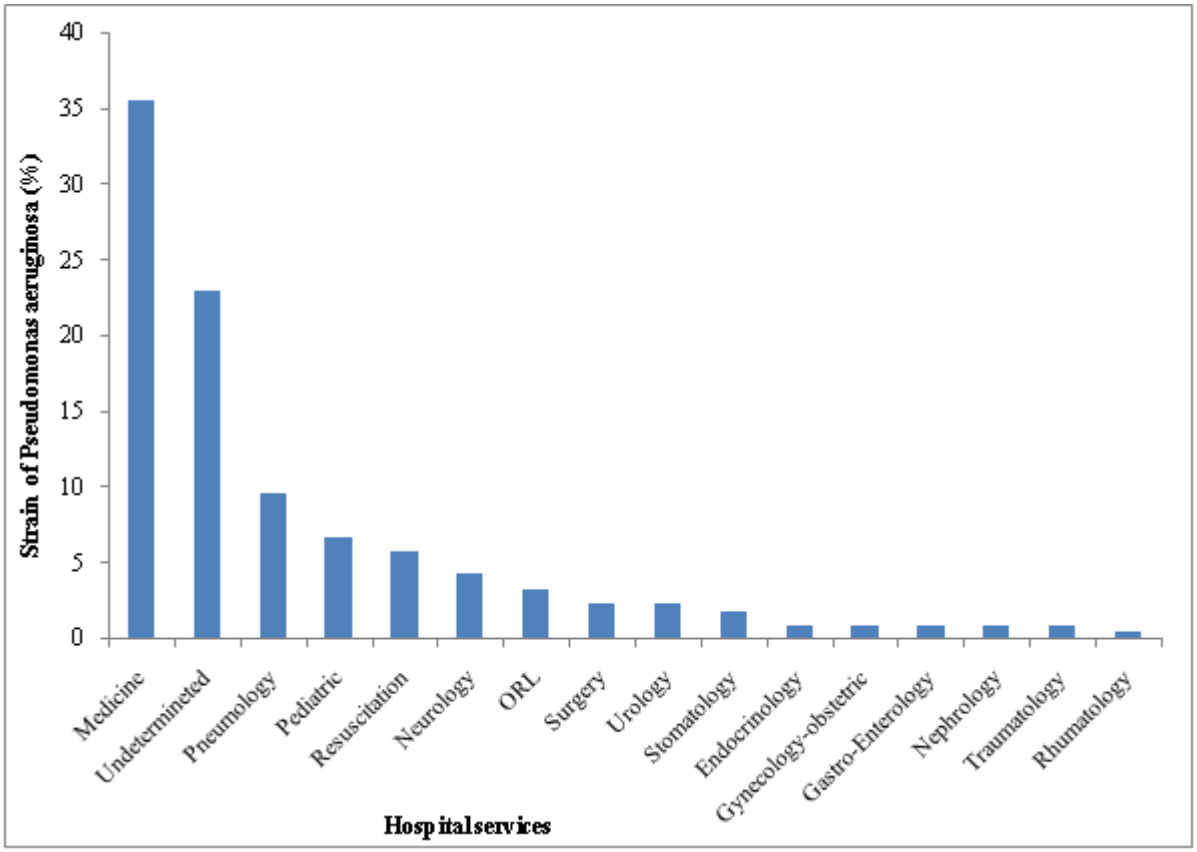

Figure 2: Frequency of *P. aeruginosa* strains isolated from hospital

3.3 Prevalence of strains of *P. aeruginosa* according to sampling sites

Among 208 strains isolated from 14 biological products of the patients in different proportions (**Figure 3**), results revealed a high prevalence of *P. aeruginosa* in abscess area. In this biological product, 100 strains of *P. aeruginosa* were isolated, ie 48.08%. On the other biological products, the occurrence *P. aeruginosa* was less than 10%. They were at unspecified sites 20 (9.62%), urine 20 (9.62%), sputum 18 (8.65%), bronchial aspirations 11 (5.29%), pleural fluid 10 (4.81%), blood 7 (3.37%), urinary catheter 6 (2.88%), wound 5 (2.40%), catheter 4 (1.92%) and cerebrospinal fluid 3 (1.44%). This low prevalence was recorded in tracheal aspiration, ascites fluid and stool. Among these three biological products, It was respectively 2 (0.96%), 1 (0.48%) and 1 (0.48%) strains of *P. aeruginosa* isolated **(Figure 3).**

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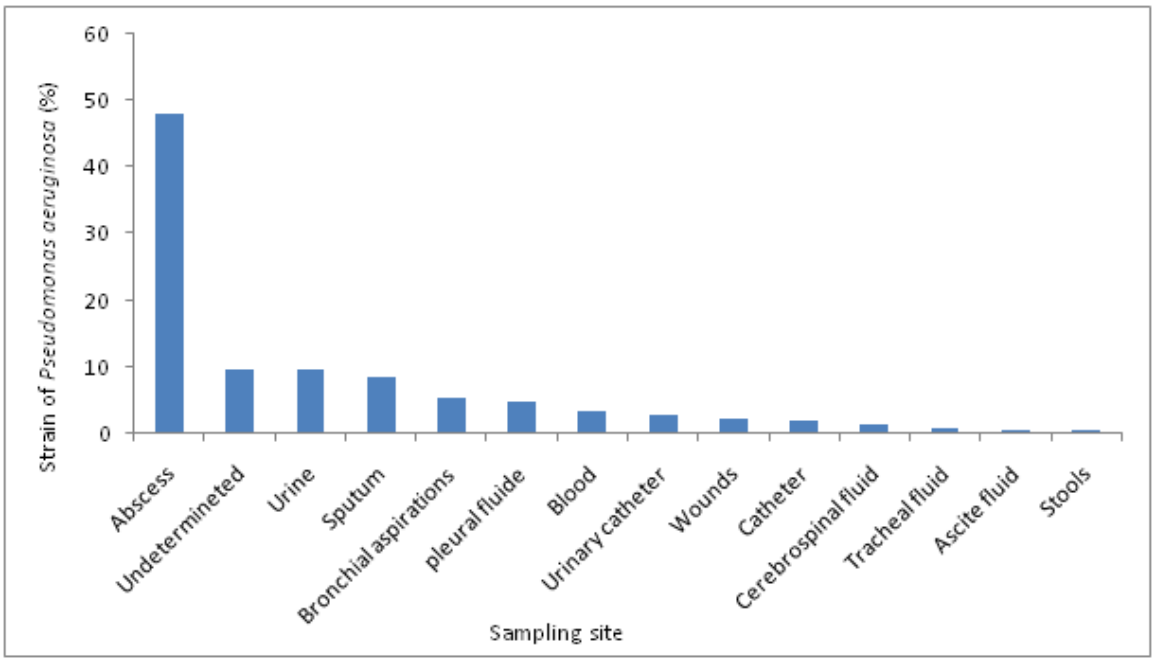

Figure 3: Frequency of strains of *P. aeruginosa* isolated by biological fluid

3.4 *Pseudomonas* antibiotic resistance profile

Exploration of the antibiotic resistance of strains from different services and biological products revealed three categories of strains including resistant, sensitive and intermediate (**Figure 4**). At the level of resistance, *P. aeruginosa* strains exhibited at least one resistance for all the tested antibiotics. The proportions of the resistant strains varied from 2.40 to 30.77%, respectively colistin and ticarcillin. Thus among 208 strains tested, colistin is the most active antibiotic on *P. aeruginosa* isolated throughout the study period from 2012 to 2015. In terms of colistin low resistance rate of 2.40% has been recorded (**Figure 4**). This strong action was also obtained with aztreonam belonging to the β-Lactam family. As far as aztreonam is concerned, a small proportion (3.85%) of resistant strains was observed. In addition, in the β-Lactam family, resistant strains were observed with ceftazidime (16.83%), cefepime 47 (22.60%), imipenem 24 (11.54%), Ticarcillin 64 (30.77%), ticarcillin + clavulanic acid 62 (29.81%) and piperacillin 41 (19.71%). Antibiotics belonging to the aminoglycoside family, amikacin were the most active antibiotic 18 (8.65%). This 8.65% resistance level recorded with amikacin was less than 42 (20.19%) and 39 (18.75%) observed with gentamicin and tobramycin. In the fluoroquinolone family, strains were resistant to ciprofloxacin and levofloxacin at 30 (14.42%) and 35 (16.83%), respectively, throughout the study period **(Figure 4).**

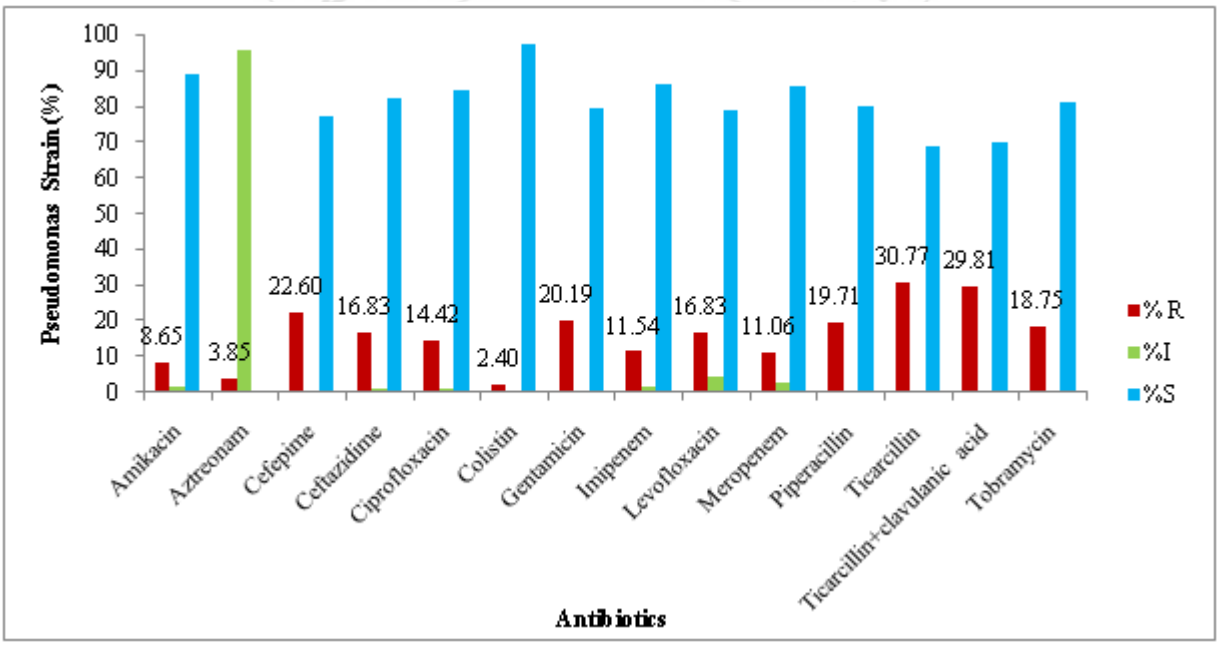

Figure 4: Antibiotic resistance profile of *P. aeruginosa*.
* According to Eucast-CASFM 2015; I = Intermediate; R = Resistant; S = Sensitive

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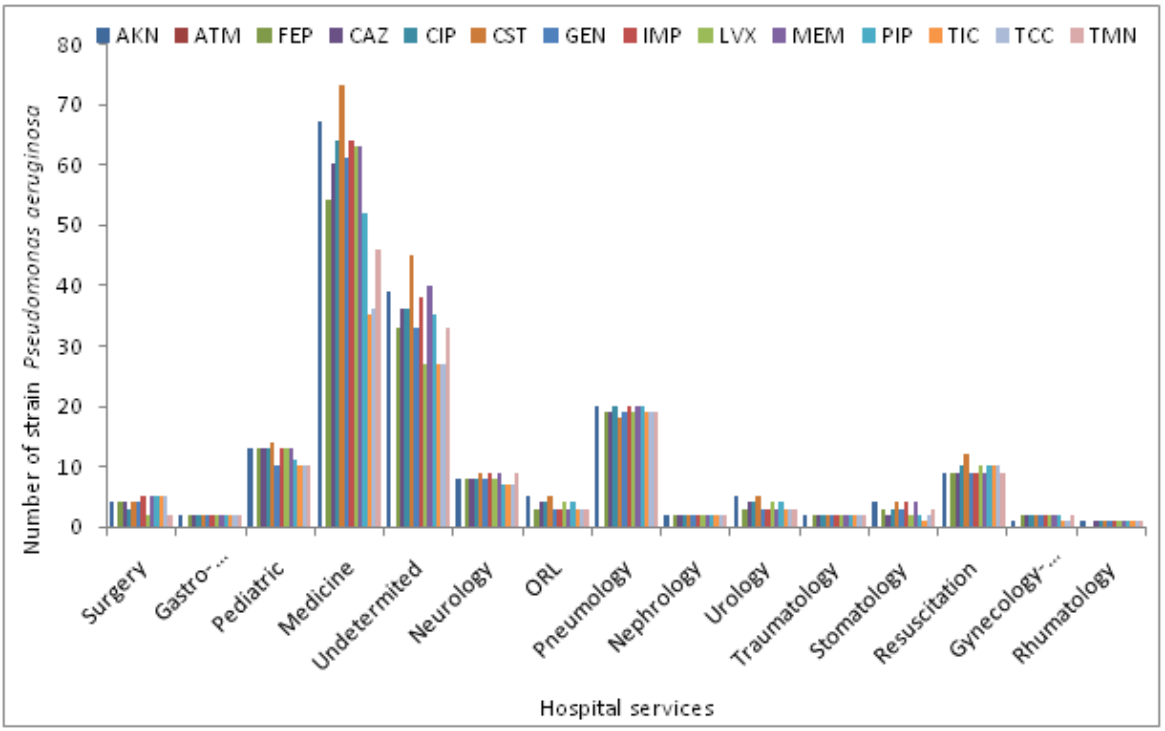
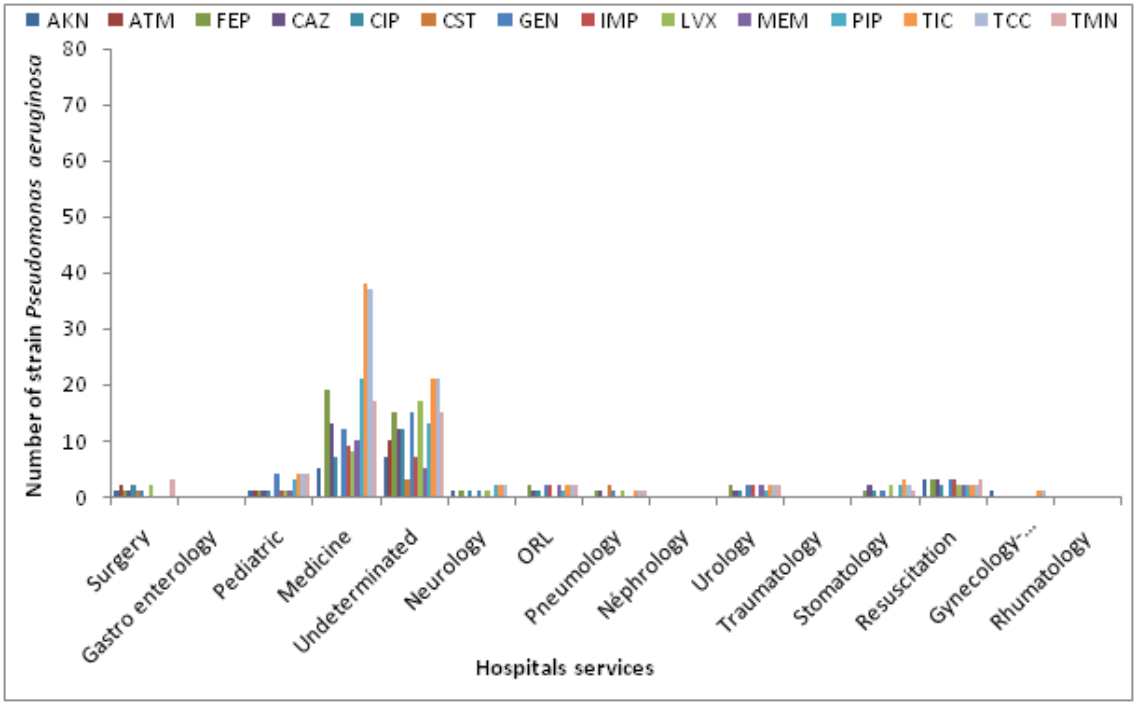
3.5 Antibiotic resistance profile of *P. aeruginosa* strains from different hospital services

Strains of *P. aeruginosa* from all departments showed sensitivity to all antibiotics tested except aztreonam (**Figure 5a**). The largest number of strains susceptible to antibiotics tested result from Medicine, Undetermined and Pneumology services. No strain from the Gastroenterology, Traumatology, and Nephrology and Rheumatology areas showed resistance to the tested antibiotics (**Figure 5b**). Strains from the Pneumology area showed 5% of resistance to various antibiotics including ceftazidime, cefepime, ticarcillin, ticarcillin + clavulanic acid, levofloxacin, gentamicin and tobramycin, and 10% to colistin. The largest number of *P. aeruginosa* showing resistance to antibiotics was observed on strains from the non-determined areas **(Figure 5b)**.



(a)



(b)

Figure 5 (a, b): Sensitive or antibiotic resistant strains of *P. aeruginosa* from different hospital services.
Caption: CAZ: ceftazidime; FEP: cefepime; ICT: ticarcillin; TCC: ticarcillin + clavulanic acid; LVX: levofloxacin; GN: gentamicin; TMN: tobramycin, AKN: amikacin; CIP: ciprofloxacin; ATM: aztreonam; CST; Colistin; MEM: meropenem; IMP: imipenem; PIP: piperacillin

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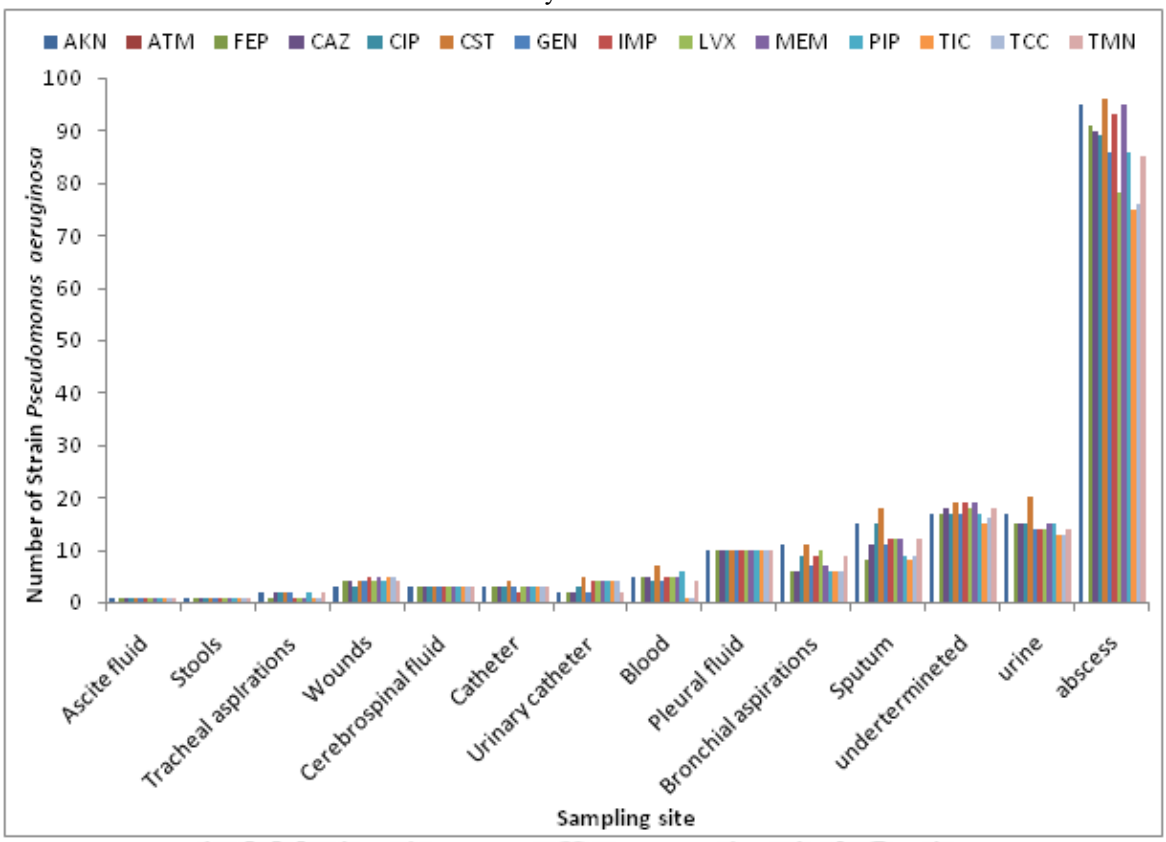
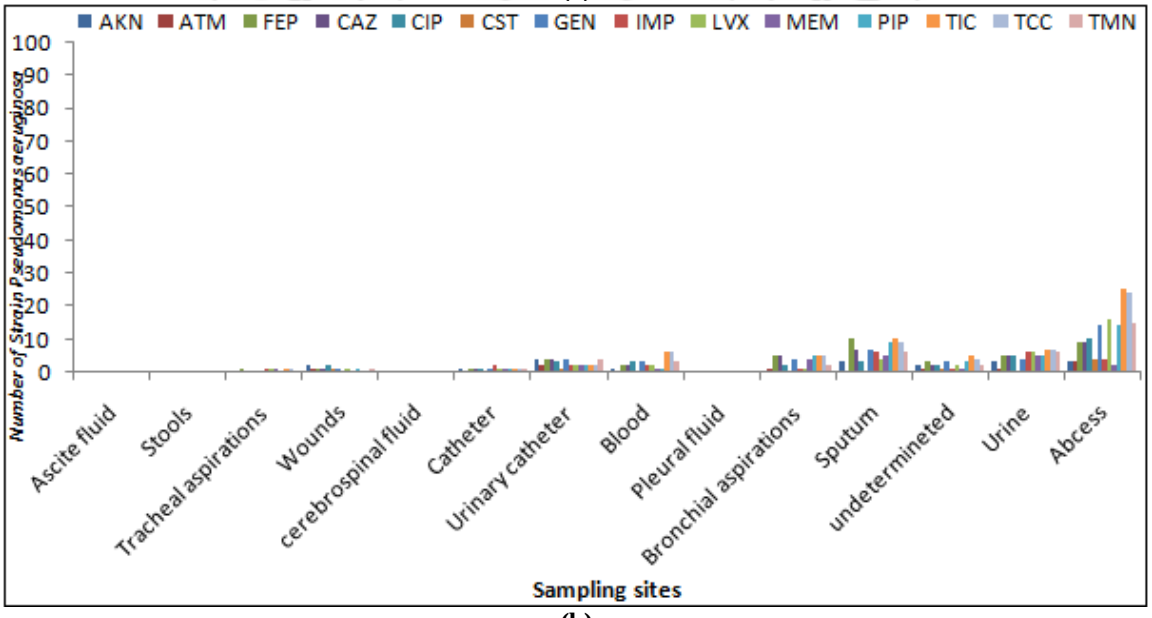
3.6 Antibiotic resistance profile of *P. aeruginosa* strains from different sampling sites

All strains isolated from the different biological products, showed sensitivity to all tested antibiotics, with the exception of aztreonam. The majority of those isolated from abscess presented a sensitivity profile to all tested disks except for aztreonam (**Figure 6a**). Strains isolated in the cerebrospinal fluid, stool, ascites fluid and pleural fluid showed no resistance to antibiotics tested in this study (**Figure 6b**). The strains of *P. aeruginosa* isolated from the catheter showed a high resistance to imipenem. Thus, 2 strains out of 4 expressed resistance to imipenem. The only active antibiotic was colistin. The majority of the strains isolated from the urinary catheters presented a resistance profile to all the antibiotics tested. 100 strains isolated from the abscess did not show much resistance profile. Strains isolated from unidentified sites, abscess and urinary probes showed a colistin resistance profile **(Figure 6b)**.



(a)



(b)

Figure 6 (a, b): Strains antibiotic sensitivity of from different biological products.

Caption: CAZ: ceftazidime; FEP: cefepime; ICT: ticarcillin; TCC: ticarcillin + clavulanic acid; LVX: levofloxacin; GN: gentamicin; TMN: tobramycin, AKN: amikacin; CIP: ciprofloxacin; ATM: aztreonam; CST; Colistin; MEM: meropenem; IMP: imipenem; PIP: piperacillin.

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3.7 Correlation between resistance to antibiotics and efflux systems

The correlation between the antibiotic resistances of *P. aeruginosa* strains and efflux system has been studied using five different antibiotics as phenotypic resistance markers of these four clinically important Mex systems tested in this study. Ticarcillin is targeted to be the antibiotic substrate of the MexAB-OprM system; Cefepime for MexCD-OprJ, imipenem and ciprofloxacin for MexEF-OprN, and gentamycin for MexXY-OprM. By RT-PCR, expression of most *mexB* (155bp), *mexD* (250pb), *mexF* (350pb) and *mexY* (445pb) genes has been observed **(figure 7)**. For ticarcillin-resistant isolates of *Pseudomonas aeruginosa,* 83% of the strains expressing the *mexB* gene of the MexAB-OprM pump were observed. In terms of expression of the *mexD* gene encoding a MexCD-OprJ pump protein, 52% have been found of the strains resistant to cefepime. The *mexF* gene was studied for the expression and function of the MexEF-OprN pump specific to the resistance of two antibiotics (ciprofloxacin and imipenem), it was detected in 62% of the cases. The *mexY* gene encoding MexXY-OprM has been detected in 59% of the strains resistant to gentamycin. Some isolates simultaneously presented the four different Mex systems as observed by conventional RT-PCR. The *mexB, mexD, mexF* and *mexY* genes were amplified by PCR in 24 (37%) isolates simultaneously, 6 (9%) isolates revealed the simultaneous presence of *mexB*, *mexF* and *mexY*. 3 (5%) isolates, revealed the simultaneous presence of the *mexB*, *mexD* and *mexF* genes. 1 (2%) isolate simultaneously had the *mexB, mexD* and *mexY* genes. The *mexB* and *mexY* genes were amplified simultaneously in 4 (6%) isolates and 3 (5%) isolates revealed the simultaneous presence of *mexB* and *mexF*. 6 (9%) had only the *mexB* gene and 1 (2%) the *mexD* and *mexF* genes respectively. In our study 11 (17%) isolates had none of the targeted genes.

Table 2: Efflux genes of resistant of *P. aeruginosa* strains

Number of strains	Antibiotics	Targeted genes	Pumps	Prevalence (%)
6	Ticarcillin	*mexB*	MexAB-OprM	9
1	Cefepime	*mexD*	MexCD-OprN	2
1	Imipenem, Ciprofloxacin	*mexF*	MexEF-OprJ	2
4	Ticarcillin, Cefepime	*mexB, mexD*	MexAB-OprM, MexCD-OprN	6
3	Ticarcillin, Imipenem, Ciprofloxacin	*mexB, mexF*	MexAB-OprM, MexEF-OprJ	5
4	Ticarcillin, Gentamicin	*mexB, mexY*	MexAB-OprM, Mexxy-OprM	6
3	Ticarcillin, Cefepime, Imipenem, Ciprofloxacin	*mexB, mexD,mexF*	MexAB-OprM, MexCD-OprN, MexEF-OprJ	5
1	Ticarcillin, Cefepime, Gentamicin	*mexB, mexD,mexY*	MexAB-OprM, MexCD-OprN	2
6	Ticarcillin, Imipenem,Gentamicin	*mexB, mexF, mexY*	MexAB-OprM, MexEF-OprJ, MexXY-OprM	9
24	Ticarcillin, Cefepime, Imipenem, Ciprofloxacin, Gentamicin	*mexAB , mexCD, mexEF, mexXY*	MexAB-OprM, MexCD-OprN, MexEF-OprJ, MexXY-OprM	37
11	Ticarcillin, Cefepime, Imipenem, Ciprofloxacin, Gentamicin	*None*	none	17

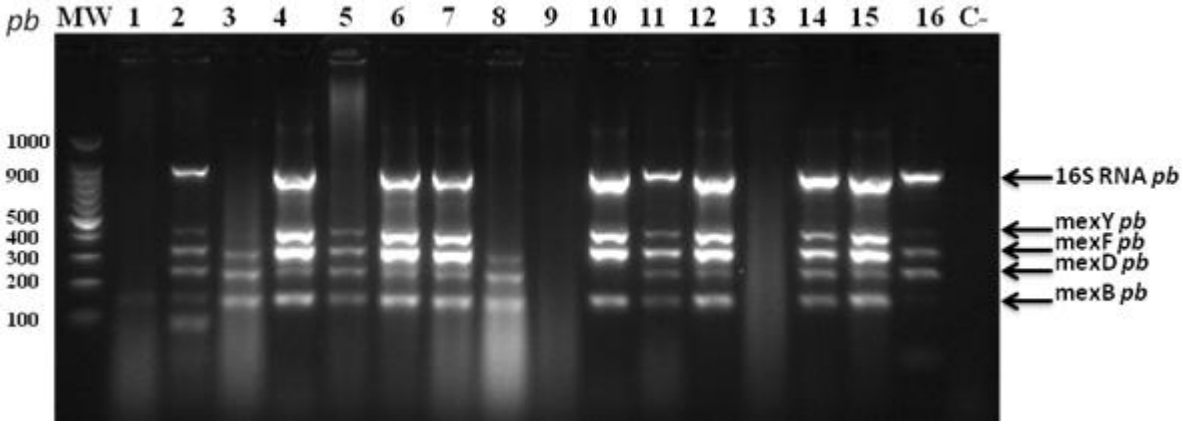

Figure 7: PCR multiplex of 16S RNA and efflux genes (*mexY, mexF, mexD* and *mexB*). Number 1 to 16 correspond to *P. aeruginosa* strains

4. Discussion

This study carried out from 2012 to 2015 revealed the existence of a high prevalence of *P. aeruginosa* strains of previously isolated in hospital centres and biological products. At the level of hospital services, the Department of General Medicine showed a greater occurrence (35.48%) than the other 15 (64.52%). In addition, the prevalence of *P. aeruginosa* strains was higher in abscess (48.08%) compared to the 13 other biological products (51.92%). For *P. aeruginosa* strains isolated from hospital services, similar results were obtained by Kamga and al., 2015 [14]. As results studies revealed a high prevalence of *P. aeruginosa* strains isolated in medicine. However, our results seem to be in contrast with those obtained by Sefraoui in Algeria [31]. It has been shown the antibiotic the prevalence of *Pseudomonas* resistance was 65.90%. As for organic products, similar results have been obtained by Chinbo and

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al., 2014 [4]. For these authors, abscess showed a high prevalence of strains compared to other biological products. However, urine showed a high prevalence of these strains in studies carried out in Cameroon [14].

In terms of the resistance profile of *P. aeruginosa* strains, the study was carried out basing on 14 antibiotics belonging to 4 different families. We have been found that colistin is the most active antibiotic on *P. aeruginosa* with a low resistance level of 2.40%. Several studies have been demonstrated similar results [4], [5], [10], [14], [31]. The work carried out by these authors revealed no strain of *P. aeruginosa* to be resisted to colistin. However, a resistance rate of 2% was recorded with the work done by Goli and al [10] on *P. aeruginosa* strains from the Tabriz hospitals in Iran. This colistin activity is due to its lesser use in therapy in infections with *P. aeruginosa* and probabilistic antibiotic therapy. Moreover, resistance rates of 48.65% were observed by Moyen and al [23], *P. aeruginosa* strains isolated from the wounds of patients hospitalized at the CHU of Brazzaville in the Congo. This difference in colistin activity can be explained by the environment of the strains, by local habits in the consumption and prescription of colistin. On the other hand, low activity (30.77%) of ticarcillin belonging to the β-lactam family was observed with respect to strains collected from 2012 to 2015 in Abidjan. A similar resistance rate was recorded for *Pseudomonas aeruginosa* isolates in a Moroccan pediatric hospital by Chinbo and al., [4]. This low ticarcillin activity would probably be related to non-enzymatic mechanisms, mainly through efflux mechanisms [3]. Recent studies have found ticarcillin resistance rates of 73% in Iran [10], 35.5% in Cameroon [14], and 41.9% In Morocco [20]. For other antibiotics in the same family, resistance to ceftazidime was 16.83% lower than in Iran (55%), Morocco (21%) and Congo (21.62%). Carbapenems, one of the best molecules of *P. aeruginosa* infections treatment, may also be affected by resistance. In our study, the resistance rate to imipenem was 11.83% close to those obtained in Lister and al [17] and Chinbo and al [4] and above 6% in Congo [23] and 8% observed in Morocco [20]. Compared to previous data from the Observatory of Microorganism Resistance to Anti-Infectives in Côte d'Ivoire, the antibiotic resistance rate of the β-lactam family decreased considerably, with the exception of imipenem. With this antibiotic, the resistance rate rose from 10.4% to 11.83% [11]. This decrease is due to an awareness of the consumption of antibiotics belonging to the β-lactam family. In the aminoglycoside family, amikacin was the most active antibiotic in pyocyanic bacilli with a resistance rate of 8.65% (www.memobio.fr/htlm/bact/ba-an-paep.htlm). Similar results have been recorded in Côte d'Ivoire with the resistance rate of 5% [9], 6% [4] and 10% by ONERBA. With this antibiotic, the resistance rate was lower than 18.92% in Congo [23] and 12.9% in Morocco [20]. Our results show that the resistance levels of tobramycin and gentamicin were 18.75% and 20.19%, respectively. These rates are half below 40.54% and 43.24%, recorded with strains of *P. aeruginosa* isolated in the Congo [23]. The difference in resistance could be explained by the origin of strains originating mainly from wounds and by the abusive use of antibiotics of this family in hospitals. In our study, resistance levels of 14.42% and 16.83% were obtained with ciprofloxacin and levofloxacin belonging to the fluoroquinolone family. Ciprofloxacin was the most active fluoroquinolone on *P. aeruginosa*. However, the resistance rate of 13% was observed by Chinbo and al [4]. Furthermore, no resistant ciprofloxacin has been observed in Cameroon in *P. aeruginosa* strains isolated from hospitals in Yaoundé [14].

At the level of the medical service, the high resistance of *P. aeruginosa* strains is observed with ticarcillin and ticarcillin + clavulanic acid. This resistance could be the formation of resistance gene reservoirs or efflux genes to these antibiotics by these strains. In addition, *P. aeruginosa* strains have a very high genetic plasticity, facilitating the acquisition of mobile elements encoding resistance mechanisms from other bacteria [22]. In addition, a 10% resistance to colistin was observed with strains of *P. aeruginosa* from pneumology service. This rate would be due to an abusive use of this antibiotic in this service. At the level of biological products, the high rate of resistance to ticarcillin, ticarcillin + clavulanic acid and piperacillin is observed with strains of *P. aeruginosa* isolated from abscess and sputum. The strains resulting from abscess and expectoration are multi-resistant. Our results contrast with those obtained by Chinbo and al [4] who showed multi-resistant strains from catheter specimens. Moreover, our strains obtained from urinary catheter, urine and bronchial aspirations were multiresistant. This multi resistance could be explained on the one hand by the production of biofilm by *P. aeruginosa* around biomedical devices and which gives it resistance to antibiotics administered parenterally [4]. On the other hand, the active efflux mechanisms would participate in the multi-resistance to the antibiotics tested.

Basing on correlation between the resistance of strains of *P. aeruginosa* and the efflux system, the study revealed that 83, 52, 59 and 62% of the strains respectively resistant to ticarcillin (*mex*B gene), cefepime (*mex*D gene), Gentamicin (*mex*Y) and imipenem and ciprofloxacin (*mex*F). These antibiotics would be indicators of efflux pumps in *P. aeruginosa*. At the origin of low-level resistances, efflux pumps in *Pseudomonas aeruginosa* can be the source of multi-resistant strains [22]. Analysis after RT-PCR showed that antibiotic resistance could be correlated with the Mex system in *Pseudomonas aeruginosa* strains. Moreover, these results tend to demonstrate the concomitant overexpression of efflux systems could superimpose their activity. The genetic study of the existence of the *mexB*, *mexD*, *mexF* and *mexF* genes representative of MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM respectively. The 24 (37%) isolates carrying the 4 target genes were resistant to at least 4 of the 5 antibiotic markers. This multi-resistance of its strains could be imvolved to the co-expression of these genes.

5. Conclusion

This work made possible to show antibiotic emergence of *Pseudomonas aeruginosa* strains from 2012 to 2015. Among 208, 14 antibiotics have been tested with *Pseudomonas aeruginosa* obtained in a clinical setting and stored at the Center for Biological Resources (CeReB) Institut Pasteur of Côte d'Ivoire. 41% of the strains exhibited antibiotic resistance phenotypes. However, colistin remained the most

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active molecule. Antibiotics such as amikacin, imipenem, meropenem and ciprofloxacin may be recommended for P. aeruginosa infections, but the combination of β-lactams / aminoglycosides or β-lactams / fluoroquinolones would be more effective. Finally, this work has shown that there is a correlation between resistance to antibiotics and the expression of the genes coding for the components of the efflux pumps.

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References

[1] Amazian K., Rossello J., Castella A., Sekkat S., Terzaki S., Dhidah L., Abdelmoumène T and Fabry J. 2010. Prevalence of nosocomial infections in 27 hospitals in the Mediterranean region. *East Mediterr Health J*. 16: 1070-1078.

[2] Askoura M., Mottawea W., Abujamel T and Taher I. 2011. Efflux pump inhibitors (EPIs) as new antimicrobial agents against Pseudomonas aeruginosa. *Libyan J Med*. 6:5870.

[3] Cavallo JD, Plesiat P, Couetdic G, Leblanc F, Fabre R. 20021997**.** Mechanisms of beta-lactam resistance in Pseudomonas aeruginosa: prevalence of OprM-overproducing strains in a French multicentre study 1997. *J AntimicrobChemother*.50:1039-43.

[4] Chinbo M., Moutachakkir M., Addebbous L., El Khoudri N., Chabaa L and Soraa N. 2014. Epidémiologie et résistance aux antibiotiques des isolats de *Pseudomonas aeruginosa* dans un hôpital pédiatrique marocain: implications thérapeutiques. *International Journal of Innovation and Scientific Research*. 11 : 283-290.

[5] Cholley P. 2010. Analyse génétique des souches multi-résistantes de Pseudomonas aeruginosa dans l'Est de le France, apport prédictif potentiel sur le risque infectieux. phD thesis, Université de Franche-comté, Besançon. France

[6] Chuanchuen R., Narasaki C.T and Schweizer H.P. 2002. The MexJK ef- flux pump of Pseudomonas aeruginosa requires OprM for antibiotic efflux but not for efflux of triclosan. *J Bacteriol*. 184:5036-5044.

[7] Comité de l'antibiogramme de la Société Française de Microbiologie. Récommandations 2015.V.2.0 Juillet

[8] Fatima A., Syed B.N., Sheikh A.K., Shaheen P and Sabahat J. 2012. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infections. *SpringerPlus* 1:70

[9] Faye-Ketté H., Kouassi M .Y., Akoua-Koffi G., Bakayoko S., Boni-Cissé C., Diallo-Touré K., Dosso M and Lambin Y. 2008. Epidémiologie Microbienne des Infections de Sites Opératoires (ISO) dans un service de Traumatologie à Abidjan et sensibilité des germes aux antibiotiques. *Bio-Africa.* 6 : 25-31

[10] Goli H.R., Mohammad R.N., Mohammad A.R., Alka H., Hossein S.K and Mohammad A. 2016. Emergence of colistin resistant *Pseudomonas aeruginosa* at Tabriz hospitals, Iran. *Iranian Journal of Microbioligy*. 8 : 62-69

[11] Guessennd K.N. 2014. Emmergence de métallo- β-lactamase chez *Pseudomonas aeruginosa* en Côte d'Ivoire. Communication, Dakar 10 juin. hptt/WWW.pasteur.sn/dmdocuments.

[12] Hogardt M., Hoboth C., Schmoldt S., Henke C., Bader L and Heesemann J. 2007. Stage specific adaptation of hypermutable *Pseudomonas aeruginosa* isolates during chronic pulmonary infection in patients with cystic fibrosis. *J Infect Dis.* 195:70–80

[13] Husson M.O., Izard D and Hansen W. 1995. Pseudomonas et genres apparentés. In : Manuel de Bactériologie Clinique. éd. J. Freney, F Renaud, W Hansen, C Bollet. *Collection Option Bio, Elsevier, Paris, 2ème Edition*; 3 : 1141-1159

[14] Kamga H.G., Toukam M., Sando Z., Ngamba J.M.N., Mbakop C.D and Adiogo D. 2015. Caractérisation phénotypique des souches de *Pseudomonas aeruginosa* isolées dans la ville de Yaoundé (Cameroun). *African Journal of Pathology and Microbiology*. 4 Pages.

[15] Kienlen J. 1998. Infections à pyocyaniques en Réanimation. Conférences d'actualisations, *Elsevier, Paris, et SFAR*. : 551-567

[16] Kohler T., Epp S. F., Curty L.K and Pechere J.C. 1999. Characterization of MexT, the regulator of the MexE-MexF-OprN multidrug efflux system of *Pseudomonas aeruginosa*. *J Bacteriol*. 181 : 6300–6305.

[17] Lister P.D., Wolter D.J and Hanson N. D. 2009. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol* . 22: 582-610.

[18] Livermore D. M. 2001. Of Pseudomonas, porins, pumps and carbapenems . *J Antimicrob Chemother*. 47, 247–250.

[19] Livermore D. M. 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clin Infect Dis*. 34 : 634–640.

[20] Louzi L., Boughalem M., Charra B and Jana M. 2003. *Pseudomonas aeruginosa* : Profils de résistance aux antibiotiques : A propos de 62 souches.. Conférence Nationale d'Anesthésie et de Réanimation. Paris (18-21 septembre2003). *J. Magh. A. Réa SFAR*. 191-196

[21] Masuda N., Sakagawa E., Ohya S., Gotho N., Tsujimoto H and Nishino T. 2000. Substrate specificities of MexAB-OprM, Mex- CD-OprJ, and MexXY-OprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 44:3322-3327.

[22] Mesaros N ., Nordmann P., Plésiat P., Roussel-Delvallez M., Van Eldere J and Glupc- zynski Y et al. 2007. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect.13: 560–578.

[23] Moyen R., Ahombo G., Nguimbi E., Niama R. F., Ontsira N.E., Yala G .C., Obengui and Louembe D. 2012. Résistance aux antibiotiques des souches d'isolées des infections de plaies au Centre Hospitalier et Universitaire de Brazzaville *Pseudomonas aeruginosa*. *Rev.CAMES-Série A*.13 (2): 98-101

[24] Navon-Venezia S., Ben-Ami R and Carmeli Y. 2005. Update on *Pseudomonas aeruginosa* and *Acinetobacter*

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baumannii infections in the healthcare setting. *Curr Opin Infect Dis*. 18: 306–313.

[25] Oliver A., Canton R., Campo P., Baquero F and Blazquez J. 2000. High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection. *Science*. 288: 1251-1254.

[26] Ouattara N.D., Boby B., Guessennd N., Guinan J.C and Dosso M. 2013. Rôle du Laboratoire dans la Surveillance des bacteries multi- resistantes d'origine infectieuses en Côte d'Ivoire de 2007 A 2011. *Bio-Africa*. 11 : 35-42

[27] Pier G and Ramphal R. 2005. *Pseudomonas aeruginosa*. In: Mandell G., Bennett J., Dolin R, editors. Principales and practice of infectious diseases. Philadelphia, PA. *Elsevier Churchill Livingstone*. 2587–2615.

[28] Poole K., Gotoh N., Tsujimoto H ., Zhao Q., Wada A., Yamasaki T., Neshat S., Yamagishi J., Li. X. Z and Nishino, T. 1996. Overexpression of the mexC-mexD-oprJ efflux operon in nfxB-type multidrug-resistant strains of Pseudomonas aeruginosa. *Mol Microbiol*. 21 : 713–724.

[29] Poonsuk K and Chuanchuen R. 2014. Detection of the Mex Efflux Pumps in *Pseudomonas aeruginosa* by Using a Combined Resistance-Phenotypic Markers and Multiplex RT-PCR. *Open Journal of Medical Microbiology*. 4 :153-160.

[30] Sacha P., Wieczorek P., Ojdana D., Hauschild T., Milewski R and Czaban S. 2014. Expression of MexAB-OprM and suceptibility to antibiotics of different Pseudomonas aeruginosa clones isolated from patients hospitalized in two intensive care units at University Hospital in Bialystok (northeastern Poland) between January 2002 and December 2009. *APMIS*. 931 – 940.

[31] Sefrauoi I. 2015. Etude de la résistance aux antibiotiques de *Pseudomonas aeruginosa* au niveau des différents hôpitaux de l'Ouest algérien. Thèse, Doctorat de l'université Abou Beka Belkaid, Tlemcen, Algérie.

[32] Vettoretti L., Floret N., Hocquet D ., Dehecq B., Plesiat P and Talon D. 2009. Emergence of extensive-drug-resistant Pseudomonas aeruginosa in a French university hospital.Eur *J Clin Microbiol Infect Dis*.

[33] WWW. memobio.fr/html/bact/ba-an-pap.html

[34] Zinzendorf N.Y., Ouassa T., Agbessi B.T., Kouassi K.M., Ekra D and Loukou Y.G. 2009. Facteurs de Risque et Étiologies Microbiennes des Infections Nosocomiales au CHU de Treichville, Abidjan (Côte d'Ivoire). *Sci. pharm. biol*. 10(2), 56-64.

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