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Carbapenem Resistant Pseudomonas Aeruginosa: A Multicenter Study on Prevalence, Distribution, and Risk Factors across Six Health Facilities in Yaoundé, Cameroon

Georgette Njila Kameni [1,2], Emilia Lyonga Mbamyah [3,4], Esther Del Florence Moni Ndedi [1], Ines Dalia Nganou Kamgang [1,2], George Mondinde Ikomey [2,4], Yengo Clauvis Kunkeng [5], Martha Tongo Mesembe [4], Neline Yameni Elimbi [6], Mary Ngongang Kameni [1], Jean Bosco Taya Fokou [1,3], Justice Ohene Amofa[7], Albert Noubissi Toyim [1] and Maximilienne Ascension Nyegue[1]

[1]Department of Microbiology, Faculty of Science, University of Yaoundé I, Cameroon.

[2]Department of Public Health, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon.

[3] Department of Microbiology, Parasitology, Hematology and Infectious Diseases, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon.

[4] Centre for the Study and Control of Communicable Diseases, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon.

[5] Department of Biochemistry, Faculty of Science, University of Yaoundé I, Cameroon.

[6] Centre for Health Promotion and Research, Cameroon.

[7] Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana.

ORCID iD: 0009-0008-0200-7800

ORCID record: https://orcid.org/0009-0008-0200-7800

ABSTRACT

Carbapenem-resistant *Pseudomonas aeruginosa* is a significant public health threat, especially in low and middle-income countries. These strains are known to cause outbreaks, and their isolation continues to increase, further limiting therapeutic options. In Cameroon, however, there is limited data on the resistance profiles of circulating Carbapenem-resistant *Pseudomonas aeruginosa* strains. This study aimed to determine the prevalence, distribution and susceptibility profile of Carbapenem-resistant *Pseudomonas aeruginosa* isolates from six health facilities in Yaoundé, Cameroon. An analytical cross-sectional study was conducted over nine months (November 2023–July 2024). Carbapenem-resistant *Pseudomonas aeruginosa* isolates from clinical specimens were collected and re-identified. After culturing on Cetrimide and Nutrient Agar, biochemical identification was done using the API 20 NE system. Antimicrobial susceptibility testing was performed to assess resistance to Carbapenems and other antibiotics. Carbapenemase and Extended spectrum beta-lactamases production in Carbapenem resistant *Pseudomonas aeruginosa* was detected using the Carbapenem inhibition and combined disc methods. Of the 217 isolates, 125 (representing 57.6%) were confirmed as *Pseudomonas aeruginosa*, with 31.2% (39/125) resistant to carbapenems. Among these, 48.7% (19/39) were resistant to Imipenem, and 51.3% (20/39) were resistant to Meropenem. Carbapenemase production was observed in 46.2% (18/39), while coproduction of carbapenemase and Extended spectrum beta-lactamases was observed in 28.20% (11/39). High resistance was also seen to Cephalosporin (54.4%) and Penicillin (59.2%). A majority of Carbapenem-resistant *Pseudomonas aeruginosa* isolates had a multiple antibiotic resistance index ≥ 0.2. This study underscores the growing public health concern posed by Carbapenem-resistant

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Pseudomonas strains. Enhanced surveillance is crucial to curb the dissemination and spread of these strains.

KEYWORDS: Distribution, Susceptibility, *Pseudomonas aeruginosa*, Carbapenems, Carbapenemase, Extended spectrum beta-lactamases

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I. INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa), a Gram-negative bacillus, non-Enterobacteriaceae, is a ubiquitous opportunistic pathogen with little or no virulence in healthy humans but is mostly encountered in hospital environments [1]. Infections due to *P. aeruginosa* typically arise from a combination of factors, including patient susceptibility, hospital-acquired exposure, the presence of indwelling devices, and other invasive medical procedures [2]. These risk factors are particularly pronounced in immunocompromised patients due to their underlying conditions and more frequent exposure to healthcare settings [3]. This bacterium can infect almost any site in the body and can be isolated from various clinical specimens, including respiratory secretions, wounds, urine, blood, cerebrospinal fluid, ear secretions, skin and soft tissue infections [2]. The increasing isolation in healthcare settings of *P. aeruginosa* strains non-susceptible to most anti-pseudomonal agents is driven by multiple factors, including its innate resistance to a variety of antimicrobial agents, its ability to acquire resistance determinants and the increased use of antibiotics, which fosters the emergence of resistant strains [3]. In addition, *P. aeruginosa* is notorious for its ability to form biofilms, which are complex communities of microorganisms that adhere to surfaces and are embedded in a protective matrix [4].

Carbapenems are a last line of defense against many drug-resistant bacterial infections. These powerful broad-spectrum-β-lactam antibiotics are commonly used to treat *P. aeruginosa* infections and are currently the main antibiotics used for treating multidrug-resistant (MDR) *P. aeruginosa* infections. Unfortunately, the rising emergence of Carbapenem resistant *P. aeruginosa* strains (CRPAs) seriously threatens this class of lifesaving drugs [4]. Carbapenem-resistant *P. aeruginosa* ranges from 10 to 50% in most countries. Data from European countries, covered by the European antimicrobial resistance surveillance system, showed an increasing trend in the prevalence of carbapenem-resistant *P. aeruginosa* strains varying between 4.4 and 58.5% among different countries [5]. Recent studies in Tanzania and Uganda have shown that this prevalence could be as high as 22.4-35% with the circulation of CR genes blaVIM, blaOXA, blaKPC and blaNDM predominantly among *P. aeruginosa* strains [5]. In Nigeria, 20.5 % of *P. aeruginosa* isolates were carbapenem non-susceptible (resistant) [6]. In Cameroon, retrospective studies carried out by Yangouo in 2020 at the Yaoundé University Teaching Hospital showed a considerable increase in the resistance of *P. aeruginosa* to carbapenems from 2010 to 2020 [7].

P. aeruginosa isolates have been reported to contain a wide variety of carbapenemases globally. These include essentially KPC (*Klebsiella pneumoniae* Carbapenemase), GES (Guiana Extended-Spectrum), IMP (active-on-Imipenem), VIM (Vietnam Extended-spectrum beta-lactamase), NDM (New Delhi Metallo-beta-lactamase), and SPM (Sao Paulo metallo-β-lactamase) in Latin America [8]; IMP, and NDM in the Arabian Peninsula; [9] and KPC, NDM, VIM, and IMP in the United States [10]. The diversity and emerging prevalence of carbapenemases producers among carbapenem-resistant strains of *P. aeruginosa* (CR-PA) have been recently highlighted in the multi-national ERACE-PA Surveillance Program [11]. Recent studies reported by Cecile *et al* in 2023 showed a prevalence of 25.07 % carbapenemase producing *P. aeruginosa* in three regions of Cameroon [12].

Resistance to beta lactams occurs through the production of restricted or Extended spectrum beta-lactamases (ESBLs), including TEM (Temoneira) type ESBL, SHV (Sulfhydryl Variable), CTXM (Cefotaximase Munich), PER (*Pseudomonas* extended resistance), VEB (Vietnam Extended spectrum beta-lactamases), GES (Guyana Extended spectrum), and OXA (Oxacillinase) [8].

CRPA strains that produce carbapenemases and ESBLs are of significant concern, as they exhibit resistance to multiple antibiotics, including: Penicillins, Cephalosporins, Aztreonam and Carbapenems, often considered the last resort of treating infections [8]. The coproduction of ESBLs and Carbapenemases in *P. aeruginosa* can lead to pan drug resistance, limited treatment options, increased risk of treatment failure and mortality [11].

Despite this increasing rate of *P. aeruginosa* carbapenem resistance, there is still little surveillance data available, combining prevalence, molecular and epidemiological information on CRPA strains in Cameroon. Continuous surveillance of this high-risk pathogen and understanding its resistance mechanisms are therefore, important to effectively guide clinical treatment and support infection control programs, as well as to prevent its dissemination in Cameroon. Consequently, the objective of this study was to determine the prevalence, distribution and susceptibility profile of Carbapenem-resistant *P. aeruginosa* isolates recovered from six health facilities in Yaoundé, Cameroon.

II. METHODS

A. Type, Site, and Duration of Study

A cross-sectional and analytical study was carried out during a nine-month period, from November 2023 to July 2024. Isolates were collected from six health facilities in Yaoundé: (The Yaoundé Military hospital, the Saint Martin de Porres Dominican Hospital Centre, Yaoundé University Teaching Hospital, Yaoundé Central Hospital, Yaoundé Bethesda Hospital and Yaoundé General Hospital).

B. Sampling Method and Selection Criteria

During the study period, all *P. aeruginosa* or suspected *P. aeruginosa* isolates obtained from pathological specimens including pus, wound swabs, probe tips, urine, blood, effusion fluid, and prostatic fluid were systematically collected from the bacteriology laboratories of the six participating hospitals. These isolates were re-identified to confirm their species and included in the study. Isolates that could not be confirmed as *P. aeruginosa* during re-identification as well as those lacking relevant clinical information were excluded from the analysis. Re-identification of isolates and downstream analyses were carried out at the Bacteriology Laboratory of the Centre for the Study and Control of Communicable Diseases.

C. Re-identification and subculture of collected isolates

In an aseptic environment, the isolates were initially inoculated on Cetrimide agar, followed by Nutrient Agar using the streaking method. The resulting colonies underwent a thorough macroscopic examination to assess characteristics such as size, color, and morphology. Subsequently an oxidase test was performed on a single pure colony, with a positive result indicating oxidase activity. Biochemical identification was then carried out using the miniaturized API 20 NE system (BioMerieux, Lyon). The latter was performed on a bacterial suspension (using the 0.5 Mc Farland standard) [13-14].

D. Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was conducted using the disc diffusion method on Mueller– Hinton agar (MHA) in accordance with the guidelines of the Antibiogram Committee of the French Society of Microbiology (CA-SFM-2021v.1.0). A total of 13 antibiotics discs from various classes (BIORAD, Marnes-la-coquette, France) were tested, representing a broad spectrum of antimicrobial agents: Carbapenem: Imipenem (10 ug) and Meropenem (10 ug), Penicillins: Tircacillin (75 ug), Piperacillin (30 ug),and Amoxicilline (36 ug), Cephalosporines: Ceftazidim (30 ug) and Cefepime (30 ug), Monobactam: Aztreonam (30 ug), Aminosides; Amikacin (10 ug) and Gentamicin (10 ug), Fluoroquinolones: Ciprofloxacin (5 ug), Netilmicin (30 ug) and Fosfomycin (10 ug). These antibiotics were selected based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2023 recommendations. A pure bacterial suspension was seeded on Mueller–Hinton Agar by the swab method, and antibiotic discs were then placed 25 to 30 mm apart, and the plates were incubated at 37 °C for 24 hours for susceptibility assessment [14-15]. Diameters of inhibitions were interpreted according to the European committee on antimicrobial susceptibility testing [14] recommendations as follows: Susceptible (S), Resistant (R), or Intermediate (I).

Detection of Extended Spectrum β-lactamase (ESBL):

ESBL production was evaluated according to the Clinical and Laboratory Standards Institute guidelines [15]. All 125 *P aeruginosa* isolates were tested for resistance to Ceftazidime, Aztreonam, and Cefepime both with and without Clavulanic Acid. A difference of ≥5 mm in the inhibition zone diameters between cephalosporin disks or their corresponding Cephalosporin–clavulanate disc was considered indicative of ESBL production [16]. Ceftazidime (100 µg), Cefepime (30 µg), Aztreonam (30 µg), and Ceftazidime/clavulanic Acid (10/4 µg) disks were used.

Detection of Carbapenemase producing Pseudomonas aeruginosa:

The Carbapenem Inactivation Method (CIM) was used to differentiate between carbapenemase producing and non-producing *P. aeruginosa* isolates. To perform CIM, a suspension was prepared by inoculating a full 10 µl loop of culture into 400 µl of distilled water. A susceptibility-testing disc containing either Meropenem/Imipenem (10µg) was immersed in the suspension and incubated for two hours at 37 °C. After incubation, the disc was transferred from the suspension using an inoculation loop, placed on Mueller-Hinton Agar plate inoculated with a susceptible *Escherichia coli* (*E. coli*) indicator strain (ATCC 29522) and subsequently incubated at 37°C. Inoculation of the MHA plate with the indicator strain was done with a suspension of 0.5 Mc Farland, streaked in the three directions using a sterile cotton swab. If the *P aeruginosa* isolate produced carbapenemases, the carbapenem in the susceptibility disc was inactivated, allowing uninhibited growth of the *E. coli* strain. Results were read after overnight incubation. CRPA isolates were then grouped into carbapenemase producing and carbapenemase non-producing isolates [16].

Detection of Specific Carbapenemases:

The combine disc test was used for the detection of specific carbapenemases. A suspension of morphologically similar *P. aeruginosa* colonies was prepared to match the 0.5 McFarland turbidity standard. The inoculum was streaked onto Mueller-Hilton Agar plates, and the following cartridges were applied; Meropenem/Imipenem 10µg (MRP10), Meropenem/Imipenem 10µg + Dipicolinic Acid, Meropenem/Imipenem 10µg + EDTA, Meropenem/Imipenem 10µg and Meropenem/Imipenem 10µg + Boronic Acid. Plates were incubated at 37 °C for 18 - 24 hours [14-16], and the results were read after incubation.

E. Statistical Analysis

The data collected from the study were systematically recorded in Excel 2021 software and subsequently analyzed with the Statistical Package for Social Sciences (SPSS) version 27) and GraphPad Prism 10 for statistical computations and graph creation. The analyses included one-way ANOVA to determine significant differences in the multiple antibiotic resistance indexes (MAR) and *P. aeruginosa* susceptibility profiles, independent t-tests to compare the means across different groups, and Chi-square tests to examine the associations between carbapenem resistance and clinical factors such as health care units, sample types, and frequency of hospitalization.

F. Ethical and Legal Considerations

This study received ethical approval from the Ethical Committee of the Delegation of Public Health for the Centre Region Cameroon, under approval number 00213/CRESHC/2023. A research authorization was also obtained from the directors of each participating hospital. Anonymity of participants and confidentiality of results were scrupulously respected.

III. RESULTS

A. Socio-demographic Characterization

Of the 217 isolates initially collected, 125 (74.14%) were confirmed as *P. aeruginosa* following re-identification (Table I). Most of the isolates were sourced from the Yaoundé University Teaching Hospital, accounting for 40% (50/125), with 64% (80/125) from male patients. Approximately three-quarters of the study participants were hospitalized, with hemodialysis and internal medicine units being the most represented, with 24% (30/125) and 15.2% (19/125), respectively (Table I).

Table I: Socio-demographic distribution of *Pseudomonas aeruginosa* isolates

Characteristics		**Yaoundé Bethesda Hospital**	**Yaoundé General Hospital**	**Yaoundé Central Hospital**	**Yaoundé Military Hospital**	**Saint Martin Porres**	**Yaoundé University Teaching Hospital**	**Total n (%)**
Sex	Female	3	5	10	8	2	17	**45 (36%)**
	Male	2	5	5	12	23	33	**80 (64%)**
Total n (%)		**5 (4%)**	**10 (8%)**	**15 (12%)**	**20 (16%)**	**25 (20%)**	**50 (40%)**	**125 (100%)**
Age	0 – 13	0	0	0	2	0	3	**5 (4%)**
	13– 25	0	1	0	0	2	4	**7 (5.6%)**
	25 – 50	5	1	7	12	6	19	**50 (40%)**
	50 – 65	0	6	8	0	10	16	**40 (32%)**
	65 – 100	0	2	0	6	7	8	**23 (18.4%)**
Healthcare unit	Intensive care	0	0	4	0	0	0	**4 (3.2%)**
	Maternity	0	0	0	1	0	3	**4 (3.2%)**
	Gastrology	2	0	0	0	0	3	**5 (4%)**
	Dental	0	0	2	4	2	0	**8 (6.4%)**
	Gynecology	0	4	0	2	0	4	**10 (8%)**
	Surgical	0	0	0	2	9	0	**11 (8.8%)**
	External	3	0	1	1	11	0	**16 (12.8%)**
	Emergency	0	0	0	5	0	13	**18 (14.4%)**
	Internal Medicine	0	1	8	5	3	2	**19 (15.2%)**
	Hemodialysis	0	5	0	0	0	25	**30 (24%)**
Hospitalization	Yes	5	6	10	20	10	43	**94 (75.2%)**
	No	0	4	5	0	15	7	**31 (24.8%)**

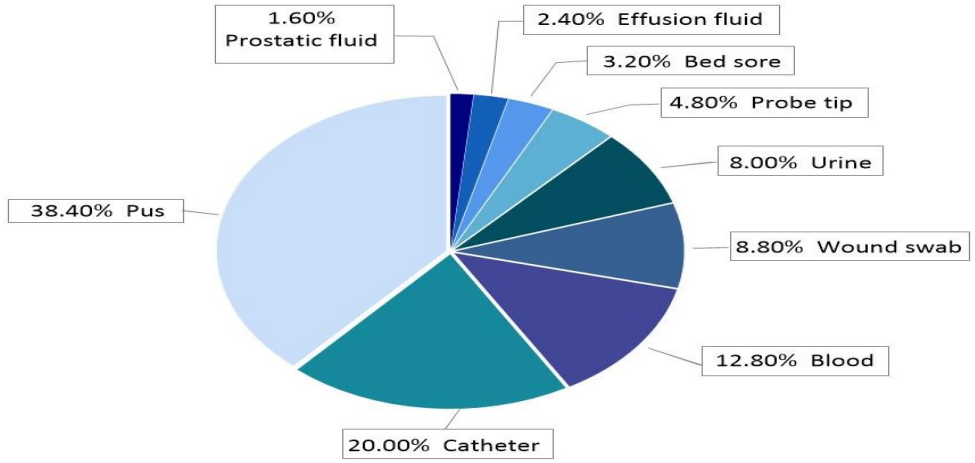

Figure 1: Proportion of *P. aeruginosa* isolates according to sample type

The most common sample types included; pus, 38.40% (48/125), catheter 20.0% (25/125), and Blood, 12.80% (16/125) (Figure 1).

B. Antimicrobial susceptibility profile of Pseudomonas aeruginosa Isolates

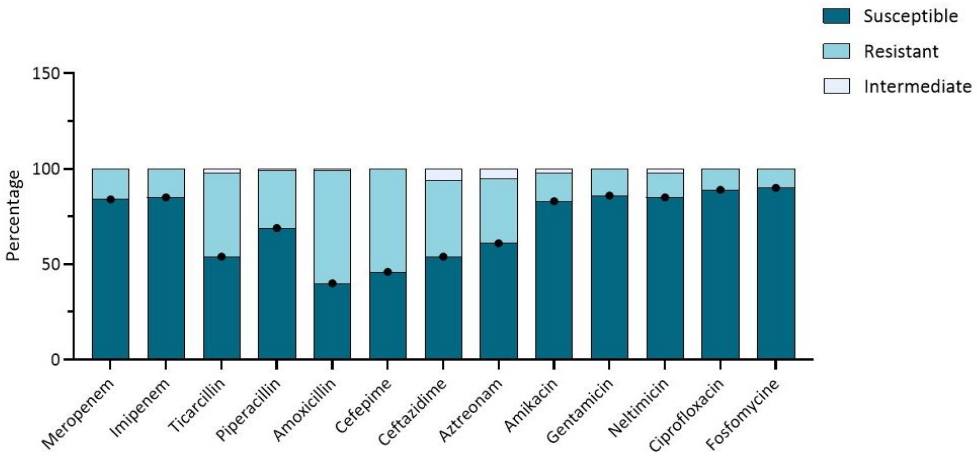

Figure 2: Susceptibility profile of *P. aeruginosa* isolates to various antibiotics

The antimicrobial susceptibility profile of all 125 *P. aeruginosa* isolates to the tested antibiotics is presented in Figure 2. The resistance profile revealed a notably high rate of resistance to Penicillins, with Amoxicillin showing 59.2% resistance and Ticarcillin 44.8%. Similarly, the Cephalosporins demonstrated significant resistance, with Cefepime exhibiting 54.4% and Ceftazidime 40%. In contrast, the sensitivity profile revealed a high susceptibility to both Ciprofloxacin (89.2%) and Fosfomycin (89.5%).

All *P. aeruginosa* isolates (N=125) tested by the Kirby-Bauer disc diffusion susceptibility test were divided into two major groups. The first group consisted of 39 (31.2%) Carbapenem resistant *P. aeruginosa* strains (resistant to Imipenem or Meropenem) and the second group comprised of 86 (68.8%) Carbapenem sensitive *P. aeruginosa* strains (Table II). Table III displays the distribution and proportion of Carbapenem-resistant *Pseudomonas aeruginosa* isolated from various health centers and sample types.

Table II: Percentage resistance and sensitivity of *P aeruginosa isolates*

	Susceptible (S)		Resistant (R)		Intermediate (I)		Total No. of isolates
	No. of isolates	%	No. of isolates	%	No. of isolates	%	
Carbapenem							
Meropenem	105	84	20	16	0	0	125
Imipenem	106	84.8	19	15.2	0	0	125
Penicillins							
Ticarcillin	67	53.6	56	44.8	2	1.6	125
Piperacillin	86	68.8	38	30.4	1	0.8	125
Amoxicillin	50	40	74	59.2	1	0.8	125
Cephalosporines							
Cefepime	57	45.6	68	54.4	0	0	125
Ceftazidime	68	54.4	50	40	7	5.6	125
Monobactam							
Aztreonam	76	60.8	43	34.4	6	4.8	125
Aminosides							
Amikacin	104	83.2	19	15.2	2	1.6	125
Gentamicin	108	86.4	17	13.6	0	0	
Neltimicin	100	85.5	15	12.8	2	1.709	117
Fluoroquinolones							
Ciprofloxacin	91	89.2	11	10.8	0	0	102
Others							
Fosfomycine	111	89.5	13	10.5	0	0	124

a% = percentage (number of isolates/total number of isolates tested)

Table III: Distribution of Carbapenem resistant *Pseudomonas aeruginosa* isolates according to sample type and health facilities

carbapenem resistant	**Yaounde Bethesda Hospital n(%)**	**Yaounde General Hospital n(%)**	**Yaounde Central Hospital n(%)**	**Yaounde Military Hospital n(%)**	**Saint Martin Porres n(%)**	**Yaounde University Teaching Hospital n(%)**	**Total**
No	3 (3.5%)	7 (8.1%)	11 (12.8%)	13 (15.1%)	18 (20.9%)	34 (39.5%)	86
Yes	2 (5.1%)	3 (7.7%)	4 (10.3%)	7 (17.9%)	7 (17.9%)	16 (41%)	39
Bed sore	2 (50%)	-	-	-	-	2 (50%)	4
Blood	-	-	-	-	5 (100%)	-	5
Catheter	-	-	4 (40%)	5 (50%)	-	1 (10%)	10
Effusion fluid	-	-	-	-	1 (100%)	-	1
Probe tip	-	1 (33.3%)	-	-	-	2 (66.7%)	3
Pus	-	-	-	2 (20%)	1 (10%)	7 (70%)	10
Urine	-	-	-	-	-	3 (100%)	3
Wound swab	-	2 (66.7%)	-	-	-	1 (33.3%)	3

Statistical analysis revealed a highly significant association between healthcare units and sample type with Carbapenem (Meropenem and Imipenem) resistance, according to the chi-square test ($p < 0.001$ for both) but the Fisher exact test could not be performed due to memory limitations. Hospitalization shows a weakly significant association according to the chi-square test ($p = 0.05$), but this association is strengthened by the Fisher-Freeman-Halton exact test ($p = 0.013$), confirming statistical significance (Table IV).

Table IV: Association between variables and Carbapenem resistance

	Carbapenem resistance		
	Chi-Square Tests		Fisher-Freeman-Halton Exact Test
	X2	P-value	exact P-value
Healthcare Unit	64.627	<0.001	Insufficient memory
Hospitalisation	20.9	0.05	0.013
Sample Type	86.557	<0.001	Insufficient memory

C. Distribution of CRPA isolates according to enzyme production.

This study shows a high prevalence of *P. aeruginosa*-producing carbapenemase and ESBL (Table V).

Table V: Proportion of CRPA isolates according to enzyme production

	Carbapenem resistant	**Carbapenem susceptible**	**Total n (%)**
	No. of isolates (%)	**No. of isolates (%)**	
Carbapenemase producing only	7 (18)	-	7 (5)
ESBL producing only	-	7 (8)	7 (5)
ESBL and Carbapenemase producing	11 (28)	-	11 (9)
No enzyme producing	21 (54)	79 (92)	100 (80)
Total n (%)	**39 (31)**	**86 (69)**	**125 (100)**

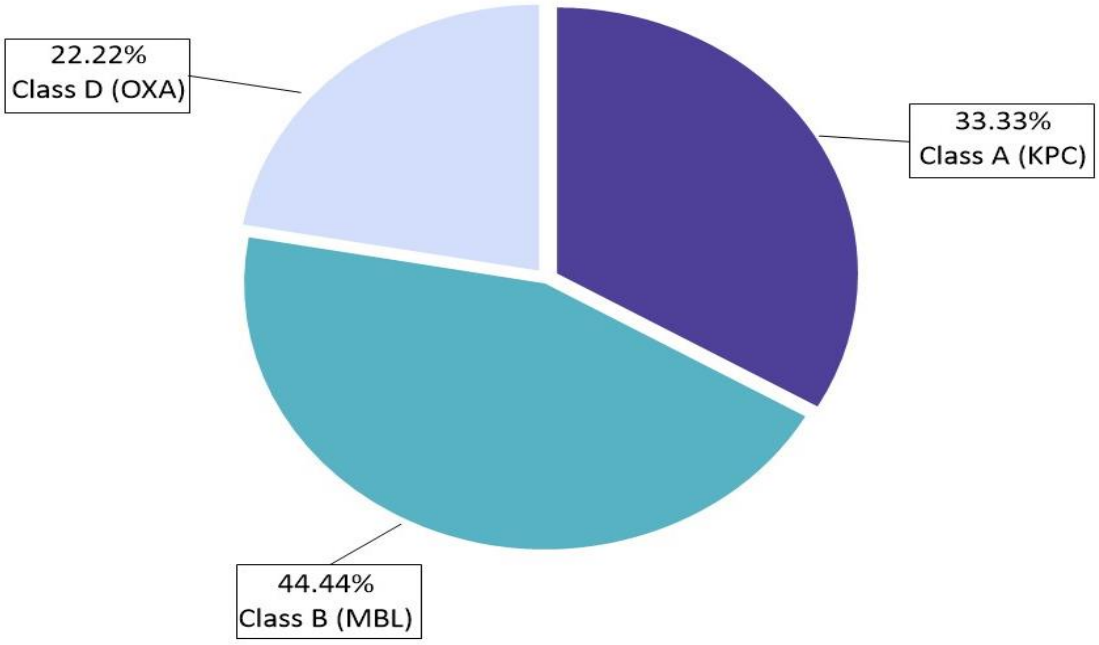

Figure 3: Proportion of specific carbapenemases amongst CRPAs

The prevalence of carbapenemase-producing *P. aeruginosa* was 46.15%) (18/39), with 33.33 % (06/18) class A, 44.44% (8/18) class B and 22.22 % (04/18) class D (Figure 3).

Table VI displays the distribution and proportion of specific carbapenemases and extended spectrum beta-lactamases amongst Carbapenem-resistant *P. aeruginosa* isolates recovered from various health centers.

Table VI: Distribution of specific carbapenemases and extended spectrum beta lactamases amongst health care facilities

Characteristics		Yaounde Bethesda Hospital n(%)	Yaounde General Hospital n(%)	Yaounde Central Hospital n(%)	Yaounde Military Hospital n(%)	Saint Martin Porres n(%)	Yaounde University Teaching Hospital n(%)	Total
Carbapenemase producing	No	3 (2.8%)	9 (8.4%)	13 (12.1%)	18 (16.8%)	21 (19.6%)	43 (40.2%)	107 (85.6%)
	Yes	2 (11.1%)	1 (5.6%)	2 (11.1%)	2 (11.1%)	4 (22.2%)	7 (38.9%)	18 (14.4%)
Class of Carbapenemase	Class A (KPC)	1 (16.7%)	-	1 (16.7%)	1 (16.7%)	1 (16.7%)	2 (33.3%)	6 (33.3%)
	Class B (MBL)	-	1 (12.5%)	1 (12.5%)	1 (12.5%)	2 (25%)	3 (37.5%)	8 (44.4%)
	Class D (OXA)	1 (25%)	-	-	-	-	3 (75%)	4 (22.2%)
ESBL producing	No	21 (19.6%)	1 (0.9%)	13 (12.1%)	10 (9.3%)	18 (16.8%)	44 (41.1%)	107 (85.6%)
	Yes	4 (22.2%)	4 (22.2%)	2 (11.1%)	-	2 (11.1%)	6 (33.3%)	18 (14.4%)

D. Occurrence of Multiple antibiotic resistance indexes (MAR)

The percentage occurrence of MAR index for *P. aeruginosa* isolates across various sample locations is presented in Table VII. The isolates exhibited MAR index from 0.00 to 0.54, representing that the *P. aeruginosa* isolates are resistant to between 0 and 7 types of the antibiotics tested.

Table VII: Occurrence of MAR index *P aeruginosa isolates*

No	Antibiotic resistant pattern	MAR index	No. of *Pseudomonas aeruginosa* isolates
1	AMX	0.08	1
2	ATM	0.08	6
3	MEM	0.08	1
4	PIP	0.08	3
5	TIC	0.08	2
6	AMX, ATM	0.15	6
7	AMX, CAZ	0.15	1
8	AMX, FEP	0.15	4
9	CAZ, ATM	0.15	1
10	MEM, FEP	0.15	1
11	PIP, AMX	0.15	1
12	TIC, AMX	0.15	2
13	TIC, FEP	0.15	1
14	IPM, FEP	0.15	2
15	MEM, AMX	0.15	1
16	AMX, CAZ, AK	0.23	1
17	FEP, CAZ, ATM	0.23	1
18	MEM, AMX, FEP	0.23	2
19	PIP, AMX, FEP	0.23	1
20	TIC, AMX, ATM	0.23	7
21	TIC, AMX, FEP	0.23	1
22	TIC, CAZ, ATM	0.23	1

23	TIC, PIP, AMX	0.23	2
24	AMX, FEP, CAZ, ATM	0.31	1
25	MEM, FEP, CAZ, ATM	0.31	1
26	MEM, TIC, PIP, ATM	0.31	1
27	PIP, FEP, CAZ, ATM	0.31	2
28	TIC, PIP, AMX, CAZ	0.31	2
29	TIC, PIP, AMX, FEP	0.31	1
30	IPM, AMX, FEP, CAZ	0.31	1
31	IPM, AMX, FEP, GEN	0.31	1
32	MEM, AMX, FEP, ATM	0.31	1
33	IPM, FEP, ATM, GEN	0.31	1
34	IPM, PIP, FEP, AK	0.31	1
35	MEM, FEP, CAZ, AK	0.31	2
36	AMX, CAZ, GEN, NET, FOS	0.38	2
37	IPM, AMX, FEP, CAZ, CIP	0.38	1
38	IPM, TIC, AMX, FEP, ATM	0.38	1
39	MEM, TIC, PIP, FEP, CAZ	0.38	1
40	MEM, TIC, PIP, FEP, CIP	0.38	1
41	PIP, AMX, CAZ, AK, CIP	0.38	1
42	TIC, AMX, FEP, CAZ, GEN	0.38	4
43	TIC, PIP, AMX, FEP, CAZ	0.38	1
44	IPM, PIP, FEP, ATM, AK	0.38	1
45	MEM, PIP, FEP, CAZ, AK	0.38	1
46	MEM, TIC, FEP, CAZ, AK	0.38	4
47	IPM, TIC, AMX, FEP, CAZ, ATM	0.46	1
48	PIP, AMX, FEP, AK, NET, CIP	0.46	1
49	TIC, AMX, CAZ, GEN, NET, FOS	0.46	1
50	TIC, AMX, FEP, CAZ, AK, GEN	0.46	1
51	TIC, AMX, FEP, GEN, NET, CIP	0.46	1
52	TIC, AMX, FEP, GEN, NET, FOS	0.46	1
53	TIC, PIP, AMX, CAZ, NET, FOS	0.46	2
54	TIC, PIP, AMX, FEP, NET, CIP	0.46	1
55	TIC, PIP, AMX, FEP, NET, FOS	0.46	1
56	IPM, PIP, AMX, FEP, CAZ, ATM	0.46	3
57	IPM, TIC, FEP, CAZ, ATM, FOS	0.46	2
58	IPM, TIC, PIP, AMX, FEP, CAZ	0.46	1
59	MEM, TIC, PIP, FEP, CAZ, AK	0.46	1
60	MEM, TIC, PIP, AMX, FEP, CAZ, AK	0.54	1
61	PIP, AMX, FEP, AK, GEN, NET, CIP	0.54	2
62	TIC, AMX, FEP, CAZ, AK, GEN, CIP	0.54	1
63	TIC, AMX, FEP, GEN, NET, CIP, FOS	0.54	1
64	TIC, PIP, AMX, FEP, CAZ, NET, FOS	0.54	1
65	TIC, PIP, AMX, FEP, NET, CIP, FOS	0.54	1
66	IPM, TIC, PIP, AMX, FEP, CAZ, ATM	0.54	3
67	MEM, TIC, FEP, CAZ, AK, GEN, FOS	0.54	1

AMX, amoxicillin; ATM, aztreonam; MEM, meropenem, PIP, piperacillin; TIC, ticarcillin; FOS, fosfomycin; NET, netilmicin; IPM, imipenem; CIP, ciprofloxacin; FEP, cefepime; CAZ, ceftazidime; AK, amikacin; GEN, gentamicin

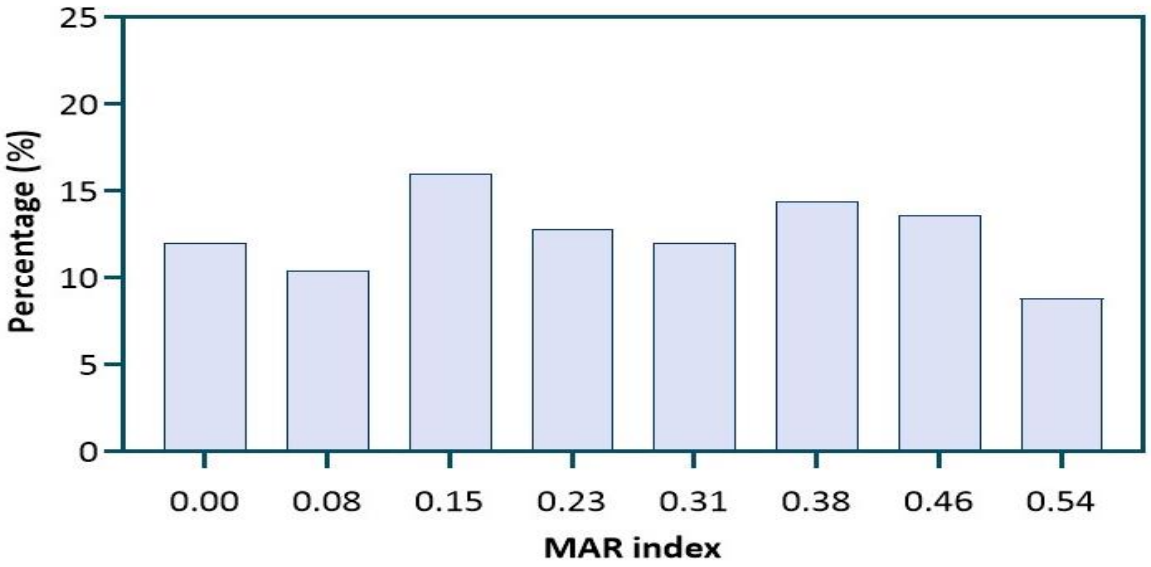

Figure 4: Percentage occurrence of MAR index for P. aeruginosa isolates

Carbapenem-resistant isolates exhibited a significantly higher MAR index compared to Carbapenem-sensitive strains (p <0.001), indicating that they are resistant to a greater number of antibiotics. The mean difference between the two groups was 0.1588, with the 95% confidence interval for the difference ([0.0989, 0.2188]) not including zero, further reinforcing the statistical significance (p <0.001). This clear and significant difference in means suggests a strong association between carbapenem resistance and a higher MAR index (Table VIII).

Table VIII: Association between MAR index and carbapenem resistance

Test	Carbapenem Resistant	N	Mean	Std. Deviation	Std. Error Mean	P-value ANOVA ONE WAY	P-value t-test Student independent	t-test 95% Confidence Interval of the Difference
	Resistant	39	0.3905	0.1371	0.022	<0.001	<0.001	[0.0989, 0.2188]
	Sensitive	86	0.2317	0.165	0.0178			

IV. DISCUSSION

In view of contributing to the mitigation and surveillance of multi resistant bacteria, our study aimed to determine the distribution, prevalence and susceptibility profile of carbapenem resistant *P. aeruginosa* recovered from six health facilities in Yaoundé. Out of 217 collected isolates, 74.16 % were confirmed *P. aeruginosa* isolates. Most isolates came from the Yaoundé University Teaching Hospital, 40.00% (50/125), this could be explained by the problem of overcrowding and poor sanitation at this hospital. Up to 75.00 % of the isolates were recovered from hospitalized patients. Hospitalized patients may have underlying conditions, such as cystic fibrosis, burns or surgical wounds that make them more prone to *P. aeruginosa* and often receive broad-spectrum antibiotics, which can disrupt the balance of their microbiome and select for antibiotic-resistant bacteria like *P. aeruginosa* [4]. *P. aeruginosa* has been shown to colonize the hospital environment and to be resistant to many antibiotics, making its elimination difficult [17]. Additionally, about 38.40 % of the samples in our study were pus. *P. aeruginosa* is commonly isolated from pus due to its ability to thrive in environments with high levels of nutrients, moisture, and low oxygen [18]. Gonsu *et al*., in their study carried out in the city of Yaoundé in 2015, also found that *P. aeruginosa* were more isolated in hospitalized patients, with 17.6 % from pus samples [19]. Drug susceptibility testing revealed that, *P. aeruginosa* had a high resistance to Penicillins; with 59.2% to Amoxicillin and 44.8% to Ticarcillin. They also showed a high resistance to fourth and third generation Cephalosporins; Cefepime 54.4% and Ceftazidime 40 %. Indeed, Penicillins and third-generation Cephalosporins are currently increasingly used in humans and animals and are easily accessible in street pharmacies at very low costs [19]. Noteworthy is the result that, 31.2% of the *P. aeruginosa* isolates were carbapenem resistant (resistant to imipenem or meropenem) and 68.8% carbapenem susceptible. This high prevalence of *P. aeruginosa* resistant to carbapems is explained by the fact that, *P. aeruginosa* has the ability of developing acquired antimicrobial resistance to nearly all available antipseudomonal agents through the selection of

chromosomal mutations [18,20], low outer membrane permeability, expression of efflux pumps that expel antibiotics out of the cell, and the production of antibiotic-inactivating enzymes [21]. These high resistance rates obtained poses a great challenge given that they are antibiotics of last resort and are used as the last therapeutic options in cases of antibiotic treatment failure. Furthermore, the isolates exhibited MAR index from 0.08 to 0.54, highlighting that *P. aeruginosa* isolates are resistant to 0–7 types of antibiotics tested. Similar trends were reported by Moctar *et al*. in Cameroon in 2019 [22]. Several studies have suggested that mutations play very crucial roles in developing drug resistance and cross-resistance through the selection of chromosomal mutations [20, 23]. Intrinsic resistance, efflux pumps, genetic adaptability, horizontal gene transfer, biofilm formation and overuse/misuse of antibiotics have contributed to *P. aeruginosa* high MAR, making it a significant challenge in clinical settings. Moreover, the accumulation of several chromosomal mutations leads to the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), or even pan-antibiotic-resistant (PDR) strains, which can be responsible for notable epidemics in the hospital setting [23,24]. Of the 39 isolates of *P. aeruginosa* resistant to carbapenems, the carbapenemase producers represented 46.15%, of which 44.44 % were class A (KPC) carbapenemase, 33.33 % were class B (MBL) and 22.22 % class D (OXA). These results are higher compared to the study of Castanhiera *et al.* in 2014 who found 20 % of *P. aeruginosa* producing carbapenemases [25] and that of Alkudhairy *et al.* in 2020 who found 10.3% of *P. aeruginosa* producing class B carbapenemases [26]. The indiscriminate use of carbapenems in health care settings can exert selective pressure, favoring the emergence and spread of carbapenemase producing resistant strains. In addition, 14.4% of the *P. aeruginosa* isolates produced ESBL. The sharing of genetic material between bacteria can lead to the acquisition of ESBL encoding genes. This finding is lower than the results reported by Cecile *et al* in 2023, and could be due to the difference in sample size [12]. Of the 39 *P. aeruginosa* isolates resistant to carbapenems, 28.20% produced both carbapenemase and ESBLs. The presence of specific determinants, such as plasmids or intergrons, can carry genes encoding for both ESBLs and carbapenemases. Also, antibiotics, such as Cephalosporins and Carbapenems, can select for bacteria that produce both ESBLs and carbapenemases. In the statistical analysis, it was found that there are significant associations between carbapenem resistances and these three variables (Health care unit, hospitalization, and sample type). Different healthcare units and hospitals may serve distinct patient populations, which can influence the prevalence of resistance. In fact, infection control practices, such as hand hygiene, sterilization, and isolation protocols can vary between health care, influencing the spread of resistance [23]. Also, variations in antibiotic usage patterns between health care units and hospitals can contribute to the development and spread of resistance. These factors can interact with each other and with other variables to create complex relationships between resistance, health care unit, hospitalization and sample type [24]. Several studies have found higher mortality rates of *P. aeruginosa* infection to be related to patients' comorbidity, the site of primary infection, disease severity, multidrug resistance and inappropriateness of empirical therapy [27,28].

V. CONCLUSION

These findings evidence the evolution of carbapenem resistant strains of *P. aeruginosa* species in Yaoundé, Cameroon and could be attributed to factors such as self-medication; high population density, overcrowded hospitals, and poor sanitation in our hospital settings. Our study highlights the need for continuous surveillance, antimicrobial stewardship, and targeted infection control measures in healthcare settings.

Author Contributions: G.N.K. M.A.N., and E.L.M.., conceived the project and designed the study. N.Y.E searched relevant literature, scrutinized all relevant information, and drafted the manuscript. G.N.K. and I.D.N.K. conducted and coordinated the field study. G.N.K., and I.D.N.K., collected and processed the samples. A.N.T. and M.N.K analyzed the data. G.M.I., C.Y.K., M.T.M. and E.D.F.M.N. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT: All data generated or analyzed in the course of this study are included in this manuscript.

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