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Antimicrobial Resistance of *Pseudomonas aeruginosa* Isolated from Human Infections in N'Djamena, Chad

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Abstract

Background: Urinary Tract infections and pus are major public health problems. The evolution of bacterial resistance to antibiotics makes the treatment of these infections problematic. This is why this study is undertaken to identify and evaluate the resistance of *Pseudomonas aeruginosa* to antibiotics. **Methods:** This is a prospective study carried out from December 2020 to November 2021. The germs were isolated on the agar supplemented with cetrimide and identified by the API 20 NE gallery method according to the manufacturer's protocol. The strains' resistance profiles were determined by the diffusion method on Mueller-Hinton according to the criteria EUCAST-2021. **Results:** A total of 46/1467 (3.13%) *Pseudomonas aeruginosa* were identified, of which 29/1008 (2.87%) were urinary tract infections and 17/459 (3.70%) were pus. The high resistances were: 97.8% to ceftazidim, 91.3% to aztreonam, 93.5% to cefepim, 82.6% to piperacillin, 58.7% to levofloxacin, 52.2% to amikacin, 47.8% to tazobactam-piperacillin, 47.8% to tobramycin and 43.5% to ciprofloxacin. Low resistance was only 2.2% to fosfomycin, 2.2% to colistin and 15.2% to imipenem. **Conclusion:** This study reveals the considerable resistance of *Pseudomonas aeruginosa* to commonly used antibiotics, and thus compromises the empirical treatment practiced in hospitals. This result motivates the need to carry out susceptibility testing of isolates before any prescription of antimicrobials.

Keywords

Pseudomonas aeruginosa, Resistance to Antibiotics, Urine, Pus, N'Djamena

1. Introduction

Urinary tract infections and pus are common in Africa and are caused mainly by bacteria. They represent a significant burden for public health due to their high frequency, their cost of treatment, and the treatment failures often observed due to multiple bacterial resistances [1] [2]. In recent decades, *Pseudomonas aeruginosa* has established itself as a major hospital pathogen, responsible for a large number of infections remarkable for their severity [3]. Thanks to its ability to use different organic compounds as energy substrates. This strict aerobic Gram-negative bacillus lives in a wide variety of environments for long periods of time, as long as they are sufficiently humid [3]. Infections caused by *Pseudomonas aeruginosa* are often difficult to treat due to both the species' natural resistance and its remarkable ability to acquire other mechanisms of resistance to several groups of antimicrobial agents. According to the World Health Organization, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Escherichia coli*, and *Klebsiella pneumoniae* have become resistant to a large number of antibiotics, including carbapenems and third-generation cephalosporins, the best antibiotics available for treat multi-resistant bacteria [4]. This phenomenon means that patient care has become a major concern in infectious therapy due to multi-drug resistance. These bacteria circulate on all continents and it is recommended that each country strengthen surveillance of bacterial resistance by collecting data in microbiology laboratories and carrying out targeted research on antimicrobial resistance [4]. In Chad, very little data is available on bacterial resistance to antibiotics. The published works focus in particular on *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase (ESBL) [5] [6] [7]. Antibiotic resistance data for *Pseudomonas aeruginosa* are not available at this time. This is why this first study was undertaken to assess the resistance of *Pseudomonas aeruginosa* to antibiotics in the major hospitals of N'Djamena. The results of the work could be used as preliminary data to inform healthcare personnel in charge of care about the circulation of these formidable bacteria and the need to readjust treatments in the event of treatment failure in hospitalized patients who are often exposed to the risks of nosocomial *Pseudomonas aeruginosa* infections.

2. Materials and Methods

2.1. Setting, Type of Study, and Period

This is a prospective study carried out from December 2020 to November 2021 in N'Djamena. Urine and pus samples from hospitalized and non-hospitalized patients were collected in three major hospitals, namely the Mother and Child

University Hospital (CHU-ME), the National Reference University Hospital (CHU-RN), and the Hospital of Friendship Chad/China (HATC). The microbiological analyzes were carried out at the CHU-ME. The patient variables considered were age, sex, patient origin, and collection center.

2.2. Sample Size Calculation

Due to the absence of data on the prevalence of *Pseudomonas aeruginosa* on human infection in Chad, it was taken the prevalence (P = 25.5%) reported by Kamga [8] for *Pseudomonas aeruginosa* in Cameroun around Chad. Thus, on the basis of this estimation, the following Lorenz formula was applied:
$N = \varepsilon^2 P(1-P)/j^2$. This gave a minimal sample size of 392 individuals, by opting for ε value of 1.96, a confidence level of 95%, and a margin of error (j) of 5%. This number was increased to find more bacteria to analyze.

2.3. Inclusion and Non-Inclusion Criteria

The population studied consisted of patients consulted for a cytobacteriological examination of urine and pus, without distinction of sex and age. Urine from patients who complied with the standard collection conditions for bacteriological culture was accepted and included, and samples not complying with the conditions were not included. Similarly, closed and open pus collected using syringes and swabs performed by medical personnel in compliance with clinical microbiology procedures were included. Pus samples that did not follow collection and transport procedures were not included.

2.4. Samples Collection and Processing

These samples were collected in accordance with standard microbiological procedures for sample collection and transport. Each sample was streaked onto cetrimide agar and incubated at 42˚C for 18 - 24 hours [9]. Isolated strains underwent Gram staining and oxidase tests for orientation. The identification of isolates was based on biochemical characters using the API 20 NE gallery (BioMérieux, Marcy l'Etoile, France).

2.5. Determination and Antibiotic Susceptibility

Antibiotic susceptibility testing was performed by the Mueller-Hinton (MH) agar antibiotic disk diffusion method according to the method according to Bauer and collaborators [10]. The reading and interpretation of the susceptibility tests were carried out according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing [11]. The antibiotics studied are: ceftriaxone (30 µg), piperacillin (75 µg) fosfomycin, piperacillin-tazobactam (75/10µg), ceftazidime (30 µg), imipenem (10 µg), aztreonam (30 µg), amikacin (30 µg), tobramycin (10 µg), gentamicin (15 µg), ciprofloxacin (5 µg), levofloxacin (30 µg), cefipim (30 µg), colistin (10 µg). *Pseudomonas aeruginosa* American Type Culture Collection (ATCC10145) was used as a quality control strain.

2.6. Phenotypic Detection of ESBL Production

The detection of ESBL production was studied by the double disc synergy test [12]. Discs of ceftazidim (30 µg), cefepim (30 µg), cefotaxim (30 µg) and aztreonam (30 µg) were placed next to a disc containing amoxicillin/clavulanic acid (20/10µg) at a distance of 20 mm (center to center) on a Mueller-Hinton II agar plate previously inoculated with *Pseudomonas aeruginosa*. After incubating at 37˚C, an enhancement of the zone of inhibition around at least one of these discs towards the disc containing clavulanic acid indicates the presence of ESBL.

2.7. Metallo-β-Lactamase

Isolates classified as resistant (R) to imipenem were subjected to the metallo-β-lactamase (MBLs) phenotypic test using the imipenem-EDTA (ethylene diamine tetraacetyl) inhibition method [13]. The principle of the test is based on the potential of EDTA to inhibit metallo-β-lactamase activity. In order to perform the test, the EDTA was prepared by adding 24.193×10^3 µg of disodium EDTA-$2H_2O$ to 130×10^3 µL of sterile distilled water so that 4 µL of the solution corresponds to 750 µg of EDTA 0.5 M per imipenem disc [13]. The pH of the EDTA solution was adjusted to 8 with the NaOH solution. Bacterial suspensions were prepared in a 0.8% (w/v) saline solution to obtain a turbidity equivalent to 0.5 McFarland standard and 100 µL aliquots were inoculated onto MHA plates by the technique of spread plate. Two discs of imipenem (10 µg) were placed 30 mm apart on the inoculated Mueller-Hinton plates. A 4 µL aliquot of EDTA solution corresponding to 750 µg of EDTA was added to one of the discs [13]. The inoculated dishes were incubated aerobically at 37˚C for 24 h. The diameter of inhibition produced by the imipenem discs (10 µg) alone and the imipenem + EDTA discs (10 + 750) µg was measured to determine the potential of the isolates to produce MBLs. For producers of MBLs, the diameter of inhibition of imipenem + EDTA should be ≥7 mm of that produced by imipenem alone.

2.8. Data Analysis

All data collected were introduced into Microsoft Excel 2010 and imported into Statistical Package for Social Sciences (SPSS) software version 18.0 for the calculations. All variables calculated were presented as numbers of cases or effective, and percentages.

2.9. Ethical Considerations

The study received authorization from the Ministry of Public Health and the Dean of the Faculty of Medicine of Chad. In addition to authorizations, individual consent was obtained for the collection of samples for research.

3. Results

3.1. Prevalence and Distribution of *Pseudomonas aeruginosa*

A total of 1467 samples including 1008 urine and 459 pus were analyzed during

the study. The overall prevalence was 3.13% (46/1467) including 2.87% (29/1008) in urine samples and 3.70% (17/459) in pus. Table 1 shows the distribution of *Pseudomonas aeruginosa* by patient age groups, genders, and sample collection sites. The frequencies of the isolates were maximum and equal to 56.52% (26/46) in the age group of 30 - 39 years, compared to the other age groups. The rates of isolates were higher than CHU RN (41.30%) and lower than CHU ME (34.78%) and HATC (23.91%). The infection rates by gender were 54.35% (25/46) in males and 45.65% (21/46) in females with a sex ratio M/F of 1.19. But, 58.62% (17/29) of germs were isolated from females and 41.38% (12/29) from males and pus, 76.47% (13/17) were from males, and 23.53% (4/17) from females.

3.2. Distribution of Germs by Department

Table 2 shows the distribution of isolates by hospital department. We observe that 45.65% of *Pseudomonas* were isolated from patients in internal services and 54.35% from external services. In the internal services, the infection rates were maximum and equal to 26.32% in urology (26.32%) at the CHU RN, 18.75% in Gynecology at the CHU ME, and 27.27% in Medicine and Pediatrics at HATC.

3.3. Antibiotic Resistance Profile

Table 3 presents the antibiotic resistance profile of *Pseudomonas aeruginosa* strains. High resistance rates were observed with β-lactams such as ceftriaxon

Table 1. Distribution of *Pseudomonas aeruginosa* isolated from urine and pus.

Variables	Urine (N = 29)		Pus (N = 17)		Total (N = 46)	
	N	%	N	%	N	%
Age						
[0 - 9]	3	10.34	2	11.76	5	10.87
[10 - 19]	0	0.00	3	17.65	3	6.52
[20 - 29]	5	17.24	3	17.65	8	17.39
[30 - 39]	18	62.07	8	47.06	26	56.52
[40 - 49]	2	6.90	0	0.00	2	4.35
[50 and et +]	1	3.45	1	5.88	2	4.35
Sex						
Male	12	41.38	13	76.47	25	54.35
Female	17	58.62	4	23.53	21	45.65
Hospital						
CHU-ME	11	37.93	5	29.41	16	34.78
CHU-RN	9	31.03	10	58.82	19	41.30
HATC	9	31.03	2	11.76	11	23.91

Table 2. Distribution of *Pseudomonas aeruginosa* by department.

Service	CHU-RN (N = 19)		CHU-ME (N = 16)		HATC (N = 11)		Total (N = 46)	
	N	%	N	%	N	%	N	%
Medicine	0	0.00	0	0	3	27.27	3	6.52
Intensive care	0	0.00	2	12.5	0	0.00	2	4.35
Pediatrics	0	0.00	1	6.25	3	27.27	4	8.70
surgery	1	5.26	0	0	1	9.09	2	4.35
Gynecology	0	0.00	3	18.75	0	0.00	3	6.52
Urology	5	26.32	0	0	0	0.00	5	10.87
ORL	1	5.26	0	0	0	0.00	1	2.17
Traumatology	1	5.26	0	0	0	0.00	1	2.17
Total Internal	8	42.11	6	37.5	7	63.64	21	45.65
Total external	11	57.89	10	62.5	4	36.36	25	54.35

ORL: Oto-Rhino-Laryngology.

Table 3. Resistance profile of *Pseudomonas aeruginosa* to antibiotics.

Antibiotics	Urine (N = 29)		Pus (N = 17)		Total (N = 46)	
	R + I (N)	%	R + I (N)	%	R + I (N)	%
PIP	25	86.2	13	76.5	38	82.6
TZP	17	58.6	7	41.2	24	52.2
CRO	29	100.0	17	100.0	46	100.0
CAZ	28	96.6	17	100.0	45	97.8
CPM	27	93.1	16	94.1	43	93.5
AZT	26	89.7	16	94.1	42	91.3
IMI	2	6.9	4	23.5	6	13.0
GMN	20	69.0	8	47.1	28	60.9
AK	17	58.6	5	29.4	22	47.8
TOB	19	65.5	5	29.4	24	52.2
CIP	16	55.2	10	58.8	26	56.5
LEV	11	37.9	8	47.1	19	41.3
FOS	0	0.0	1	5.9	1	2.2
COL	0	0.0	1	5.9	1	2.2

N = Number of bacteria; R + I = réSistance and Intermediate; PIP = Piperacillin; TPZ = Piperacillin + Tazobactam; CRO = Ceftriaxon; CAZ = Ceftazidim; CPM = Cefepim; AZT = Aztreonam; IPM = Imipenem; GMN: Gentamicin; AK: Amikacin; TOB: Tobramycin; CIP = Ciprofloxacin; LEV = Levofloxacin; FOS = Fosfomycin; COL = Colistin.

(100%), ceftazidim (98.8%), cefepim (93.5%), aztreonam (91.3%), piperacillin (82.6%), piperacillin/tazobactam (52.2%) and only weak at imipenem (13.0%). They were average with gentamicin (60.9%), amikacin (47.8%), tobramycin (52.2%), ciprofloxacin (56.5.0%), levofloxacin (41.3%). However, fosfomycin (2.2%) and colistin (2.2%) were the most active antibiotics on the isolates.

3.4. Phenotype of Enzyme Suspected

Table 4 shows the phenotypes of enzymes suspected in germ resistance to antibiotics. The rates observed were 52.2%, 15.2%, 8.69%, and 23.91%, respectively for EBLSs, CRPAs, MBLs, and other enzymes that were not classified.

Table 4. Enzyme produced by *Pseudomonas aeruginosa*.

Enzyms	Urine (N = 29)		Pus (N = 17)		Total (N = 46)	
	N	%	N	%	N	%
EBLSs	16	34.78	8	17.39	24	52.2
CRPA	5	10.87	2	4.35	7	15.2
MBLs	3	6.52	1	2.17	4	8.69
No detected	5	10.87	6	13.04	11	23.91

ESBL = Extended-Spectrum β-lactamase, CR = Carbapenemase-producing, MBLs = Metallo-β-lactamase, PA = *Pseudomonas aeruginosa*.

4. Discussion

Pseudomonas aeruginosa has become a pathogen of major public health importance, in particular, because of its frequent involvement in nosocomial and community-acquired infections. The plasticity of its genetic material allows it to acquire mobile genetic elements and to develop the various mechanisms of resistance to antibiotics. The present study showed a prevalence of 3.1% of this germ associated with urinary infections and pus. This prevalence is lower than that obtained by Yasmeen in Bangladesh (4.39%) [14], Ouedraogo in Burkina Faso (6.02%) [15], Frikh in Morocco (7.4%) [16] and Qayoom in India (9.16%) [17]. Higher rates were obtained by Srivastava in Egypt (21.85%) [18] and Gad in India (18%) [19]. These results show variations in infection rates by country and year. According to the types of samples, the proportions of urinary tract infections (2.87%) were almost similar to that of pus (3.70%). This similarity could justify the fact that opportunistic pathogens such as *Pseudomonas aeruginosa* are often involved in abscesses, the various pus at the start of urinary tract infections [20].

The age of the patients studied was 30 - 79 years (Table 1). The individuals with high proportions of infections were those of 30 - 39, compared to the extremities of ages. These results are similar to those of Sissoko in 2006 in Mali [21], showing a high rate of urinary tract infections among women in the age groups 16 - 35 and 36 - 65. Our results could be explained by the fact that indi-

viduals of the age group of 30 and 39 years are young and more sexually active and represented in this study. However, the frequency of urinary tract infections was higher in the female sexes than in males (**Table 1**). This difference can be justified by the peculiarity of the female anatomy characterized by its short, wide, straight urethra close to the perianal region and which, during sexual intercourse, causes the opening of the urethral meatus and favors the transfer of commensal bacteria in the bladder [22]. Unlike men who have a fairly long urinary tract and limit the ascent of bacteria to the urethra and the bladder. This result corroborates the work of Ouedraogo and collaborators [15] in Burkina Faso, who note that women in this age group are very sexually active and more exposed to urinary tract infections, and some opt for the use of contraceptives in order to avoid pregnancies and others, on the other hand, use tampons during menstruation, and these practices could increase the risk of urinary tract infection [23]. On the other hand, for the pus, on the contrary, the infection rates were higher in males (76.47%) than in females (23.53%). This result is similar to that reported by Qayoom and collaborators highlighting the dominance of *Pseudomonas aeruginosa* in men (66.19%) than in women (33.80%) [17].

According to the services, the strain of *Pseudomonas aeruginosa* was isolated from internal (45.65%) and external (54.35%) patients and testifies to the significant circulation of germs in the community. This paradigm loading would result from a significant transformation in microbial genetics. Also, strains of hospital origin could colonize the community environment during the discharge of hospitalized patients or by health personnel who also live in the community. The level of hygiene of populations and hospitals could also explain these differences. Some authors maintain that the prevalences generally increase with the level of technicality and the size of the establishments [17] [24]. Others report that community-acquired *Pseudomonas aeruginosa* infections are localized opportunistic infections resulting from local conditions favorable to their development and are often associated with contact with contaminated water or antiseptic solutions [25].

Disparities were also observed depending on the services. Infection rates were highest in urology at CHU RN (26.32%), in gynecology at CHU, ME (18.75%), and in medicine and pediatrics at HATC (27.27%) (**Table 2**). These rates, which vary by service and hospital, could be related to the patient profile and the medical devices which are different in the three hospitals sampled [26]. Women and children are consulted at the CHU ME, adolescents and adults at the CHU RN, and at HATC. Of the three sites, only HATC does not have a laboratory for bacterial culture and antibiotic susceptibility testing. Other studies have reported similar disparities with 15% of nosocomial infections in intensive care units and 10% in medicine and surgery units [3]. The low level of hygiene in the hospital can also be the factor that favored the bacteria circulation. The causative germs most often come from the patient himself, but they are transported to the infectious site through personnel or medical devices [26].

Regarding the resistance of *Pseudomonas aeruginosa* to *β*-lactams, our results revealed a proportion greater than 80% to ceftriaxon (97.8%), ceftazidim (97.8%), aztreonam (91.3%), cefepim (93.5%), piperacillin (82.6%), except piperacillin-tazobactam (52.2%) (**Table 3**). The *β*-lactamine resistance phenotypes were essentially ESBL, cephalosporinases, and carbapenemases. These alarming rates of bacterial resistance could be justified by the bacterial selection pressures induced by the inappropriate use of the latest generation antibiotics such as ceftriaxon, cefotaxim, and others of dubious quality which are sometimes sold in pharmacies, on the side of the streets, in markets and by street vendors [27]. Similar resistances have also been reported by other authors but in sometimes medium and high proportions to ceftazidim (52.31%), aztreonam (52.11%), cefepim (46.45%), piperacillin + tazobactam (47.15%), ticarcillin (70.86%) and cefotaxim (83.58%) [28]. On the other hand, resistance to imipenem was low in the present study (13.0%). It is similar to data provided by Arab countries such as Egypt (10%), Libya (11.1%), Iraq, and Jordan (0%) [29]. He noted that the inactivity of most *β*-lactamine was linked to the production of ESBL, with the exception of the imipenem-resistant strain whose phenotype of production of a metallo-enzyme was proven by the partial restoration of the sensitivity of imipenem by the action of EDTA. Compared to our study, low proportions of resistance of isolates were obtained to piperacillin-tazobactam at 19% in South Africa [30], 21% in France [31], and 30% in India [17]. High rates of imipenem resistance were found in the Philippines (31.1%), Singapore (23.3%), Thailand (28.7%) [32], and Japan (28.5%). These results show resistance rates of *P. aeruginosa* vary according to countries, regions, and years.

Concerning aminoglycosides, resistances were observed to gentamicin (60.9%), amikacin (47.8%), and tobramycin (52.2%). Saudi Arabia, Iraq, and Israel showed low aminoglycoside resistance of 25%, 38.7%, and 33.3%, respectively. In addition, a very small proportion (10%) of *Pseudomonas aeruginosa* previously isolated in Cameroon were resistant to aminoglycosides [33]. This result, although high, remains lower than an Egyptian ratio (91%) [33]. Moreover, the potential of *P. aeruginosa* to resist killing by a variety of antimicrobial agents results from the frequent use of several drugs albeit at low doses against diseases caused by these strains [34].

Regarding fluoquinolones, *Pseudomonas aeruginosa* was resistant to ciprofloxacin (56.5%), and levofloxacin (41.3%). Superior results (68%) were obtained in a previous study on various bacteriological cultures in N'Djamena [7]. Similar resistances have been reported by Al-Orphaly and collaborators in Saudi Arabia (25%), Iraq (50%), and Israel (44.4%) showed low resistance to aminoglycosides [29]. It should be noted that resistance to fluoroquinolones has been described by many authors. Plasmid genes (*Qnr* and *AAC* (6') *Ib-cr*) have been described in *Enterobacteriaceae*. Resistance to ciprofloxacin in this species is exclusively chromosomal [35] [36].

Fosfomycin and colistin were very active on the isolates. These antibiotics are not currently available for the treatment of bacterial infections in the country.

Colistin in particular has been withdrawn from the treatment of human infections on the grounds of nephrotoxicity. This context justifies the preserved efficiency of these molecules. Mirroring our study, it is not surprising because while the use of colistin in humans is rare in Africa, its use in livestock is unregulated [37]. The case of resistance observed could come from the veterinary sector and spread to other ecosystems [37]. This indicates that for all antimicrobial agents tested other than colistin, at best, only fosfomycin could serve as an empirical treatment option for *P. aeruginosa* infections.

According to the enzymes suspected, medium and low rates of EBLSs (52.2%), CRPA (15.2%), MBLs (8.69%), and other enzymes (23.91%) were observed (**Table 4**). This lower prevalence of CRPAs and MBLs observed in Chad could be explained by the limited access to the use of carbapenems in human medicine. Other studies reported higher rates of positive isolates for EBLSs ranging from 52.4% to 82.3% in Poland [38], 63% in Iran [39], and 55% of MBLs in burn patients in Turkey [40].

At the end of this study, we can identify limitations such as the use of molecular methods to detect antibiotic resistance genes in order to better understand the circulation of these dangerous bacteria in the population.

5. Conclusions

This study made it possible to identify the strains of *Pseudomonas aeruginosa* involved in urinary tract infections and pus in the National Reference University Hospital, the Mother and Child University Hospital, and the Chad-China Friendship Hospital. It highlights the extent of the problem of *Pseudomonas aeruginosa* infections which are becoming increasingly difficult to treat because of their ability to resist antibiotics. The isolated strains showed strong resistance to β-lactamines, quinolones, and aminoglycosides. Faced with this worrying problem, global awareness is needed. The best ways to fight against urinary tract infections and suppurative pathologies are essentially prevention through population hygiene measures, carrying out antibiotic sensitivity tests before treating patients, respecting the prescription of antibiotics, and public awareness to avoid self-medication in healthcare settings and communities.

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Conflicts of Interest

The authors declare no competing interests.

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