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Resistance of Strains of *Pseudomonas aeruginosa* to Antibiotics in N'Djamena in Chad

Ahmat Mahamat Ahmat[1], Adoum Fouda Abderrazzack[2], Abdelsalam Tidjani[3], Brahim Boy Otchom[4], Aly Savadogo[5]

[1]Laboratory of the Mother and Child Hospital, University of N'Djamena, N'Djamena, Chad

[2]Sectoral Program for the Fight Against HIV/AIDS and Sexually Transmitted Infections, N'djamena, Chad

[3]Department of Public Health, Faculty of Sciences and Human Health, University of N'Djamena, N'Djamena, Chad

[4]Biomedical Sciences, Faculty of Sciences, Toumai University, Ndjamena, Chad

[5]Biochemistry Microbiology, Faculty of Sciences, Joseph Ki-Zerbo, Ouagadougou, Burkina Faso

Email address:

razzackadoum@yahoo.fr (A. F. Abderrazzack)

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Abstract: The objective of this study is to determine the resistance profile of *Pseudomonas aeruginosa* strains towards antibiotics in N'Djamena (CHAD). This a cross-sectional and prospective study, conducted at the Mother and Child Hospital (MCH) in N'djamena, from February to July 2016 on 550 urine samples. Uroculture and antibiotic susceptibility testing were performed according to MCH laboratory standards. Germ isolation was performed on Cetrimide agar and identification by API 20 NE gallery. The antibiogram was determined by the microgram method on Mueller-Hinton agar. A total of 15/550 (2.72%) strains of *Pseudomonas aeruginosa* were identified. These strains showed low resistance between 6.66% and 13.33% to Cefixime, Colistin, Gentamycin, Ciprofloxacin and Tobramycin. On the other hand, a high resistance (100%) was observed with Augmentin, Ampicillin, Cefotaxime, Ceftriaxone, Imipenem, Vancomycin, Oxacillin, Erythromycin and Tetracycline. The emergence of multiresistant bacterial strains is a major public health problem that requires adequate and adapted antibiotic therapy. *Pseudomonas aeruginosa* is one of the bacteria whose monitoring is more than necessary to defect the appearance of multidrug resistant strains. Given the involvement of *Pseudomonas aeruginosa* in several infectious pathologies, a monitoring of its profile with respect to antibiotics must be systematically carried out in all the health structures that have adequate technical platforms allowing them to perform an antibiogram.

Keywords: Resistance, Antibiotics, Urine, *Pseudomonas aeruginosa*

1. Introduction

Worldwide, nosocomial infections have become common with rapid and increasing emergence of multidrug-resistant microbial strains, often in intensive care units [15] Among the multiresistant strains responsible for nosocomial infections, *Pseudomonas aeruginosa* predominates with a frequency of approximately 9.2%, placing it in 3rd place just after *Escherichia coli* and *Staphylococcus aureus* [5].

Indeed, the main factors in the emergence of this resistance are related to the increasing and unjustified use of antibiotics, the pressure of selection exerted by the use of antibiotic therapy and the epidemic spread of resistant strains, and this becomes more and more a major problem in the hospital environment. [28].

As a general rule, the resistance of microorganisms to antibiotics may be considered as a therapeutic failure. However, *P. aeruginosa* also has a high level of natural resistance to antibiotics. This also results from the accumulation of resistance mechanisms related to chromosomal mutations and the acquisition of exogenous genes. This opportunistic pathogen is characterized by its ability to persist and resist in the hospital environment, and by its rapid acquisition of resistance to antibiotics, leading to

a therapeutic impasse.

In spite of the therapeutic progress, the mortality related to *Pseudomonas aeruginosa* infections remains high because of the therapeutic difficulties caused by this bacterium and because of the associated pathologies [4].

In order to evaluate the resistance of strains of *Pseudomonas aeruginosa* to antibiotics in order to improve the quality of nosocomial infections in hospitals, we opted to isolate, identify and record the sensitivity strains of *Pseudomonas aeruginosa* in urine collection in hospitalized patients at the Mother and Child Hospital (MCH) in N'Djamena, Chad.

2. Material and Methods

2.1. Field Investigation

It was a cross-sectional and prospective study conducted at the Mother and Child Hospital in N'Djamena from February to July 2016.

Urine sampling was performed using adequate and sterile equipment. It consists of sterile pot of 10 ml. All patients received practical advice for the respect of hygienic conditions in order to achieve a better sampling. As a general rule, the urine was taken in the middle of the jet in the morning from the non-probed patient and by direct puncturing of the lid of the probes in the patient being probed. The samples were stored at 2-8°C before handling.

2.2. Microbiological Analyzes

Microbiological analyzes were performed using standardized techniques from the Maternal and Child Hospital Laboratory in N'djamena.

For this purpose, the cetrimide medium was used for the culture and isolation of the germ. Seeding was performed by the tight streak technique next to a bunsen burner. The dishes are incubated at 42°C in an oven for 18 to 24 hours (NF ISO 22717).

2.3. Biochemical Identification of Germs

The biochemical characteristics of the germs were made by conventional tests (Véron M. 1982).

Pseudomonas aeruginosa is characterized by Urea (-), Oxidase (+), Gelatin (+), ADH (Arginine) (+), TDA (Tryptophan) (-). The figure below summarizes its biochemical characteristics.

Figure 1. *Biochemical characteristics of Pseudomonas aeruginosa.*

2.4. Study of Antibiotic Resistance

Antibiotic resistance was determined by the Mueller-Hinton agar microscopic susceptibility test method, in accordance with the recommendations of the antibiogram committee of the French Microbiology Society (CA-SFM2011) and the HME laboratory. Thus, the resistance profile of each bacterial strain with respect to the antibiotics tested is determined according to the reference to a CASFM reading table, (2011) giving the correlation between the inhibition diameter and the Minimum Inhibitory Concentration (MIC).), which will classify bacteria as sensitive (S), intermediate (I) or resistant (R).

The antibiotic discs that have been tested are Augmentin (AUG), Ampicillin (AMP), Amikacin (AK), Cefotaxime (CTX), Ciprofloxacin (CIP), Cotrimoxazole (SXT), Ofloxacin (OFX), Gentamycin (CN), Imipenem (IMI), Vancomycin (VA), Oxacillin (OX), Erythromycin (E), Colestin Sulfate (CS), Norfloxacin (NOR), Piperacillin (PIL), Tobromycin (TOB), Tetracycline (TE) Cefipime (FEP), Levofloxacin (LEV) and Ceftriaxone (CTX).

2.5. Statistical Analyzes

Statistical analyzes were performed using Epi Info version 2007. The probability was set at $p < 0.05$.

3. Results

3.1. Microbiological Analyzes

A total of 550 samples were collected and analyzed, fifteen (15) strains of *Pseudomonas aeruginosa* were identified in the urine of patients hospitalized at the Hospital of the Mother and Child in N'Djamena a prevalence of 2.72%.

3.2. Distribution of Pseudomonas Aeruginosa Strains

Table 1 shows the proportion of samples analyzed in relation to the number of samples received in the different departments.

Table 1. *Proportion of samples analyzed.*

Services	Number of samples	Percentage (%)
Gynecology	172	31.27
Obstetrics	94	17.09
Pediatry I	80	14.54
Pediatry II	67	12.18
Pediatry Emergency	116	21.09
Resuscitation	21	3.81
Total	550	100

Table 2. *Proportion of strains of Pseudomonas aeruginosa.*

Services	*Pseudomonas aeruginosa*	Percentage (%)
Gynecology	6	40
Obstetrics	1	6.66
Pediatry I	1	6.66
Pediatry II	1	6.66
Pediatry Emergency	5	33.33
Resuscitation	1	6.66
TOTAL	15	2.72

The high number of urinary samples analyzed came from the gynecology department (172) (31.27%), followed by pediatric emergency department 1 (16) (21.09%).

According to Table 2, the most important proportions of *Pseudomonas aeruginosa* strains were identified in the departments of Gynecology (40%) and Pediatric Emergency (33.33%).

The other services each have a proportion of 6.6% (Pediatrics II, Pediatrics I, Resuscitation and Obstetrics).

3.3. Antibiotic Susceptibility of Pseudomonas Aeruginosa Strains

The details of the susceptibility profile of *Pseudomonas aeruginosa* strains are shown below (Figure 2).

The three categories used for the interpretation of in vitro susceptibility tests are: Sensitive (S) = probability of acceptable therapeutic success; Resistant (R) = expected therapeutic failure; Intermediate (I) = unpredictable therapeutic success (CASFM, 2011).

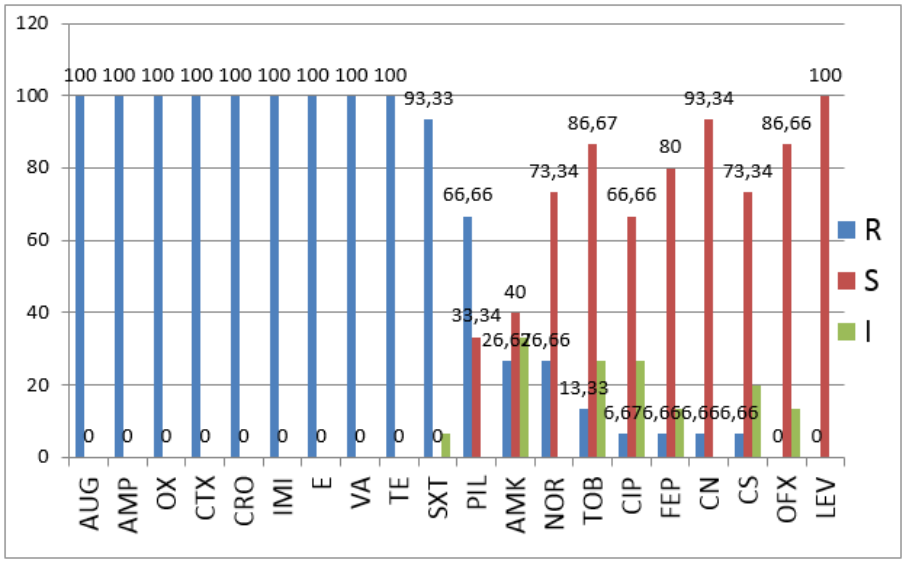

Figure 2. *Resistance of strains of Pseudomonas aeruginosa to antibiotics.*

From Figure 2, strains of *Pseudomonas aeruginosa* exhibited varied resistance to the twenty (20) antibiotics tested.

4. Discussion

Women are mainly exposed to urinary tract infections (UTIs), and the main causative organisms are enterobacteria (2). Our results also showed that women are mostly infected (46%) with *Pseudomonas aeruginosa*. This female predominance is confirmed by other authors, it could be explained that anatomically their urethra is short, wide, straight and close to the perianal region and also during sexual intercourse could cause an opening of the urethral meatus which favor easily the penetration of germs in the bladder. [20, 3]

P. aeruginosa is one of the major bacteria that causes hospital-acquired urinary tract infections (12%). According to the results of a study, the prevention of this infection would be established by a mechanical, physical or chemical barrier between pathogenic microorganisms and the potential host represented by the patient or hospital staff [29].

Based on our results, we found an ITU frequency of 2.72% which is much lower than the results reported in 1999 by CIAFU (22%). However, our results are comparable to those demonstrated in 2011 by Medboua (7.86%) and Drissa in 2013 (2.40%). Compared to the distribution of this bacterium in the gynecology department, our propitiations oscillate between the frequencies reported by ZieDrissa, 2013 (28.8%) and Medboua (4.13%) in 2011. By cons in the intensive care unit, the frequency of *Pseudomonas aeruginosa* represents (6.66%) in our study, which is consistent with those obtained by Ben (2010) (5.6%) but decayed results of Medboua in 2011 (0.98%). In pediatric I and II, infections also accounted for 6.66%, which are similar to Ben's 2006 results (6.5%); however, Hmamouchi's results in 2005 (21.9%) are higher. These important identification frequencies for resuscitation and pediatrics are linked, on the one hand, to the frequent nosocomial character and, on the other hand, to the use of the central venous pathways as well as to the immune depression.

With regard to the resistance of strains to antibiotics, our results show a 100% resistance of strains of *Pseudomonas aeruginosa* against Augmentin, Ampicillin, Cefotaxime, Ceftriaxone, Imipenem, Vancomycin, Oxacillin, Erythromycin and Tetracycline but a resistance that oscillates between 66.66% and 93.33% against the molecules of Cotrimoxazole and Piperacillin.

Our results corroborate with the data of some authors who reported resistance of these strains to ampicillin, tetracycline, and Erythromycin [23, 16, 25, 19, 6, 12]. The high resistance of strains of *Pseudomonas aeruginosa* to the antibiotics mentioned above can be explained on the one hand because of their excessive prescription and on the other hand their

anarchic use, and also by the impermeability of the bacterial wall and is especially the production of beta-lactamase (Phillips et al., 2012 and Cooksey, 1991).

Despite the multiple resistance of *P. aeruginosa* strains to reported antibiotics, our results showed that they had a sensitivity of 66.6% to 100%. Faced with Cefipime, Colestine, Gentamycin, Ciprofloxacin and Tobramycin molecules.

5. Conclusion

The present study identified resistance of P. aeruginosa strains to antibiotics in hospitalized patients at N'Djamena (CHAD).

Indeed, infections caused by *Pseudomonas aeruginosa* are formidable given their resistance to antibiotics.

Our work shows that the resistance of P. aeruginosa to antibiotics is beginning to emerge at MCH N'Djamena. Thus, our study lays a milestone for a large-scale research to determine the resistance of microorganisms to N'Djamena.

We call on the hospital health authorities of Chad in general and those of HME in particular to monitor and control the misuse of antibiotics, to put effective hygienic measures in hospital structures to reduce the risk of nosocomial infections.

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