

Full Length Research Paper

Antimicrobial susceptibility of *Pseudomonas aeruginosa* strains in Bamako, Mali

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***Pseudomonas aeruginosa* is generally susceptible to antibiotics of the families of Beta-lactam, aminoglycosides and quinolones. The aim of this study was to evaluate the antimicrobial susceptibility of *P. aeruginosa* strains in Bamako, Mali. *P. aeruginosa* strains were isolated on Drigalski agar. Antimicrobial susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar. Among 317 non repetitive strains recovered from 2010 to 2019, there were 246 (77.6%) hospital strains and 71 (22.4%) extra-hospital strains. Colistin (100%), imipenem (98.4%), ceftazidime (89.3%), amikacin (85.2%) and piperacillin (72.3%) were the most active antibiotics against our *P. aeruginosa* strains. Of the strains 11 (3.5%) were multi-drug resistant (MDR) and 5 (1.6%) were extensively drug-resistant (XDR). The extra-hospital *P. aeruginosa* strains were more susceptible to aztreonam (91.5% vs 60.6%; P = 0.000018), piperacillin (84.5% vs 68.7%; P = 0.013), gentamycin (84.5% vs 62.2%; P = 0.00071), netilmicin (56% vs 32.5%; P = 0.0045) and ciprofloxacin (79% vs 65.4%; P = 0.0455) than the hospital strains. Colistin, imipenem, ceftazidim, amikacin and piperacillin have a high-level activity against *P. aeruginosa* in Bamako.**

Key words: *Pseudomonas aeruginosa*, antimicrobial susceptibility, Bamako, Mali.

INTRODUCTION

Pseudomonas aeruginosa is a strictly non-fermenting aerobic Gram-negative bacillus that belongs to the Pseudomonadaceae family. *P. aeruginosa* is involved in various infections: urinary tract infections, abscesses, bacteremia, pulmonary infections, bone and joint infections, eye infections, infections of the otolaryngological sphere, meningeal infections, skin infections, enteritis, and endocarditis Avril et al., 2000;

Wu et al., 2015). *P. aeruginosa* which is catalase and oxidase-positive, generally produces two pigments: pyocyanin (blue-green) and pyoverdine (yellow-green and fluorescent), Avril et al., (2000). *P. aeruginosa* is susceptible to carboxypenicillins, ureidopenicillins (mezlocillin, piperacillin), some 3rd generation cephalosporins (cefsulodine, cefoperazone, ceftazidime, cefepime, and ceftipime), carbapenems (imipenem),

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monobactams (aztreonam), certain aminoglycosides (gentamicin, netilmicin, and amikacin), fluoroquinolones, and colistin (Avril et al., 2000). *P. aeruginosa* has a natural resistance to aminopenicillins, 1st and 2nd generation cephalosporins, cefotaxime, cotrimoxazole, tetracyclines, chloramphenicol and nalidixic acid (Avril et al., 2000).

In addition to this natural resistance, there are acquired resistances which constitute the whole problem with this bacterium, particularly in hospitals and increasingly in community settings. These acquired resistances could be found in β -lactam (cephalosporin), aminoglycosides, fluoroquinolones (Avril et al., 2000; Kouamé et al., 2016; Weldaghen et al., 2003).

In Mali, there are very limited data on the susceptibility of *P. aeruginosa* to antibiotics, and given the increasing antibiotic resistance worldwide, the aim of this study was to evaluate the susceptibility of *P. aeruginosa* to antibiotics in Bamako.

MATERIALS AND METHODS

Study site and setting

This was a retrospective study carried out in the Medical Biology and Hospital Hygiene Laboratory of the University Teaching Hospital of the Point G, Bamako, Mali from January 1st, 2010 to December 31st, 2019. The University Teaching Hospital of the Point G is the third-pyramidal reference in Mali, and has 522 beds divided between the surgical, intensive care and medical departments.

Bacterial strains

While patients admitted to the different departments of the University Teaching Hospital of the Point G, were hospitalized patients (in-patients), those who were coming to the hospital, for medical consultation, laboratory tests and/or X-Ray were not hospitalized and were called out-patients.

The hospital strains of *P. aeruginosa* were isolated from samples from hospitalized patients at the Point G University Teaching Hospital.

The extra-hospital strains of *P. aeruginosa* (community strains) were isolated from samples from out-patients.

The 317 non-repetitive strains isolated from samples collected from in-and out-patients visiting the University Teaching Hospital of the Point G. The strain isolated was done on Drigalski agar (Bio-Rad, France) at 37°C.

The identification of the strains was made either on the production of pyocyanin and pyoverdine on respectively King A and King B (Bio-Rad, France) media, on oxidase (Bio-Rad, France) and catalase (bioMérieux, France) positive reactions, and by the API 20 NE systems (bioMérieux, France).

Susceptibility to antibiotics test

The antimicrobial susceptibility testing was carried out on Mueller-Hinton agar (Bio-Rad, France) by the disc method (Agar diffusion method). The strains of *P. aeruginosa* were classified as "susceptible", "intermediate" or "resistant" according to the

recommendations of the Antibiotic Committee of the French Society of Microbiology/European Committee on Antimicrobial Susceptibility Testing in 2015 (CA-SFM/EUCAST) (Jehl et al., 2015). The strains of *P. aeruginosa* classified as <<intermediate>> to the antibiotics tested were considered resistant.

Laboratory procedure

The antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Bio-Rad, France) poured into a Petri dish. A colony isolated from an 18-24 h culture of *P. aeruginosa* was suspended in 5 ml of sterile saline solution which was calibrated to 0.5 MacFarland. Two drops of this suspension are then added in 10 ml of sterile distilled water. This second suspension is poured over the entire surface of the Mueller-Hinton agar poured into a Petri dish. The excess is poured into bleach. The seeded agar is left to dry for 15 min at 37°C inside the incubator. Please kindly note that the incubator is not used to dry seeded agar plate. Instead, the plates are allowed to stand on the bench for a while, before the antibiotic discs are introduced. After the seeded agar has dried, the blotting paper discs impregnated with the antibiotics to be tested are placed on the surface of the agar using a disc dispenser according to manufacturer's instructions (Bio-Rad, France). After a first diffusion of the antibiotics in 30 min at room temperature, the Petri dish is incubated at 37°C for 18 to 24 h, in the inverted position (cover down). The reading is performed in measuring the diameter of inhibition of each antibiotic disc using a caliper in contact of growth.

Antibacterial agents tested

The antibiotics tested were ticarcillin (75 μ g), piperacillin (75 μ g), ceftazidime (30 μ g), aztreonam (30 μ g), imipenem (10 μ g), gentamicin (15 μ g), tobramycin (10 μ g), netilmicin (30 μ g), amikacin (30 μ g), ciprofloxacin (5 μ g), and colistin (50 μ g) (Bio-Rad, France).

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes

The strains of *P. aeruginosa* intermediate or resistant to at least one molecule in three groups of antibiotics active against *P. aeruginosa*: (1) β -lactams except imipenem (ticarcillin, piperacillin, ceftazidime, and aztreonam), (2) imipenem, (3) aminoglycosides, and (4) ciprofloxacin were considered to be MDRs. The strains of *P. aeruginosa* intermediate or resistant to at least one molecule in each group of antibiotics have been considered as XDR (Barbier and Wolff, 2010; Magiorakos et al., 2012; Horcajada et al., 2019).

Ethics statement

The clinical specimens included in this manuscript were collected under public health surveillance of antimicrobial testing, and not as human subject research. Thus, submission to institutional review boards was not applicable. Participants were explained, and they consented to use the results. In addition, permission was received from Hospital Director for this manuscript.

Statistical analysis of data

The samples were collected under public health surveillance of antimicrobial resistance, and thus an estimated sample size was not previously determined. The data were entered and analyzed using Epi Info software 7.1 version. For the comparison of the results, we used the test of χ^2 with a significance level $P \leq 0.05$.

Annual Frequency of *P. aeruginosa* from 2010 to 2019

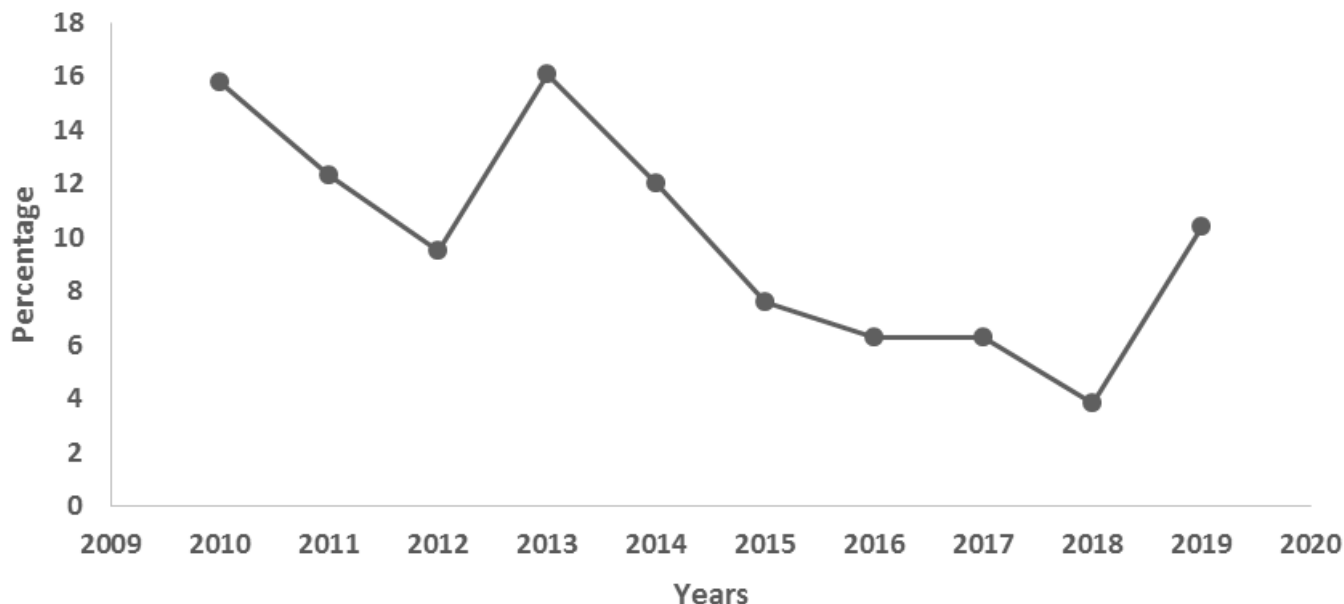


Figure 1. Distribution of the frequency of *P. aeruginosa* in the Point-G University Teaching Hospital between 2010 and 2019.

RESULTS

A total of 317 non-repetitive strains of *P. aeruginosa* were identified from 317 persons between 2010 and 2019. The mean age of patients was 48.77 ± 18.5 years old, and the sex ratio (male/female) was 1:4 ratio.

The annual frequency of strains during the ten-year period is presented in Figure 1. Among these strains, 246 (77.6%) were of hospital and 71 (22.4%) of extra-hospital origin. The hospital strains were isolated in the wards of medicine ($n = 185$), surgery ($n = 49$) and intensive care unit ($n = 12$).

The distribution of *P. aeruginosa* strains according to the samples is shown in Table 1. The strains of *P. aeruginosa* have been isolated primarily from urine 143 (45.1%), pus 81 (25.6%), vaginal swabs 41 (12.9%), and/or sputum 26 (8.2%).

The antibiotic susceptibility of *P. aeruginosa* is reported in Figure 2. Thus, colistin, imipenem, ceftazidime, amikacin and piperacillin were the most active antibiotics against *P. aeruginosa*. Of the 317 strains 11 (3.5%) were MDR and 5 were XDR (1.6%). More specifically, 7 (2.8%) MDR-strains of *P. aeruginosa* were isolated in in-patients, and 4 (5.6%) in the out-patients setting, while 3 (1.2%) XDR-strains were isolated in in-patients, and 2 (2.8%) in the out-patients setting.

The susceptibility of antibiotics to *P. aeruginosa* strains isolated either from in- or out-patients is reported in Table 2. The strains isolated from out-patients were statistically more susceptible to aztreonam ($P < 0.0000$), piperacillin

($P = 0.0130$), gentamicin ($P = 0.007$), netilmicin ($P = 0.0045$) and ciprofloxacin ($P = 0.0455$) than in-patients' strains.

DISCUSSION

This study was carried out to evaluate the antimicrobial susceptibility of different *P. aeruginosa* strains isolated in our laboratory between 2010 and 2019. To the best of our knowledge, this study is the first study of its kind conducted in Bamako, Mali.

The identification of our strains of *P. aeruginosa* was based on their morphological and biochemical characteristics (Avril et al., 2000). The interpretation of the results was done with regard to international recommendations (CA-SFM/EUCAST) (Jehl et al., 2015).

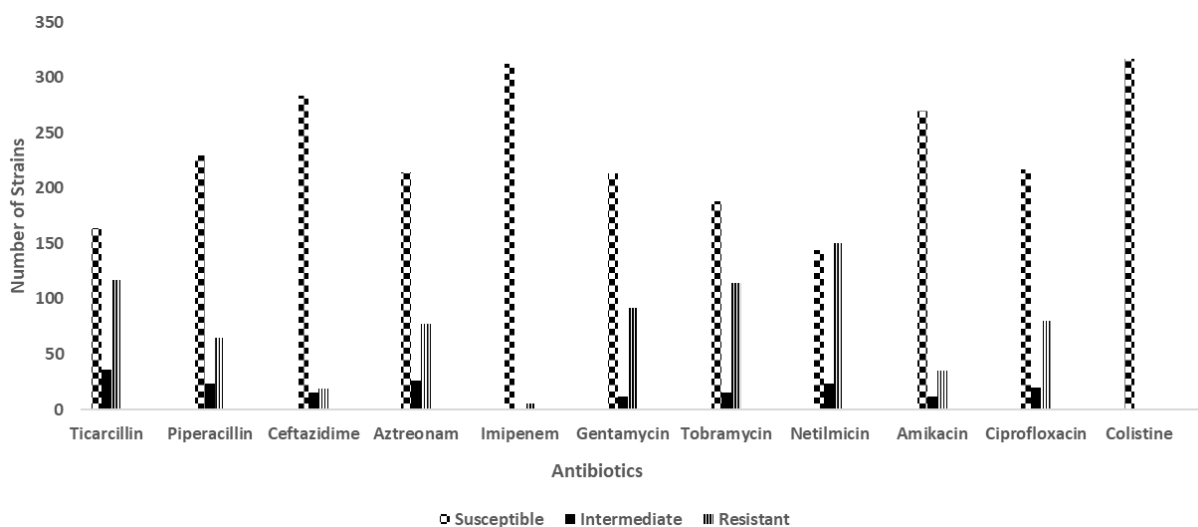
This has public health implication in Mali as it was a surprise to find out that we have resistance to some antibiotics such as imipenem, ceftazidime, and amikacin which are not used in routine care. Thus, both in hospital area and outside hospital, the use of antibiotics should be guided by antimicrobial susceptibility testing results.

In this study, the strains of *P. aeruginosa* were of hospital and non-hospital origin, and the hospital strains were mainly from the medical and surgical departments. In Monastir in Tunisia, *P. aeruginosa* strains were isolated in intensive care, surgery and ear, nose, and throat (ENT) departments, mostly (Ben Abdallah et al., 2008).

This difference in the study site may explain the difference between the two studies.

Table 1. Distribution of 317 *Pseudomonas aeruginosa* strains according to the specimen and the patients' origin.

Specimen	Hospital strains (No. of strains)	Extra-hospital strains (No. of strains)	Total (Rate in %)
Urines	119	24	143 (45.1)
Pus	70	11	81 (25.6)
Vaginal secretions	12	29	41 (12.9)
Sputums	19	7	26 (8.2)
Blood cultures	12	0	12 (3.8)
Pleurisy	4	0	4 (1.3)
Catheters	4	0	4 (1.3)
Cerebro-spinal fluid	1	0	1 (0.3)
Prostatic fluid	1	0	1 (0.3)
Peritoneum fluid	1	0	1 (0.3)
Broncho-alveolar fluid	1	0	1 (0.3)
Articular fluid	1	0	1 (0.3)
Gastric fluid	1	0	1 (0.3)
Total	246	71	317(100)

**Figure 2.** Distribution of 317 *Pseudomonas aeruginosa* strains according to the antimicrobial susceptibility.

In the present study, *P. aeruginosa* strains were isolated from different samples: urine, pus, vaginal samples, and blood cultures (Table 1), while in Monastir in Tunisia the strains of *P. aeruginosa* were isolated from pus (52.9%), respiratory samples (19.5%), urine (10.6%) and blood cultures (5%) (Ben Abdallah et al., 2008). The *P. aeruginosa* strains of Abdou-Souley Lié Moustapha (2002) were isolated in 2002 at the Point G University Teaching Hospital in the same samples as the present study. The sampling sites fit well with the pathogenicity of *P. aeruginosa* which determines various infections (Avril et al., 2000; Wu et al., 2015; Michel-Briand, 1992).

In Europe, 74% of *P. aeruginosa* strains were

susceptible to ticarcillin, 80% to ceftazidime, 73% to aztreonam and 82% to imipenem (Rossolini and Mantengoli, 2008). Ticarcillin, ceftazidime and aztreonam were, respectively active in 46.9, 89.6 and 67.5% of the strains of *P. aeruginosa* in this study.

Piperacillin was not active in 27.7% of the strains. The proportion of *P. aeruginosa* strains resistant to piperacillin varies from one country to another: it was 48.5% in Germany, 38.4% in France and 5.4% in the United Kingdom (Nordmann and Naas, 2012). This difference could be explained by the previous exposition to this antibiotic. Generally, this ureidopenicillin is not available in Mali. The susceptibility of the strains to imipenem is

Table 2. Comparative antimicrobial susceptibility of *Pseudomonas aeruginosa* hospital and extra-hospital strains.

Antibiotics	Hospital strains (in-patients)		Extra-hospital strains (out-patients)		P value
	S [n (%)]	I+R [n (%)]	S [n (%)]	I+R [n (%)]	
Ticarcillin	121 (49.2)	125 (50.8)	43 (61)	28 (39)	0.091
Piperacillin	169 (68.7)	77 (31.3)	60 (84.5)	11 (15.5)	0.013
Ceftazidime	218(88.6)	28 (11.4)	65 (91.5)	6 (8.5)	0.482
Aztreonam	149 (60.6)	97 (39.4)	65 (91.5)	6 (8.5)	0.0000018
Imipenem	243 (98.8)	3 (1.2)	69 (97)	2 (3)	0.341
Gentamycin	153 (62.2)	93 (37.8)	60 (84.5)	11 (15.5)	0.00071
Tobramycin	143 (58.1)	103 (41.9)	42 (59)	29 (41)	0.985
Netilmicin	80 (32.5)	166 (67.5)	40 (56)	31 (44)	0.0045
Amikacin	210 (85)	36 (15)	60 (84.5)	11 (15.5)	0.857
Ciprofloxacin	161 (65.4)	85 (34.6)	56 (79)	15 (21)	0,0455

S = Susceptible; I = intermediate; R = resistant; P= probability.

almost the same (Figure 2). The proportion of *P. aeruginosa* strains resistant to ceftazidime is 18.6% in France and 21.8% in Monastir in Tunisia (Ben Abdallah et al., 2008; Nordmann and Naas, 2012). This proportion was 10.4% in the present study (Figure 2).

The proportion of *P. aeruginosa* strains resistant to carbapenems (imipenem or meropenem) is 18.4% in France (Nordmann and Naas, 2012). In Monastir in Tunisia, the resistance rate of *P. aeruginosa* to imipenem was 19.6% (Ben Abdallah et al., 2008), while resistance to imipenem was 1.6% in the present study (Figure 2).

In this study the prevalence of *P. aeruginosa* strains MDR and XDR were low regardless of origin. Usually, the prevalence of MDR strains of *P. aeruginosa* varies from 15 to 30% in many regions (Horcajada et al., 2019). In 2017 in Spain, a multicenter study of *P. aeruginosa* infections found 26% of MDR strains and 17% XDR strains (Ben Abdallah et al., 2008). In the United States, out of 7,868 strains of *P. aeruginosa* isolated in 94 hospitals between 2013 and 2016, 1,562 (19.8%) were MDR and 717 (9.1%) XDR (Sader et al., 2017).

In 1990 at Henri Mondor Hospital in France, the resistance rate of *P. aeruginosa* strains to gentamicin and amikacin was 39.81 and 12.03%, respectively (Caron and Humbert, 1993). This rate is close to the present study with regards to gentamicin (Figure 2 and Table 2). This difference of resistance to specific and/or MDR strains could be explained by the low prevalence of *P. aeruginosa* and imipenem is rarely prescribed in setting of the present study.

The *P. aeruginosa* strains of Ben Abdallah et al., (2008) isolated in Monastir were more resistant to gentamicin (39.3%) as the present study. This strains of *P. aeruginosa* appear to be more resistant to amikacin than those from the Henri Mondor Hospital in France (Figure 2 and Table 2), and the strains of Ben Abdallah et al. (2008) isolated in Monastir, Tunisia, were resistant to amikacin at 19.2%.

In France, the *P. aeruginosa* resistance rate to

ciprofloxacin was stable at around 25 to 30% according to Soussy (2012). The resistance rate of our extra-hospital strains to ciprofloxacin was identical to that of Soussy (2012) (Figure 2), and probably due to the baseline prescription of ciprofloxacin to many other bacterial diseases such as typhoid fever which is very common in the setting of the present study.

The strains of *P. aeruginosa* were susceptible to colistin (Avril et al., 2000), and the susceptibility of the strains of the present study to colistin was constant, because there was no resistance to this polymyxin (Figure 2).

This study has some limitations; first the data were retrospectively collected, and also were limited by the number of discs to be purchased for a complete full profile of antimicrobial resistance to the *P. aeruginosa* strains.

Despite these limitations, the study is unique as it collects consecutive strains of the *P. aeruginosa* isolated in both hospital and outside-hospital area in Bamako, Mali.

Conclusion

Colistin, imipenem, ceftazidime, amikacin and piperacillin were the most active antibiotics against *P. aeruginosa*. Ciprofloxacin and ticarcillin were active in every other strain in hospital area. The frequency of multidrug-resistant strains of *P. aeruginosa* is low in Bamako. Out-patients' strains were more susceptible to aztreonam, piperacillin, gentamicin, netilmicin and ciprofloxacin than hospital strains.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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