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ORIGINAL ARTICLE

Less effect of wells physicochemical properties on the antimicrobial susceptibility *Pseudomonas aeruginosa* isolated in equatorial region of Central Africa

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Abstract

This study aimed at assessing the impact of some wells abiotic factors on the antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated. A total of 14 well waters of Douala and Yaoundé towns (Cameroon, Central Africa) were chosen. Fifteen antibiotics were used to test antimicrobial susceptibility with the majority belonging to β-lactam, aminoglycoside and quinolone groups. Result showed that in Douala, electrical conductivity of water ranged from 145.38 to 559.69 µS/cm in well, while in Yaoundé, it ranged from 172.5 to 710.2 µS/cm. Dissolved oxygen ranged from 2.5 to 5.5 mg/l and from 3.9 to 5.1 mg/l, respectively, in Douala and Yaoundé. Organic matter varied from 2.3 to 7.2 mg/l and 1.4 to 5.2 mg/l in Douala and Yaoundé, respectively. As for pH, it fluctuated between 5.56 and 6.66 CU and between 5.1 and 7.0, respectively, in Douala and Yaoundé. The sampled waters harbour multi-drug-resistant *P. aeruginosa* strains. About 20.40% of strains from wells in Yaoundé metropolis expressed resistance to at least 5 antibiotics, whereas from wells in Douala town, up to 33.33% of strains were resistant to at least 8 antibiotics including ticarcillin, ceftazidime and gentamicin. The effect of abiotic factors on the sensitivity to antibiotics was assessed using the multiple regression tests. In both towns, less than 5% in the changes of physicochemical parameters of well waters explained the variance in inhibition diameter values of antibiotics ($p \leq 0.05$). The pH significantly impacts on antimicrobial susceptibility. Environmental conditions are less stressful for *P. aeruginosa* and fairly affect its antimicrobial susceptibility.

Keywords Wells · *P. aeruginosa* · Antimicrobial susceptibility · Abiotic factors

Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium implicated in cutaneous and ophthalmic infections, gastrointestinal attacks leading sometimes to diarrhoeic in newborns. In most cases, these infections are associated with contaminated water or contaminated antiseptic solutions (Mérens et al. 2013). Infections caused by *P. aeruginosa* are sometime difficult to treat because of its high level of intrinsic resistance to antibiotics (Hancock and Speert 2000). This opportunist bacterium can also exhibit high adaptive response to changing environmental conditions (Mérens et al. 2011). In developing countries where access to potable water and sanitation is limited, the presence of this resistant bacterium in aquatic environments is a serious public health threat (Satoru and Phan 2012).

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Antimicrobial susceptibility must not be considered just as a medical issue but also as an ecological matter. To understand the process by which antimicrobial susceptibility is governed in environmental bacteria, it is necessary to consider the ecology of microorganisms as well as the potential impact of environmental conditions (Macfarlane et al. 2000). Just like many microorganisms, *P. aeruginosa* is frequently exposed to environmental stresses such as UV radiation, acid stress (pH), nutrient starvation and oxygen limitation related to organic pollution (Poole 2012a). These stresses elicit a variety of specific and highly regulated adaptive responses that not only protect the bacterium from the offending stress, but also alter gene expression patterns (Wood and Ohman 2009) and cell physiology in ways that it can profoundly influence antimicrobial susceptibility (Macdonald and Kuehn 2013). According to Poole (2012b), the physiological changes in a bacterium exposed to environmental stresses are often transient but occasionally permanent depending on the adaptive changes in the genetic architecture of the microorganism. McMahon et al. (2007) added that some pathogens continued to express higher levels of antibiotic resistance after removal of stress, suggesting that in some cases sublethal stress may induce stable increases in antibiotic resistance.

In Cameroon, data on the antimicrobial susceptibility of bacteria originated from aquatic environments are very fragmentary (Akoachere et al. 2013). Investigations on drug resistance are mostly performed with clinical strains because of their implication in nosocomial infections (Ateba et al. 2013; Kamga et al. 2015). Yet the presence of resistant bacteria in aquatic ecosystems increases the risks during infection by water since the therapeutic possibilities are reduced. In urban areas such as Douala and Yaoundé, populations depend mainly on groundwaters which are consumed sometimes without efficient treatment (Drouart and Vouillamoz 2012; INS 2013). These groundwaters harbour diverse bacterial microflora primarily consisting of faecal bacteria, which are commensal or pathogenic (Nola et al. 2001; Nougang et al. 2011). The opportunist bacterium *P. aeruginosa* isolated from wells of Douala often expressed acquired resistances against several antibiotics such as doxycycline, cotrimoxazole, tetracycline and ampicillin (Akoachere et al. 2013). Otherwise, groundwaters of Douala and Yaoundé are acidic with low dissolved oxygen and nutrients contents (Nola et al. 2001; Nougang et al. 2011). These conditions are potentially stressful for the local bacterial microflora. The impact of these environmental conditions on the occurrence of resistant *P. aeruginosa* strains has not been yet undertaken. Although the reasons and the factors of the resistances remain poorly documented in natural water, it is well known that increasing resistance in aquatic environments in urban areas is mainly assigned to anthropogenic pollution. The implication of some anthropogenic ecosystems (hospital effluents, wastewater treatment stations, farms) in the dissemination of the resistance has been demonstrated through several works which characterized these ecosystems as reservoirs of multi-drug resistant bacteria, resistance genes and mobile genetic elements, but also as the platforms of genetic exchanges between bacterial species (Czekalski et al. 2012; Fuentefria et al. 2011; Gaze et al. 2011). However, little is known about the possible impact of physicochemical characteristics of water on the occurrence of multi-drug-resistant *P. aeruginosa* strains in these environments. The aim of this study was to have firsthand information about the possible relationship between water pollution and the antimicrobial susceptibility of *P. aeruginosa* strains in two urban areas.

Materials and methods

Site description

Douala town is located between 3° 5′ and 4° 15′ at latitude North and between 9° 37′ and 9° 50′ at longitude East, along the coastal plain of Cameroon. The relief is made of sedimentary formations (clays, marls, sands, sandstone, etc.) and an important proportion of hydromorphic soils (Hieng 2003). The population of this city is more of 2,000,000 inhabitants (BUCREP 2010). Several investigations showed that 2/3 of the population do not have access to the network water and rely on boring, wells and natural springs. The water quality of 3/4 of borings and wells is uncertain because of the proximity of the groundwater table and the sandy geologic plinth which favour the circulation of contaminants (Drouart and Vouillamoz 2012).

Yaoundé City is located between latitude 3° 44′ and 4° 2′ North and longitude 11° 22′ and 11° 24′ East. It has ferro-lateritic acidic soils with clayed texture (Hieng 2003). The demography of Yaoundé is of about 2,000,000 inhabitants (BUCREP 2010). Groundwaters play a fundamental role in the water supply in Yaoundé, where less than 50% of households have direct access to the drinking water delivered through pipeline (INS 2013). Studies established that groundwater pollution comes mainly from traditional pit latrines and agricultural activities. 53% of households are exposed to waterborne diseases by consuming groundwaters (INS 2013).

Water sampling

In each study area, 14 dug wells, coded WY1, WY2, ……,WY14 in Yaoundé, and WD1, WD2, ……WD14 in Douala, were selected according to their accessibility and their spatial distribution. Water samplings were done from April 2013 to April 2014 in order to explore environmental conditions leading in groundwaters during all seasons of the year. Water samples from the 14 wells of each town were collected and analysed monthly. At each sampling point, two water samples were collected, one in a 500-ml sterile glass bottle for bacteriological analysis and the other in a 1000-ml polythene bottle for organic matter analysis.

Analyses of water physicochemical parameters

At each sampling site, the water pH, dissolved oxygen and electrical conductivity were determined in situ (using a multi-parameter analyser kit HANNA Instruments) to estimate their impact on antimicrobial susceptibility. Organic matter (chemical oxygen demand) was evaluated in laboratory by the chromic acid wet digestion titrimetric method. These parameters were chosen since they are indicators of the organic pollution. Physicochemical analyses were made according to Rodier (2009) and APHA (2012).

Bacterial isolation and identification

Membrane filtration method was used to isolate and enumerate *P. aeruginosa* cells according to the standard methods (APHA 2012). For each well, raw or diluted water sample was filtered through a sterile 0.45-µm-pore-diameter, gridded membrane filter, under partial vacuum. Filter was removed and placed on cetrimide–nalidixic agar culture medium (CN, Difco Laboratories, Detroit, MI, USA) contained in Petri dish (Gosselin a Corning Brand, France). Incubation was done at 37 °C for 24 h. The typical *P. aeruginosa* colonies on CN agar medium were subsequently identified according to Holt et al. (2000) and confirmed using King B medium, acetamide and oxydase tests (Goto and Enomoto 1970).

Antimicrobial susceptibility tests

The antimicrobial susceptibility tests were carried out using Kirby–Bauer disc diffusion method as indicated by CA-SFM (2013). Three hundred and eleven and 312 *P. aeruginosa* strains were, respectively, collected in wells of Douala and Yaoundé. Fifteen antibiotics were tested belonging to 4 groups (CA-SFM 2013): β-lactam, aminoglycoside, quinolone and one non-classified group. The β-lactam group included piperacillin (75 µg), piperacillin/tazobactam (75/10 µg), ticarcillin (75 µg), ticarcillin/clavulanate (75/10 µg), ceftazidime (30 µg), cefepime (30 µg), cefsulodin (30 µg), imipenem (10 µg) and meropenem (10 µg). The aminoglycoside included tobramycin (10 µg), gentamicin (15 µg) and amikacin (30 µg). The quinolones used were ciprofloxacin (5 µg) and ofloxacin (5 µg). The non-classified antibiotic was fosfomycin (50 µg + 50 µg G6P).

Pseudomonas aeruginosa strain ATCC 27853 susceptibility was used as control. This strain was provided by the Laboratory of Microbiology and Environment of Centre Pasteur of Cameroon. Test results were only validated in the cases where inhibition zone diameters of the control strains were within performance ranges. Inhibition zone diameters of *P. aeruginosa* isolates were measured and then compared with the reference data (CA-SFM) to assess if strains were resistant (**R**), intermediate (**I**) or sensitive (**S**) to the tested antibiotics.

Data analysis

Prevalence of resistant (**R**), intermediate (**I**) or sensitive (**S**) strains to each antibiotic was plotted using SigmaPlot 10.0 software. To assess a dimensional measurement of the linear relationship between the inhibition diameter values of each antibiotic and physicochemical parameter of well waters, multiple regressions were done using the software XLSTAT 2007. The regression equation model defined was: $Y = a_0 + a_1\text{EC} + a_2\text{pH} + a_3\text{DO} + a_4\text{OM}$. Y (inhibition diameter value of antibiotic) was assigned to the depended variable. The explanatory variables were EC (electrical conductivity), pH, DO (dissolved oxygen) and OM (organic matter). The slopes parameters a_1, a_2, a_3 and a_4 showed how Y changes with one unit of corresponding physicochemical parameter. a_0 represented the intercept point. R^2 values were determined to measure the percentage of variation in the values of inhibition diameter of antibiotics that can be explained by the variation in the explanatory variables. A p value of 0.05 was assumed to be statistical significance level.

Results

Wells physicochemical characteristics

In Douala metropolis, the mean values of physicochemical parameters measured in the wells varied with respect to the sampling site (Table 1). The electrical conductivity values ranged from 145.38 to 559.69 µS/cm, meaning that mineralization rate of these waters was average (Rodier 2009). The pH values fluctuated between 5.56 and 6.66 and revealed acidic groundwaters. Dissolved oxygen concentration ranged from 2.5 to 5.5 mg/l, and concentrations in organic matter varied from 2.3 to 7.2 mg/l.

In Yaoundé, the value of physicochemical parameters measured in wells also varied with respect to sampling site (Table 2). The highest value of electrical conductivity reached 710.2 µS/cm, and the lowest value reached 172.5 µS/cm. The observed difference in electrical conductivity may be due to the local source of pollution. Well waters were slightly acidic with pH ranging from 5.1 to 7.0. Dissolved oxygen levels ranged from 3.9 to 5.1 mg/l, and the concentration in organic matter varied from 1.4 to 5.2 mg/l.

Table 1 Means (and standard deviations) of physicochemical parameters in wells of Douala

Parameters considered	Sampled wells													
	WD1	WD2	WD3	WD4	WD5	WD6	WD7	WD8	WD9	WD10	WD11	WD12	WD13	WD14
Electrical conductivity (µs. cm^{-1})	184.2 (46)	145.4 (34.8)	238.7 (82.8)	410.2 (82.6)	363.5 (76.7)	401.7 (91.8)	312.5 (85.5)	273.3 (67.4)	390.2 (70.6)	279.2 (55.7)	559.7 (153.5)	493.7 (110.3)	526.1 (195.6)	357.4 (142.9)
pH (CU)	6.1 (0.4)	5.9 (0.4)	6.4 (0.4)	6.4 (0.4)	6.4 (0.4)	6.7 (0.5)	6.3 (0.4)	6.2 (0.6)	6.2 (0.5)	5.6 (0.9)	5.9 (0.6)	6.3 (0.5)	6.6 (0.3)	6.7 (0.4)
Dissolved oxygen ($mg.l^{-1}$)	5.3 (0.5)	5.3 (0.5)	5.5 (0.8)	4.2 (1.2)	4.3 (0.7)	4.4 (1.4)	4.9 (1.0)	3.7 (1.6)	3.5 (0.9)	3.9 (1.2)	4.8 (0.9)	3.4 (1.0)	4.6 (1.3)	2.5 (1.0)
Organic matter ($mg.l^{-1}$)	4.4 (2.2)	4.0 (1.0)	2.3 (1.1)	4.6 (3.7)	3.9 (2.3)	3.3 (3.4)	3.8 (1.1)	4.5 (2.8)	2.9 (1.4)	2.6 (1.4)	4.9 (2.8)	3.6 (2.0)	3.3 (2.6)	7.2 (4.8)

Table 2 Means (and standard deviations) of physicochemical parameters in wells of Yaoundé

Considered parameters	Sampling sites													
	WY1	WY2	WY3	WY4	WY5	WY6	WY7	WY8	WY9	WY10	WY11	WY12	WY13	WY14
Electrical conductivity (µs. cm^{-1})	204.8 (36.7)	434.0 (89.6)	182.2 (32.8)	221.5 (46.3)	228.8 (149.8)	710.2 (139.4)	923.5 (280.2)	172.5 (39.9)	337.0 (56.6)	220.3 (39.0)	242.5 (39.5)	371.9 (71.7)	268.5 (54.6)	620.2 (115.1)
pH (CU)	6.1 (0.3)	7.0 (0.2)	5.7 (0.8)	5.2 (0.9)	5.1 (0.7)	6.0 (0.4)	6.2 (0.5)	6.3 (0.3)	6.0 (0.5)	5.7 (0.2)	5.2 (0.7)	5.4 (0.7)	5.5 (0.7)	6.1 (0.4)
Dissolved oxygen ($mg.l^{-1}$)	5.1 (1.1)	4.5 (1.5)	4.4 (0.7)	5.0 (0.7)	4.7 (0.8)	5.0 (1.2)	4.5 (0.9)	4.4 (1.7)	4.1 (1.0)	4.5 (0.9)	4.2 (1.1)	4.5 (0.9)	4.6 (1.3)	3.9 (1.5)
Organic matter ($mg.l^{-1}$)	2.5 (1.2)	4.0 (2.3)	2.4 (1.7)	1.9 (1.4)	2.2 (0.9)	1.4 (0.8)	5.2 (3.5)	2.8 (0.9)	1.4 (1.3)	1.6 (1.1)	1.8 (1.2)	2.0 (0.8)	1.9 (2.4)	1.7 (0.8)

Antimicrobial susceptibility patterns among *P. aeruginosa* strains and multiresistance aspect

A total of 623 *P. aeruginosa* strains were collected and tested. Out of this number, 311 and 312 strains were collected, respectively, from wells in Douala and Yaoundé. The prevalence of the resistant, intermediate and sensitive strains isolated is illustrated in Fig. 1.

In groundwaters of Yaoundé, *P. aeruginosa* strains expressed highest resistance to fosfomycin (90.09%) and ofloxacin (30%) and lowest resistance to tobramycin (0.6%) (Fig. 1a). In well waters of Douala, *P. aeruginosa* strains expressed highest resistance rates to fosfomycin (77.88%) and lowest resistance to cefsulodin (2.88%) (Fig. 1b). The prevalence of resistant strains to ceftazidime, ticarcillin, gentamicin, tobramycin and amikacin was relatively different in wells of Douala when compared to those of Yaoundé (Fig. 1). Resistant strains to cefsulodin were recorded in wells of Douala (2.88%), while in Yaoundé there were no resistant strains recorded to this drug.

In wells of both towns, the most active antibiotics against *P. aeruginosa* strains were ciprofloxacin, colistin, meropenem, imipenem and piperacillin/tazobactam (Fig. 1). The resistance level to combined antibiotics piperacillin/tazobactam and ticarcillin/clavulanate was different in Yaoundé and Douala (Fig. 1). About 17.16% of strains in Yaoundé town and 6.41% of strains in Douala town were sensitive to piperacillin/tazobactam but resistant to piperacillin alone. This is not surprising since tazobactam acts as a β-lactamase inhibitor. About 10.89% of *P. aeruginosa* strains from wells of Yaoundé and 9.29% from those in Douala were resistant to antibiotic combination ticarcillin/clavulanate but sensitive to ticarcillin alone.

Figure 2 shows the prevalence of multi-drug-resistant strains. In wells of Yaoundé, about 93.39% of *P. aeruginosa* strains were resistant to at least 3 antibiotics. The resistance prevalence to at least 4 antibiotics reached 52.14%. Up to 20.46% of *P. aeruginosa* strains were resistant to at least 5 antibiotics. It has been found that 0.66% of *P. aeruginosa* strains were resistant to at least 9 antibiotics. In wells of Douala, 85.26% of *P. aeruginosa* strains expressed resistance to at least 3 drugs (Fig. 2). The prevalence of resistant strains to at least 4 antimicrobials reached 68.58%. Up to 52.24% of strains were resistant to at least 5 antibiotics. It

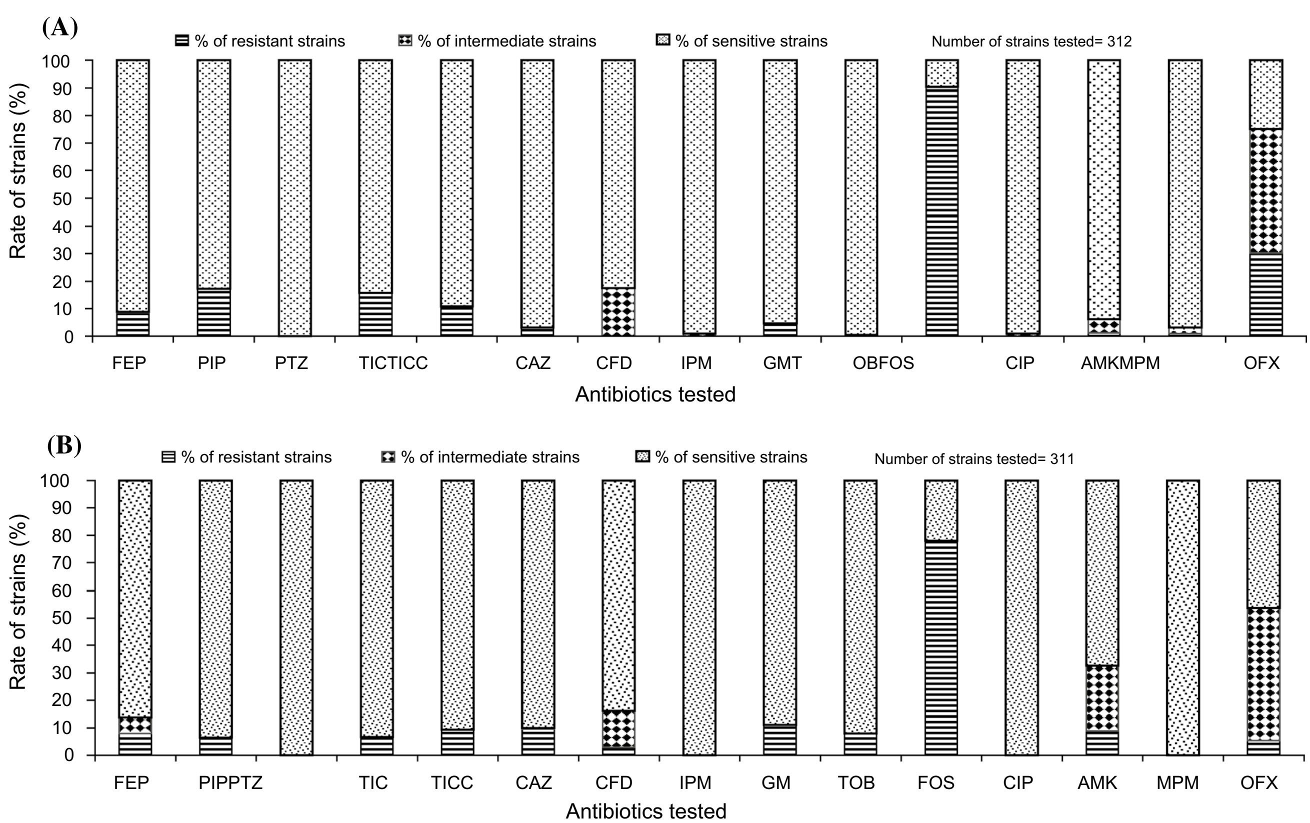

Fig. 1 Antimicrobial susceptibility patterns among *P. aeruginosa* strains isolated from wells in Yaoundé of clayey lateritic soil (**a**), and in Douala of sandy soil (**b**) (*FEP* cefepime, *PIP* piperacillin, *PTZ* piperacillin/tazobactam, *CTX* cefotaxime, *TIC* ticarcillin, *TICC* ticarcillin/clavulanate, *CAZ* ceftazidime, *CFD* cefsulodin, *IMP* imipenem, *GM* gentamicin, *TOB* tobramycin, *AZT* aztreonam, *FOS* fosfomycin, *COL* colistin, *CIP* ciprofloxacin, *AMK* amikacin, *KMC* kanamycin, *MPM* meropenem, *OFX* ofloxacin)

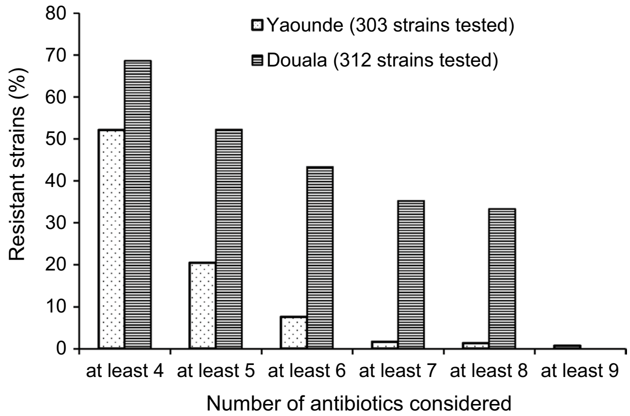

Fig. 2 Variation of the percentage of multi-drug-resistant *P. aeruginosa* isolates with the number of antibiotics they resist to and the isolation sites (Yaoundé and Douala)

is noted that up to 33.33% of strains expressed multi-drug resistance to at least 8 antibiotics.

Impact of physicochemical parameters of water on the antibiotics sensitivity of *Pseudomonas aeruginosa*

The effect of physicochemical water parameters on the inhibition diameter of *P. aeruginosa* was assessed using the multiple regression test, and the results are presented in Tables 3 and 4.

With strains isolated from wells in Yaoundé, the percentage of variation in the values of inhibition diameters of antibiotics that can be explained by the variation in the physicochemical parameters of wells oscillated between 0.7 and 4.1% ($p \leq 0.05$). The lowest R^2 values were observed with ofloxacin, meropenem, amikacin, imipenem and ceftazidime. The regression equation which showed the highest R^2 values was obtained with ticarcillin (Table 3). When examining specific slopes assigned to each physicochemical parameter, the pH of well waters seems to be the abiotic factor having the steepest slopes ranging from 0.4 to 1.8 (Table 3). These slopes showed how inhibition diameter of antibiotics changes with pH. The slopes of electrical conductivity varied between 0.00005 and 0.002, while those of dissolved oxygen oscillated between 0.006 and 0.4. The slopes assigned to the organic matter ranged from 0.01 to 0.3 ($p \leq 0.05$).

With *P. aeruginosa* strains isolated from well in Douala town, 2.5% to 4.8% of the variance in inhibition diameter values of antibiotics can be explained by the changes in physicochemical parameters (Table 4). The lowest R^2 value was observed with ofloxacin and fosfomycin, while the highest R^2 value was obtained with ceftazidime. The slopes attributed to each abiotic factor ranged from 0.1 to 4.8 with the pH, from 0.002 to 0.005 with the electrical conductivity, from 0.3 to 0.5 with the dissolved oxygen and from 0.003 to 0.2 with the organic matter.

In both towns, less than 5% of the changes in physicochemical parameters of well waters explained the variance in inhibition diameter values of antibiotics ($p \leq 0.05$). The

Table 3 Multiple regression of inhibition diameter values of antibiotics with abiotic factors in wells of Yaoundé

Depended variables (inhibition diameters of antibiotic tested)	Regression equation	R^2 value
FEP =	27 − 0.002EC − 1.4pH − 0.2DO − 0.1OM	0.014
PIP =	27 − 0.001EC − 1.4pH − 0.2DO − 0.3OM	0.014
PTZ =	25 − 0.003EC − 0.7pH − 0.004DO − 0.04OM	0.011
TIC =	21 − 0.0008EC − 1.1pH − 0.006DO − 0.2OM	0.041
TICC =	21 − 0.00005EC − 1.1pH + 0.2DO − 0.08OM	0.009
CAZ =	27 + 0.0004EC − 1.5pH − 0.1DO − 0.04OM	0.007
CFD =	26 + 0.0008EC − 1.6pH + 0.01DO − 0.02OM	0.010
IMP =	27 − 0.0001EC − 1.3pH − 0.4DO + 0.04OM	0.007
GM =	22 − 0.0003EC − 1.3pH − 0.05DO + 0.06OM	0.010
TOB =	20 − 0.0001EC − 1.2pH + 0.01DO + 0.04OM	0.008
FOS =	7.6 − 0.0007EC − 0.8pH + 0.07DO − 0.1OM	0.009
CIP =	33 − 0.001EC − 1.8pH − 0.1DO − 0.07OM	0.009
AMK =	22 + 0.0002EC − 1.2pH + 0.02DO − 0.01OM	0.007
MPM =	31 − 0.001EC − 1.4pH − 0.3DO − 0.04OM	0.007
OFX =	23 − 0.001EC − 1.07pH − 0.1DO + 0.05OM	0.007

Observations = 182; $p \leq 0.05$. *FEP* cefepime, *PIP* piperacillin, *TIC* ticarcillin, *TICC* ticarcillin/clavulanate, *CAZ* ceftazidime, *CFD* cefsulodin, *IMP* imipenem, *GM* gentamicin, *TOB* tobramycin, *FOS* fosfomycin, *CIP* ciprofloxacin, *AMK* amikacin, *MPM* meropenem, *OFX* ofloxacin, *EC* electrical conductivity, *DO* dissolved oxygen, *OM* organic matter

Table 4 Multiple regression of inhibition diameter values of antibiotics with abiotic factors in wells of Douala

Depended variables (inhibition diameters of antibiotic tested)	Regression equation	R^2 value
FEP =	40 + 0.005EC − 3.7pH + 0.4DO − 0.1OM	0.043
PIP =	41 + 0.003EC − 3.6pH + 0.4DO + 0.003OM	0.031
PTZ =	38 + 0.004EC − 3.2pH + 0.3DO + 0.03OM	0.032
TIC =	36 + 0.003EC − 3.4pH + 0.4DO − 0.005OM	0.041
TICC =	31 + 0.003EC − 2.8pH + 0.3DO − 0.02OM	0.036
CAZ =	43 + 0.005EC − 4.1pH + 0.3DO − 0.2OM	0.048
CFD =	39 + 0.005EC − 3.5pH + 0.3DO − 0.07OM	0.035
IMP =	41 + 0.003EC − 3.7pH + 0.5DO − 0.1OM	0.038
GM =	26 + 0.002EC − 2.4pH + 0.4DO + 0.01OM	0.034
TOB =	27 + 0.002EC − 2.4pH + 0.3DO + 0.008OM	0.031
FOS =	4.8 + 0.003EC − 0.1pH + 0.6DO − 0.06OM	0.025
CIP =	53 + 0.004EC − 4.8pH + 0.5DO − 0.03OM	0.037
AMK =	25 + 0.003EC − 2.3pH + 0.6DO + 0.05OM	0.037
MPM =	54 + 0.003EC − 4.7pH + 0.3DO − 0.07OM	0.034
OFX =	33 + 0.003EC − 2.8pH + 0.3DO − 0.07OM	0.025

Observations = 182*;* $p \leq 0.05$. *FEP* cefepime, *PIP* piperacillin, *TIC* ticarcillin, *TICC* ticarcillin/clavulanate, *CAZ* ceftazidime, *CFD* cefsulodin, *IMP* imipenem, *GM* gentamicin, *TOB* tobramycin, *FOS* fosfomycin, *CIP* ciprofloxacin, *AMK* amikacin, *MPM* meropenem, *OFX* ofloxacin, *EC* electrical conductivity, *DO* dissolved oxygen, *OM* organic matter

pH of water seems to be the parameter susceptible to impact the variation in the inhibition diameters values regarding the slopes of regression equations. The slopes values of pH were relatively more important with strain isolated in Douala than to those isolated in Yaoundé.

Discussion

Pseudomonas aeruginosa strains isolated from groundwaters of Yaoundé and Douala (tropical regions) were multi-drug resistant. The observed acquired resistance against ticarcillin and ceftazidime is alarming since these antibiotics are recognized to kill *P. aeruginosa* cells and they are preferentially used in hospital against *P. aeruginosa* infections (Kamga et al. 2015). The occurrence of multi-drug-resistant strains in these aquatic ecosystems has been long time questioned. During this study, the measurement of the physicochemical parameters of groundwaters showed that the concentrations of dissolved oxygen corresponded to the normal oxygenated conditions non-stressful for *P. aeruginosa*. The multiple regression tests revealed that the percentage of variation in the values of inhibition diameters of antibiotics tested can be explained by the variation in the physicochemical parameters ranging from 0.7 to 4.1% ($p \leq 0.05$) in Yaoundé and from 2.5 to 4.8% in Douala. These results mean that the relationship between antimicrobial susceptibility of *P. aeruginosa* and abiotic factors would be minimal in both regions. The reasons of this weak relation would be explained by the order of magnitude of the measured physicochemical parameters in groundwaters. Indeed, it has been established that stress is the major environmental parameter that significantly affects *P. aeruginosa*.

Stress selects in bacterium several molecular adaptation mechanisms also involved in antibiotic resistance (Macfarlane et al. 2000; Johnson et al. 2012). However, when the level of physicochemical parameter is not sufficient to induce bacterial stress, its impact on antimicrobial susceptibility would be minimal. According to Ghotaslou and Behnaz (2013), oxygen depletion of 0 to 1% (< 3 mg/l) could lead to stress in *P. aeruginosa* affecting its antibiotics sensitivity. These authors revealed that changes in oxygen pressure contributed to increased antibiotic resistance in hypoxia (1%) with penicillin, tobramycin and carbapenem drugs, while the inhibition diameter values in normoxia 21% (5–7 mg/l) remained unchanged. During the sampling campaign, the concentrations of dissolved organic matters obtained were different to those of oligotrophic groundwaters (< 1 mg/l) where organic nutrient limitation leads to nutrient stress against bacteria. According to Nguyen et al. (2011), organic nutrient limitation impacts antimicrobial resistance as a result of its activation of stress responses that promote resistance to gentamicin, meropenem and ofloxacin by activating the stringent response. Groundwaters of these two towns seem to be sufficiently charged in dissolved organic matter, and this environmental condition significantly reduces the impact of nutrient stress against *P. aeruginosa*.

Moreover, intrinsic factors linked to *P. aeruginosa* species might explain weak relation between abiotic factors of well waters and antimicrobial susceptibility. Sequencing of several *P. aeruginosa* strains genomes revealed that a large fraction (around 10%) of the genome is dedicated to gene regulation, which is consistent with its high versatility (Mathee et al. 2008; Stover et al. 2000). It has a disparate variety of metabolism; it can degrade very distinct compounds such as alcohols, fatty acids, sugars, di- and tri-carboxylic acids, aromatics, amines and amino acids, which can be used up as sources of carbon. *P. aeruginosa* has both aerobic and anaerobic metabolism. It is capable of anaerobic metabolism by converting nitrate to nitrite. This highest versatility might significantly contribute to escape *P. aeruginosa* from the stress provided by non-optimal environmental conditions (Schreiber et al. 2007).

The analysis of slopes parameters permitted to appreciate the impact of individual abiotic factors on antimicrobial susceptibility. In both towns, significant slopes ($p \leq 0.05$) were obtained only with pH. The values of pH ranged from 5.56 to 6.66 in groundwaters of Douala and from 5.1 to 7.0 in groundwaters of Yaoundé. These values were susceptible to lead to acidic stress and to affect antimicrobial susceptibility of *P. aeruginosa*. Several studies showed that the membrane transport of antibiotics such as aminoglycosides is a function of the difference transmembrane in H^{+} ion, which explains the impact that pH might have on resistance to aminoglycosides (Fraimow et al. 1991; Eisenberg et al. 1984). According to Moriarty et al. (2007), bactericidal effect of tobramycin against *P. aeruginosa* decreases when the bacterium grows in acidic condition. In addition, the sensitivity to ceftazidime is not influenced by pH. The influence of pH on acquired resistance to aminoglycoside drugs such as amikacin and neticillin has been examined by Xiong et al. (1996). These authors showed that bactericidal effect of aminoglycoside drugs depended on their concentrations at pH 7.4. At pH 6.5, the bactericidal activity of amikacin and neticillin was very weak when compared to the one observed at pH 7.4. At pH 5.5, amikacin and neticillin do not have bactericidal effect on *P. aeruginosa*.

Conclusion

Groundwaters of Douala and Yaoundé towns harbour multi-drug-resistant *P. aeruginosa* strains. The relationship between antimicrobial susceptibility and organic pollution remained weak in these regions. Only the pH was found to have an impact on the variation of inhibition diameter values of antibiotics. The values of physicochemical parameters showed that environmental conditions leading in groundwaters of Douala and Yaoundé are less stressful for *P. aeruginosa*. This might explain the weak relationship between abiotic factors and antimicrobial susceptibility.

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