Antimicrobial Multi-Resistance of *Acinetobacter baumannii* Isolated from Clinical Specimens in Douala (Cameroon)

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Abstract: Bacteria of the genus *Acinetobacter* are non-fermentative gram-negative bacilli, often considered as ubiquitous and frequently found in the environment. This work aimed to study the susceptibility of *Acinetobacter baumannii* isolated at the General Hospital of Douala. This bacterium, which is greatly involved in human pathology, demonstrated high innate and acquired resistance to multiple antibiotics classes. This was a descriptive and retrospective study over a period of ten years (2005-2014), covering all the isolates of *Acinetobacter baumannii* from in and out patients. Samples were analyzed in the bacteriology laboratory at the General Hospital of Douala. Data collection was made by extraction of the results of all susceptibility of *Acinetobacter baumannii*, carried out from 2005 to 2014, registered in the memory of the mini API™ system; this automated machine identifies and measures the susceptibility of all the bacterial strains. A total of 266 strains were isolated from suppurations, urine, urinary catheters, and all other specimens received by the laboratory during the study period. These strains obtained from samples collected from all services of the hospital and ambulatory patients. The resistance rates noted were: 67.29 % for ticarcillin, 64.28 % ticarcillin + clavulanic acid, 68.33 % for piperacillin, 56.97 piperacillin + tazobactam, 58.27 % ceftazidime, 58.01 % cefepime, 21.05 % imipenem, 33.46 % colistin, 52.63 % gentamicin, 48.49 % tobramycin, 24.43 % amikacin, 53.40 % netilmicin, 50% for ciprofloxacin and levofloxacin, 51.05 % ofloxacin. The prevalence of multi-resistant strains was 43.18 %, mostly seen in patients hospitalized in the Burns unit. Only imipenem and Amikacin showed good activity on these strains with 78.95 % and 75.57 % of susceptibility respectively. This study showed the low activity of penicillin even in combination with beta lactamases inhibitors, as well as the cephalosporins on strains of *Acinetobacter baumannii*. Therapeutic support of infections due to this pathogen is highly problematic in General hospital of Douala. However, imipenem and amikacin, and to a lesser extent colistin remains effective treatment choices.

Keywords: *Acinetobacter baumannii*, Multi-Drug Resistance, Antibiotics, Cameroon

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1. Introduction

Bacteria of the genus *Acinetobacter* are non-fermentative Gram-negative bacilli, often considered as ubiquitous and frequently found in the environment [1, 2]. These microorganisms, which are also part of the normal resident of healthy skin flora, have long been considered as contaminants of bacteriological samples, not responsible of infections [3].

Later, the *Acinetobacter* have proved their capacity to

easily spread and become pathogenic with high epidemic risk particularly in hospitals, especially among fragile patients hospitalized in intensive care units [3,4,5]. The species *A. baumannii* is particularly involved, because it has the ability to survive on dry surfaces for prolonged periods of time whereby it can be easily spread [6]. *A. baumannii* is also capable to acclimatise to stress conditions, action of detergents, and antiseptics, and to develop multiple resistance mechanisms against the majority of antibiotics. This multi-drug resistance explains the therapeutic difficulties when

support for serious infections due to this pathogen, particularly in immunocompromised patients, those with serious underlying diseases, and those subjected to invasive procedures and treated with broad-spectrum antibiotics [7,8,9].

In recent years, the rate of resistance growing of this pathogen in a wide range of antibiotics cause of serious therapeutic and clinical problems, leading to increased morbidity and mortality [10,11,12]. Microbiological surveillance is important for obtaining useful information for doctors in the empirical choice of antibiotics. The aim of this study was to determine the epidemiology and the susceptibility to antibiotics of the *Acinetobacter*, in order to limit the cost of support, and set up a suitable antibiotic therapy and appropriate preventive measures.

2. Material and Methods

This was a transversal descriptive study, carried out in the clinical biology laboratory of the General Hospital of Douala (Cameroon), and concerning all strains of *Acinetobacter* isolated from 2005 to 2014 (10 years). This multidisciplinary laboratory receives samples from outpatients and inpatients in various hospital services.

Sampling: all the isolates of *A. baumannii* obtained from diagnostic referred specimens (urine, genital samples, suppurations, blood, probes, catheter and puncture fluids) from ambulatory and in-patient received during the study period were included.

The strains considered as contaminants, as well as strains with incomplete susceptibility data were excluded.

For identification of *Acinetobacter baumannii* and the susceptibility tests to antibiotics, the urine samples were cultured on Cystine Lactose Electrolyte Deficient (CLED) agar, and the other specimens on Eosin Methylene Blue (EMB) agar; and incubated for 24 hours at 37°C. The macroscopic colony appearance were observed (shape, size, color) and biochemical and enzymatic identification were performed using the ID32GN® galleries, followed by automatic colorimetric reading on mini API™ (bio Mérieux SA, Marcy l'Étoile, France) after a period of 24 hours incubation at 37°C. The susceptibility tests were performed using the dilution technique on ATBPSE® galleries, and followed by automatic turbidi-nephelometric reading after 18 to 24 hours incubation. The results were expertise by the mini API™ system according to the breakpoints of the Committee of the Antibiogram of the French Society of Microbiology (CASFM) [13]. The following antibiotics molecules were studied in the ATB PSE® Gallery: Ticarcillin (TIC); Ticarcillin + clavulanic acid (TTC); Piperacillin (PIP); Piperacillin + tazobactam (TZP); Ceftazidime (CAZ); Cefepime (FEP); Imipenem (IMI); Meropenem (MERO); Gentamicin (GEN); Tobramycin (TOB); Amikacin (AKN); Netilmicin (NET); Ofloxacin (OFL); Ciprofloxacin (CIP); Levofloxacin (LVX); Colistin (COL); Fosfomycin (FOS); Sulfamethoxazole + trimethoprim (TSU); Rifampicin (RFA). All antibiotics were not been tested with the same frequency.

2.1. Multidrug Resistant (MDR) Bacteria

Bacterial strain was defined as multiresistant if it shows non-susceptibility to at least one agent in at least 3 antimicrobial categories of specific antibiotics active on this species [14]. Only major active antibiotics on *Acinetobacter baumannii* were considered in the profiling of multiresistant germs (ceftazidime, cefepime, imipenem, gentamicin, tobramycin, amikacin, netilmicin, ofloxacin, ciprofloxacin, levofloxacin, colistin).

Data collection consisted of the extraction of all the susceptibility results of *Acinetobacter baumannii*, carried out from 2005 to 2014 registered in the memory of the automatic miniAPI™ system (Excel format). These results included, the identification number, the date of isolation, the identified germ, the nature of the specimen, as well as antibiotics tested with their susceptibility profile ("S" for sensitive, "I" for intermediate, "R" for resistant). These data were supplemented by the service of origin of the specimen through the registers of the laboratory.

2.2. Data Processing and Statistical Analysis

In the calculation of the percentages of resistance, the "intermediary" results were included in the category "resistant". The descriptive analysis of the data was made using the software Epi info version 11 and Microsoft Excel 2010.

2.3. Ethical Considerations

We obtained the authorisation of the Ethics Committee of the General Hospital of Douala.

Limits: the specific tests for detection of beta lactamases expanded spectrum (ESBL), ampC cephalosporinase, metallobetalactamase (MBL) production was not performed. Prior antibiotic taken by patient was unknown.

3. Results

3.1. Strains Isolated

During the years 2005 to 2014, a total of 6978 non-recurring pathogenic strains have been isolated from all diagnostic referred specimens received by the laboratory of the General hospital. Among these strains, 1934 were non-fermentative Gram-negative (NFGN) bacilli and 266 were of *Acinetobacter baumannii*, representing 3.8 % of all the isolates and 27.4 % of the NFGN. Strains isolated were mostly suppurations (37.22 %), urine (28.58%) and urinary catheters (19.54%). A total of 77 strains were isolated from outpatients (28.94 %) followed by the patients hospitalized in the Department of surgery (20.30 %).

3.2. Susceptibility to Antibiotics

Resistance to the active betalactamines on *A. baumannii* strains was high for all of the molecules used, and always greater than 60 %, even when these molecules were used

singly or in combination with beta lactamases inhibitors (Table 1). The most active aminoglycoside was amikacin (Table 1). The best therapeutic choices were amikacin and imipenem (Table 1). The average prevalence of the multi resistance in *Acinetobacter baumannii* strains was 43.18 %

(Table 2). These strains resistant to at least one representative of 3 classes of antibiotics were 49.01 % intensive care unit, 44.68 % internal medicine and 62.96 % surgery (Table 3). Multi-resistant strains isolated from urinary catheter and suppurations were 63.46% and 51.51% respectively (Table 2).

Table 1. Antimicrobial resistance of *A baumannii*.

Antimicrobial category	Antimicrobial agent	N° isolates (n/N)	Rate of resistance
Penicillin + β lactamase inhibitors	TIC	179/266	67.29
	TCC	171/266	64.28
	PIP	177/259	68.33
	TZP	147/258	56.97
Extended spectrum cephalosporins	CAZ	155/266	58.27
	FEP	152/262	58.01
Carbapenems	IMI	56/266	21.05
	GEN	140/266	52.63
Aminoglycosides	TOB	129/266	48.49
	AKN	65/266	24.43
	NET	102/191	53.40
	OFL	97/190	51.05
Fluoroquinolones	CIP	133/266	50.00
	LEV	107/214	50.00
Polymyxins	COL	86/257	33.46
Rifamycines	RIF	124/252	49.20

N = total tested n = total resistant

TIC = Ticarcillin; TTC = Ticarcillin + clavulanic acid; PIP = Piperacillin; TZP = Piperacillin + tazobactam; CAZ = Ceftazidime; FEP = Cefepime; IMI = Imipenem; MERO = Meropenem; GEN = Gentamicin; TOB = Tobramycin; AKN = Amikacin; NET = Netilmicin; OFL = Ofloxacin; CIP = Ciprofloxacin; LVX = Levofloxacin; COL = Colistin; FOS = Fosfomycin; TSU = Sulfamethoxazole + trimethoprim; RFA = Rifampicin.

Table 2. Multiresistance in different specimens.

Specimen	Total isolates n (%)	MDR rate n (%)
Suppurations	99 (37.22)	51 (51.51)
Urine	76 (28.58)	21 (27.63)
Urinary catheter	52 (19.54)	33 (63.46)
Central venous catheter	24 (9.02)	11 (45.83)
Blood culture	9 (3.39)	2 (22.22)
Genital specimens	3 (1.12)	0
Probes	2 (0.75)	0
Pleural fluid	1 (0.38)	0
TOTAL	266 (100)	118 (43.18)

Table 3. Multiresistance in services.

Service	Total isolates n (%)	MDR rate n (%)
Surgery	54 (20.30)	34 (62.96)
Intensive care unit	51 (19.18)	25 (49.01)
Internal Medicine	47 (17.67)	21 (44.68)
Burns Unit	18 (6.77)	13 (72.22)
Paediatrics	9 (3.39)	2 (22.22)
Gynaecology	8 (3.00)	4 (50.00)
Neonatology	2 (0.75)	0 (0.00)
External	77 (28.94)	19 (24.67)
Total	266 (100)	118 (43.18)

4. Discussion

A. baumannii has a prevalence of 3.81 % of all the isolates in 10 years, less than *Pseudomonas aeruginosa*, the most isolated non-fermentative gram-negative bacilli in bacteriology laboratory of General Hospital of Douala. This is in agreement with data from the literature [15,16]. The prevalence of *A. baumannii* in our study is also less than that of Njall carried out at the Laquintinie hospital ICU, and

results of ICU in France, which were 12% and 14.4% respectively [17, 18]. Higher rates (19.1%) were found in another study in Benin [19].

The majority of these strains came from suppurations, urine and urinary catheters. Bharath Lexan in Madagascar isolated 88.6% of the strains of *Acinetobacter baumannii* in suppurations, urine and respiratory specimens [20]. In our sample, respiratory specimens were little represented, which can lead to an under-estimation of the prevalence, especially in the ICU in intubated patients.

Multidrug resistance was observed for 43.18 % of isolates, especially surgery, which was not the case elsewhere, where this phenomenon occurs especially in the intensive care unit [21, 22]. Vladimir Krcmery isolated 66% of multi-resistant strains of central venous catheters, which was close enough to our study [23].

This phenomenon was 97.5% in an intensive care in Italy, 100% in an intensive care unit in Pakistan, 56% in a study in Slovakia, 72% in a Department of surgery in the United States. These rates varying, but overall very high (greater than 50%) bear witness to the rapid spread of strains, and can justify the severity of infections with *Acinetobacter baumannii* [22, 23, 24, 25].

We observed high rates of multidrug resistant strains of *Acinetobacter baumannii* in the beta-lactams with a resistance rate still above 50 % (59.97 to 67.29 %). Gangoue's rate in Central hospital in Cameroon was already 62% for ceftazidime, the Morocco and Tunisia resistance by 50% and 60% respectively in blood cultures [26, 27, 28]. In Belgium, this rate of resistance varies from 35.1% to 46.5% in the regions of Flanders, Wallonia and Brussels [29].

Indeed these cephalosporins are the most commonly used antibiotics in the HGD.

Our study also shows higher activity of amikacin compared to other aminoglycosides, which is 24.43% of resistant strains. This can be explained by the fact that this molecule is little used in this hospital. Data from the literature postulate that this molecule loses over the years its effectiveness on the strains of *Acinetobacter baumannii* [15, 16, 30, 31].

Concerning fluoroquinolones, the rates of resistance of the strains of *Acinetobacter baumannii* (50 %) were high as in the other African studies [27, 28].

The low activity of cephalosporins and fluoroquinolones in our study only serves to confirm the general tendency of the behaviour of these molecules to face multi-resistant strains [20, 24, 32, 33].

Imipenem showed good activity in strains of *Acinetobacter baumannii*. But increasingly imipenem demonstrated a decrease in its activity especially in the intensive care units or as part of the related infections in healthcare [21, 30, 31, 34, 35]. Our rates are very high (21.05) compared with the study of Goly in Spain and an investigation in Belgium that were respectively 0% and 8.7% resistant strains [29, 36].

Some studies have also shown good activity of amikacin on multi-resistant strains of *Acinetobacter baumannii* [32].

The strains tested showed level of 33.46 % resistance to colistin. The good *in vitro* activity of this molecule is found elsewhere [32, 37, 38]. Colistin in particular is often the last resort in many African health institutions, European and American [20, 24, 32, 39].

Rates of susceptibility to all the molecules at the General hospital of Douala, is still far below the average. These multi-resistant strains are a genuine therapeutic problem. Indeed at the General Hospital in Douala, only amikacin and imipenem retain a good activity.

It is necessary to implement quickly the measures for preserving the effectiveness of these molecules that are expensive and which usually constitute the last therapeutic lines.

5. Conclusion

This study has shown the low activity of penicillin even in combination with beta lactamases inhibitors, as well as the cephalosporins on strains of *Acinetobacter baumannii*. Therapeutic support of pathogenic infections is very problematic in the General Hospital of Douala. Imipenem and amikacin, to a lesser extent colistin remains the effective treatment choices. High rates of antibiotic resistance should at least impose a bi-antibiotherapy for fighting against infections due to this pathogen, and an exclusion of the beta-lactams in the treatment when multi-resistant strain is suspected. This high strength affects both the community and nosocomial isolates without significant difference and is variable depending on the services and specimens.

Contribution of Authors

COE, ERM and MDT participated in the collection and analysis of data. JPNM and RN have analyzed and interpreted the data. NB coordinated the study. The manuscript was designed by COE and all authors have contributed to the revision and approved the final version.

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