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Frequencies and phenotypes of antibiotic resistance in non-fermentating bacteria isolated at the China-Guinea Friendship Hospital of Kipé in Conakry, Guinea

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Abstract

Introduction: Multidrug-resistant bacterial infections constitute a major global public health problem, particularly in developing countries.

Objective: To determine the diversity and antibiotic resistance phenotypes of non-fermentative Gram negative bacilli (NFGNB) isolated at the China-Guinea Friendship Hospital (HASIGUI).

Methodology: This is a nine-month prospective study, carried out in patients at the HASIGUI Biomedical Laboratory from May 1, 2019 to February 1, 2020. Bacterial identification and antibiogram were carried out using a machine. Vitek 2 Compact.

Results: A total of 86 NFGNB strains isolated from different biological fluids were analyzed. The average age of the patients was 45.08 years with the range from 1 year to 98 years. The female gender was dominant with 59.30%. The sex ratio (M/F) was 0.6. Fourteen NFGNB species belonging to seven bacterial genera were identified. Sphingomonas paucimobilis was the predominant species (33.72%), followed by Acinetobacter baumanni complex (22.10%), Pseudomonas aeruginosa and Pseudomonas fluorescens each representing 10.46%. Cases of multi-resistance were observed in all families of antibiotics tested. Resistance was common in some antibiotics: oxacillin (100%), ampicillin (62%), norfloxacin (66%), nalidixic acid (66%), cotrimoxazole (59.42%), cefoxitin (50%), and cefuroxime (50%). On the other hand, the most active antibiotics on BGNnf were: vignecycline (100%), imipenem (97.23%), meropenem (88.24%), minocycline (85%), piperacillin-tazobactam (83.87%), amikacin (93.87%), gentamicin (84%), tobramycin (81.43%), levofloxacin (64.28%), ofloxacin (61.70%), ciprofloxacin (60%).

Conclusion: Antibiotic resistance phenotypes of BGNnf indicate the need for antibiotic therapy based on antibiogram results.

Keywords: Non-fermentating bacteria, antibiotics, multidrug resistance, Conakry

Introduction

Bacterial resistance to antibiotics is a worrying public health problem around the world today. This situation is particularly worrying in hospitals because of the selection exerted by the abusive use of antibacterial molecules. The emergence of new forms of resistance concerns different species of bacteria, including non-fermenting Gram-negative bacilli (NFGNB), whose epidemic spread is a factor in the development of bacterial resistance to antibiotics ^[1]. Strict aerobic, non-fermenting Gram-negative bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation, found in humid environments or on plants. They are generally opportunistic bacteria involved in nosocomial infections ^[2]. They can also be involved in community infections. in community infections in immunocompromised subjects. Pseudomonas aeruginosa (P. aeruginosa) and Burkholderia cepacia (B. cepacia) are classic agents of colonization and secondary pulmonary infections in patients with cystic fibrosis. These two species are characterized by a natural resistance to many antibiotics, restricting the therapeutic possibilities to a limited number of antimicrobial agents [2-4].

These bacteria have a high natural level of resistance, in relation to different resistance mechanisms: secretion of enzymes, membrane impermeability, active efflux of antibiotics. They can acquire resistance ^[5] which can be the result of a chromosomal mutation or the

acquisition of resistance plasmids ^[6, 7]. In recent years, increasing rates of resistance to carbapenems have been reported which are used in the treatment of infections caused by species such as *A. baumannii* and *P. aeruginosa*.

In Europe, 22.8% of *P. aeruginosa* strains are resistant to fluoroquinolones against 19.0% to carbapenems. Resistance to aminoglycosides, to piperacillin associated with tazobactam or even to ceftazidime (C3G) each affects approximately 15% of *P. aeruginosa* strains.

In the USA, resistance to fluoroquinolones is compared to that observed in Europe, with a percentage of 30.7%, followed by that to carbapenems with a percentage of 25.3%. Resistance to aminoglycosides, to piperacillin associated with azobactam or even to ceftazidime (C3G) each affects approximately 17.5% of *P. aeruginosa* strains ^[8].

In Kenya, between 2006 and 2007, an 18-month study on strains of P. aeruginosa showed high resistance to antibiotics with 13.7% to imipenem (MIC > 32 mg/l), and 53% to piperacillin and aztreonam and 100% to aminoglycosides and fluoroquinolones ^[9].

In Morocco, in 2005 a study in a hospital in Rabat showed 23% resistance of P. aeruginosa stranis to imipenem. These strains were producing metallo- β -lactamases and were also resistant to fluoroquinolones, aminoglycosides and rifampicin^[10].

Samou in his work found a resistance of 7.9% among strains of *Acinetobacter baumannii* in all infections declared in one year in Mali ^[11].

In Guinea Makanera A. *et al.*, reported in 2017 that 56.25% of *Sphingomonas paucimobilis* (*S. paucimobilis*) strains were resistant to ampicillin ^[12]. In another study in 2019, these authors showed that 89.33% of *Pseudomonas* strains were resistant to ampicillin ^[10]. The aim of this work was to evaluate the prevalence and antibiotic resistance profile of non-fermenting bacilli associated with various human infections in Guinea.

2. Material and Methods

This is a prospective study carried out from May 01, 2019 to February 02, 2020 at the Biomedical Laboratory of the Sino-Guinean Friendship Hospital (HASIGUI) and involving samples of pathological products from the various departments of HASIGUI but also other health structures in the capital Conakry.

2.1. Fresh examination

After the samples were taken, all biological fluids: urine, stools, pus, vaginal secretions, cerebrospinal fluid (CSF) and semen, were subjected to macroscopic examination with the exception of blood (taking into account the appearance, color, abundance). The macroscopic examination was followed by the microscopic examination in the fresh state and after Gram staining.

2.2. Cultures

The different samples analyzed were cultured on different agar media: Blood Agar base (Liofilchem, Italy), Nutrient agar (Liofilcham, Italy),

Mac Conkey, bioMérieux, Marcy l'Etoile, France), TCBS agar (bioMérieux Marcy l'Etoile, France), CLED agar (bioMérieux, Marcy l'Etoile, France).

For blood samples, blood cultures were carried out using the Bact/Alert machine (bio Mérieux, France), using vials of

media for adults in aerobic (Bact/Alert FA) and anaerobic (Bact/Alert FN) environments and for children (Bact/Alert Pf). Subcultures were then carried out on agar media under aerobic and anaerobic conditions for all positive blood cultures.

2.3. Bacterial identification and antibiogram

Bacterial identification and antibiograms were carried out using the Vitek 2 Compact system (bioMérieux, Marcy, l'Etoile France) and the API 20 NE system (bioMérieux, Marcy, l'Etoile France) and ATB PSE (bioMérieux, Marcy, l'Etoile France). The minimum inhibitory concentrations (MIC) were determined using the Vitek 2 Compact automated system.

VITEK 2 Compact

The Vitek 2 GN cards were used for identification and the Vitek 2 AST-N 233 cards (biomérieux, Marcy l'Etoile, France) as well as those AST-XN05 (biomérieux, Marcy l'Etoile, France) were used for identification. antibiograms and determination of MICs, following the manufacturer's instructions (biomérieux, Marcy l'Etoile, France). The ready-to-use Vitek 2 GN cards contain 64 wells corresponding to 64 reactions allowing the identification of Gram-negative bacilli (Enterobacterales, Non-Enterobacterales and highly pathogenic germs). Ready-touse Vitek 2 AST-N 233 Cards containing 18 antibiotics and combinations of antibiotics belonging to different families are intended for both fermentative and non-fermentative Gram-negative bacteria. These antibiotics are: ampicillin, amoxicillin/clavulanic acid combination. ticarcillin. piperacillin/tazobactam combination, cephalotin, cefoxitin, cefotaxime, ceftazidime, imipenem, ertapenem, amikacin, gentamicin, tobramycin, nalidixic acid, ciprofloxacin, nitrofurantoin, trimethoprim ofloxacin, and Sulfamethoxazole. The results of unnecessary antibiotics are automatically eliminated by the Vitek 2 compact 15 (biomérieux, Marcy l'Etoile, France).

The Vitek 2 AST-XN05 cards (biomérieux, Marcy l'Etoile, France) intended for multiresistant Gram-negative bacilli were subsequently used to detect possible antibiotics active on the strain of S. paucimobilis analyzed and to give a possibility of antibiotic therapy adapted to the strain. The 18 antibiotics and combinations of antibiotics used in the Vitek 2 ASTXN05 cards (Biomérieux, Marcy l'Etoile, France) were: piperacillin, ampicillin/sulbactam combination, cefuroxime axetil, cefixime, ceftriaxone, cefuroxime, cefepime, aztreonam, meropenem, levofloxacin, minocycline, tetracycline, tigrecycline, moxifloxacin, chloramphenicol, trimethoprim, colistin and the combination ticarcillin/clavulanic acid. In the compact Vitek 2 system (biomérieux, Marcy l'Etoile, France), the results of antibiograms and MICs were determined according to the criteria and interpretation standards of the Clinical and Laboratory Standards Institute (CLSI). The Advanced Expert System (AES) software compares the MICs of the instrument and the identity of the identified germ to the standard phenotypes of this germ (CLSI + Natural Resistance). The AES communicates the results obtained in the form of summary reports and proposals. Depending on the germs identified, the results of antibiotics deemed not required are deleted in the report of the antibiogram results determined by the Vitek 2 compact 15 system (bioMérieux, Marcy l'Etoile, France). Thus, for AST-N 233 cards, the sensitivity results for ertapenem are always deleted for S. paucimobilis while those for colistin and the ticarcillin/clavulanic acid combination are deleted by the machine for AST XN05.

API 20NE

The API 20 NE gallery contains 20 microtubes containing dehydrated substrates. Conventional tests are inoculated with a saline bacterial suspension which reconstitutes the media. The reactions produced during the incubation period result in spontaneous color changes or revealed by the addition of reagents. Assimilation tests are inoculated with minimal medium and the bacteria grow only if they are able to use the corresponding substrate. These reactions are read using the Reading Table and identification is obtained using the Analytical Catalog or API WEB identification software (bioMérieux).

ATB PSE EU (08)

Introduction and purpose of the test:

The ATB PSE EU (08) gallery makes it possible to determine the sensitivity of *Pseudomonas* and other non-fermenting Gram (-) bacilli to antibiotics in a semi-solid medium under conditions very close to the reference techniques of agar dilution or micro- dilution.

The ATB PSE EU gallery (08) has 16 pairs of cups. The first pair, without antibiotics, serves as a growth control. The next 15 contain antibiotics at one or two concentrations (c and C). The bacteria to be tested is suspended then transferred to the culture medium and inoculated into the gallery. After incubation, growth is read either visually or with the ATB or mini API ® automaton. The result obtained makes it possible to categorize the strain as Sensitive, Intermediate or Resistant.

3. Results

3.1. Epidemiological Profile Of Patients At The China-Guinea Friendship Hospital

Sexe	Number (N=86)	Percentage			
Female	51	59,30%			
Male	35	40,70%			
1-10	6	6,98%			
11-20	10	11,63%			
21-30	10	11,63%			
31-40	9	10,47%			
41-50	18	20,93%			
51-60	11	12,79%			
61-70	9	10,47%			
71-80	9	10,47%			
81-90	3	3,49%			
91-100	1	1,16%			
Occupation					
Farmers	4	4,66			
Teacher	1	1,17			
Entrepreneur	1	1,17			
Religious (Imam)	1	1,17			
Household	21	24,41			
Not applied	8	9,30			
Traders/Merchantss	10	11,62			
Students	10	11,62			
Administration agents	16	18,60			
Security agents	4	4,66			
Workers	8	9,30			
health workers	2	2,32			

Table 1: Distribution of patients according to socio demographic characteristics

Sex-ratio (M/F) = 0.6; Standard deviation = 22.44 years.

Table 2: Distribution of patients according to requesting Department

Requesting Department	Number	Percentage
Anesthesia/Intensive care	3	3.49
Cardiology	34	39.53
Visceral surgery	1	1.16
Endoscopy	1	1.16
Other hospitals	20	23.26
Neurosurgery	4	4.65
Neurology	1	1.16
Traumatology	1	1.16
Emergencies	21	24.42
Total	86	100

Fable 3: Distribution of	f samples	according to different	types o	of biological	fluids
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Nature of biological fluids	Number	Percentage
Cerebrospinal fluids	4	4.65
Pleural fluid	1	1.16
Pus	3	3.49
Blood	4	4.65
Sperm	1	1.16
Urine	73	84.89
Total	86	100



Fig 1: Distribution of patients according to their origin

Bacterial species	Number	Percentage
Sphingomonas paucimobilis	29	33.72
Acinetobacter baumannii complex	19	22.10
Acinetobacter lwoffii	3	3.48
Acinetobacter Haemolyticus	2	2.33
Pseudomonas aeruginosa	9	10.46
Pseudomonas fluorescens	9	10.46
Pseudomonas oryzihabitants	3	3.48
Pseudomonas luteola	3	3.48
Pseudomonas stutzerie	1	1.17
Pseudomonas putida	1	1.17
Stenotrophomonas maltophila	3	3.48
Aeromonas hydrophila/cavia	2	2.33
Burkholderia cepacia	1	1.17
Ralstonia mannitolilytica	1	1.17
Total	86	100

Table 4. I toportion of unreferr species of NI-OND Identified

3.2 Resistance profile to different families of antibiotics used

Antibiotics	Sensitivity N (%)	Intermédiate N (%)	Resistance N (%)	Not Determined
Ampicillin	3 (37.5)	0 (00.00)	5 (62.50)	78
Ticarcillin	31 (48.44)	10 (15.63)	23 (35.93)	22
Piperacillin	12 (52.17)	3 (13.05)	8 (34.78)	63
Oxacilline	0 (00.00)	0 (00.00)	4 (100.00)	82
Cefuroxim	3 (50.00)	0 (00.00)	3 (50.00)	80
Cefoxitin	2 (50.00)	0 (00.00)	3 (50.00)	82
Cefotaxime	36 (65.45)	8 (14.55)	11 (20.00)	31
Cefepim	23 (74.20)	2 (6.45)	6 (19.35)	55
Ceftazidime	38 (53.52)	8 (14.08)	23 (32.39)	15
Imipenem	70 (97.23)	2 (2.77)	0 (00.00)	14
Meropenem	15 (88.24)	2 (11.6)	0 (00.00)	69
Aoxicillin/Clavulanic acid	3 (75.00)	0 (00.00)	1 (25.00)	82
Piperacillin/tazobactam	52 (83.87)	2 (3.23)	8 (12.90)	24
Amikacin	46 (93.87)	2 (4.22)	16 (34.05)	39
Gentamicin	63 (84.00)	5 (6.66)	7 (9.34)	11
Tobramycin	57 (81.43)	8 (11.42)	5 (7.15)	16
Nalidixic acid	2 (33.33)	0 (00.00)	4 (66.66)	80
Ciprofloxacin	42 (60.87)	5 (7.25)	22 (31.88)	11
Ofloxacin	29 (61.70)	2 (4.25)	16 (34.05)	39
Norfloxacin	2 (33.33)	0 (00.00)	4 (66.66)	80
Levofloxacin	18 (64.28)	5 (18.51)	4 (14.18)	59
Tetracycline	5 (35.72)	3 (21.43)	6 (42.85)	72
Tigecycline	9 (100.00)	0 (00.00)	0 (00.00)	77
Minocycline	18 (85.17)	1 (4.76)	2 (9.53)	65
Nitofurantoine	8 (80.00)	0 (00.00)	2 (20.00)	76
Trimethoprime/sulfamethoxazole	28 (40.58)	0(00.00)	41 (59.42)	17

Table 5: Antibiotic sensitivity profile of the different NFGNB strains isolated

Table 6: Results of antibiograms and minimum inhibitory concentrations (MIC in µg/ml) of some identified bacterial strains

N° patient /Sexe/Age	13/F/20	18/M/75	31/F/75	34/F/45	43/F/80		
Antibiotics							
AMP				R(>16)			
TIC	R(>64)		R(>64)	R(>64)	R(>64)		
TZP	S(16)		R(>64)		R(>64)		
CFT					R(>64)		
CTX	S(4)		R(>64)				
CAZ	R(>32)			R(>32)			
ERT			R(32)	I(16)			
IMI	R(0.25)				R(>32)		
AKN	S(≤2)						
GEN	I (8)		S(0.5)	S(0.5)			
ТОВ	I(8)			S(≤2)	S(1)		
NALF			I(8)	S(≤1)			
CIP	R(>2)		I(8)		R(>8)		
OFL	R(>4)				I(8)		
FUR			R(>2)	I(2)			
TMP/SMX	R(>160)				R(>2)		
OXA			R(>160)	R(160)			
PIC		I(64)			R(>160)		
FED		R(32)					
MERO				S(≤1)			
PER		I(8)			R(>32)		
FOS		R(>4)					
Ticarcillin/Clavulanic Acid				R(>4)			
TIG		R(>8)					
ERY		R(>4)					
LIN		R(>32)					

The table 6 shows the multi-antibiotic resistance profiles with respective minimum inhibitory concentrations (MICs). Indeed, most minimum inhibitory concentrations are high are high.

4. Discussion

This study was carried out on a total of 86 strains of nonfermenting Gram-negative bacilli isolated at the China-Guinea Friendship Hospital in Kipé / Conakry.

4.1. Sociodemographic aspects

It emerges from this study that 59.30% of patients were female and 40.70% male with a sex ratio (M/F) of 0.6 in favor of women. The mean age of the patients was 45.08 years with the extremes of 1 year to 98 years and a standard deviation of 22.44 years. Our results are similar to those found by Benbella I (2016) in Morocco with 59.93% female and 39.97% male ^[13].

The most represented socio-professional category was that of housewives, ie 24.41%, followed by administrative staff 18.60% and shopkeepers 11.62%. The predominance of housewives could be explained by the higher number of females.

The distribution according to origin showed that the majority of patients (82.56) were from the city of Conakry, and particularly from the commune of Ratoma (45.35%) where HASIGUI is located, followed by that of Matoto (24.42%). This could in part be explained by the proximity of the hospital to users.

According to the requesting services, cardiology and emergencies were the most represented with respectively 39.53% and 24.42%. They are followed by the external service (23.26%) not affiliated with a HASIGUI service, but come from other health structures in the cities of Conakry.

3. 4.2. Frequencies of biological fluids

Out of 86 patients, body fluids were mostly urine with 84.89% (73/86), followed by cerebrospinal fluid and blood which each represented 4.65% (4/86) of samples, pus with 3.49% (3/86), semen and puncture fluid each 1.16% (1/86). The high frequency of urine isolations is believed to be due in part to the high frequency of requests for cytobacteriological examinations of the urine compared to all the bacteriological examinations performed. Our results are similar to those of Makanera *et al.*, Who reported in their studies on diversity and antibiotic sensitivity of different species of *Pseudomonas*, that 69.3% of their strain was isolated from urinary tract infections ^[14].

4.3. Frequencies of isolation of different species of nonfermenting Gram-negative bacteria

In our study, bacterial identification made it possible to determine 14 species of BGNNF with *Sphingomonas paucimobilis* as the majority species (33.72%), followed by *Acinetobacter baumannii* complex (22.10%), *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* which corresponded each at 10.46% of total cash. Our results are different from those of Bebabza M (2016) Algeria who found the majority species *Pseudomonas aeruginosa* with a rate of 58.33%, followed by *Burkholderia cepacia* (20%), *Stenotrophomonas maltophilia* (16.67%), *Acinetobacter baumannii* (5%).

4.4 Resistance Profile Of Non-Fermenting Gram-Negative Bacteria As A Function Of Antibiotics 4.4.1. Resistance to β-Lactamns

These results show total resistance of NFGNB strains to oxacillin (100), followed by ampicillin (62.5) and ticarcillin (35.93). Compared to Morocco (Ait Miloud, 2011) resistance to ticarcillin varies between 66.7% and 100%, to Piperacillin between 57.6% and 100% and to Ceftazidine between 30.3% and 100% ^[15].

4.4.2. Fluoroquinolone resistance

Resistance is high to nalidixic acid and norfloxacin at 66.66% each followed by ofloxacin (34.05%). In contrast to the study carried out by Baaziz S. (2018) in Constantine which reveals a low acquired resistance of 6% for Ciprofloxacin and Levofloxacin ^[16].

4.4.3. Resistance to Aminoglycosides

NFGNB aminoglycosides with amikacin (34.05%), followed by gentamycin (9.34%) and tobramycin (7.15%). Compared to the study by Baaziz S. (2018) Constantine which found an acquired resistance of 6% to gentamycin and tobramycin ^[16].

4.4.4. Cyclin resistance

We note that BGNNF sensitivity to the cyclin showed generally a high sensitivity to minocycline (85.17%) and tigecycline (100%), but a low sensitivity of these strains to tetracycline (35.72%). However, the resistance of these bacteria to tetracycline was 42.85%.

4.4.5. Resistance to Nitrofurans

The nitrofuran class is presented by a single antibiotic which is nitrofurantoin of the 86 samples analyzed, sensitivity to nitrofurantoin was tested in only ten (10) bacterial strains. The results of the antibiograms thus showed that eight (8) of the ten (10) bacterial strains were sensitive to nitrofurantoin (ie 80%), against only two strains which were resistant to this molecule (ie 20%). These results are almost identical to those found by Odjadjare *et al.*, (2012) ^[17] who reported that 80% of their strains were sensitive to this molecule ^[17]. On the other hand, these results are very different from those found by Makanera *et al.* (2019), in which they reported that 57.33% of their strains were resistant to nitrofurantoin ^[14]. This could be justified by the low number of strains tested for susceptibility to nitrofurantoine in the present study (Table 5).

4.4.6. Resistance to sulfonamides

It is noted that sulfonamides (trimethoprim / sulfamethoxazole) have been shown to have little activity on BGNNFs. In fact, 59.42% of these strains were resistant to sulfonamides. Our results are thus comparable to those found in 2017 by Makanéra *et al.*, Who reported in 2017 a frequency of 62.5% resistance of *Sphingomonas paucimolis* strains to amoxicillin / clavulanic acid ^[18].

5. Conclusion

Non-fermenting Gram-negative bacilli are germs responsible for various, frequent and sometimes formidable pathologies. In recent years uncontrolled antibiotic therapy has led to the emergence of resistant and multi-resistant bacteria, the increase in this resistance must be monitored especially in the hospital environment.

Our study, which was carried out at the China-Guinea Friendship Hospital of Kipé in Conakry, allowed us to determine the frequency of non-fermenting Gram-negative bacteria and to monitor resistance to antibiotics.

Among 86 samples, 73 were diagnosed in favor of urinary tract infections, with a percentage of 84.89%. Our study shows a female predominance with 51 (59.30%) being a sex ratio (M/F) of 0.6. The most affected age groups were 41 to 50 and 51 to 60.

This study shows that Sphingomonas paucimobilis

predominates with a frequency of 33.72%, followed by *Acinetobacter baumanni* complex 22.10%, Pseudomonas aeruginosa and *Pseudomonas fluorescens* each 10.46%.

Antibiotic results showed that NFGNB was resistant to almost all families of antibiotics tested, however tetracyclines were the least resistant.

6. Conflict of Interest

Not available

7. Financial Support

Not available

8. References

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