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Phenotypic Characteristics of *Klebsiella pneumoniae* Extended Spectrum β-Lactamases Producers Isolated in Hospitals in the Littoral Region, Cameroon

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Abstract: Background: the resistance of enterobacteriaceae to antibiotics is experiencing a worrying worldwide development with an increasing impact of β-lactamases. Klebsiella pneumoniae is one of the bacteria responsible for nosocomial infections. Hence the need to look for these enzymes in the hospitals of the Littoral region, Cameroon. Method: cross-sectional and descriptive study during 2016 and early 2017, isolates of intermediate sensitivity or resistant to third generation cephalosporins (C3G) collected in the laboratories of the Littoral referral hospitals and in Diagmed laboratory. Confirmation of the strains made by the API 20E™ gallery, the study of antibiotic sensitivity by the method of diffusion of discs on agar (Mueller Hinton). The double synergy test for the search of extended spectrum β-lactamases (ESBL), and confirmation by the Eurobio ™ kit (France). Results: a total of 412 strains of klebsiellasp (species) were isolated and 122 Klebsiella pneumoniae included. The majority age group is that ≤1 year with 26.0%, the germs were isolated in the urine at 54.6% and in neonatology and pediatrics at 35.8%. Klebsiella pneumoniae pneumoniae identified at 82.4%. The production of BLSE was 86.9%, against that of AmpC at 3.30%. The sensitivity to imipenem and amikacin was 96.2% and 98.1%, respectively. Conclusion: the study shows a high frequency of ESBL and a low presence of ampC in the Littoral region, Cameroon; practitioners must make a rational prescription from a correctly performed antibiogram.

Keywords: *Klebsiella pneumoniae*, Resistance, β-Lactamase

1. Introduction

Infections remain a major concern in the various services of hospitals. *Klebsiella pneumoniae* is a species, also responsible for nosocomial infections where the digestive tract of hospital patients and the hands of staff are two main sources [1], The spread of resistant bacteria is the cause of the considerable increase in mortality, morbidity and the cost of treatment in hospitals [2].

The empirical use of antibiotics such as beta-lactams for the management of bacteria has enabled the bacteria to develop resistance through the production of ESBL (Extended spectrum beta-lactamase) and cephalosporinases (ampC) which are β-lactamases [3]. Studies carried out in some countries in Africa [4, 5] demonstrated the presence of enterobacteria producing Extended spectrumbeta-lactamases,

particular in Cameroon in the cities of Yaoundé [6, 7], NGaoundéré [8] and Douala [9].

The aim of this work was to identify strains of Klebsiella pneumoniae multi-resistant to beta-lactams, as well as resistant genes in several referral health facilities in the Littoral region of Cameroon in 2016 and 2017.

2. Materials and Methods

2.1. Location and Type of Study

This is a cross-sectional and descriptive study carried out in several health structures in the Littoral region, namely:

1. The laboratories of Douala referall hospitals: General Hospital (HGD), Laquintinie Hospital (HLD), Military Hospital (HMD), Gyneco-Obstetric and Pediatric Hospital (HGOPED). Saint John of Malta Hospital in Njombé (HSJM) and the Diagmed Laboratory for strain collection.
2. The central laboratory of Laquintinie Hospital for microbiological analyzes.
3. The Institute for Infectious Diseases, University of BERN in Switzerland for the determination of MICs (minimum inhibitory concentration).

It was carried out over a period of 14 months, from January 2016 to February 2017.

The strains of Klebsiella sp or Klebsiella pneumoniae isolated and identified in the laboratory and of intermediate sensitivity or resistant to a third generation cephalosporin were collected in a transport media (Mueller Hinton sloped in glass and screw tubes), were kept in a NOVALAB ™ plastic biosafety package (ISO 15189 compliant), and placed in a cooler with cold accumulator. They were sent to the central laboratory of the hospital within 2 hours.

2.2. Methods

The strains arrived at the laboratory of Laquintinie hospital, data relating to the patient and recorded on the technical file were recorded in a register and then followed the confirmation of the identification of strains which was done with the API 20E ™ gallery of BioMérieux. The antibiogram was performed according to the recommendation of the Antibiotic Committee of the French Microbiology Society (CASFM, 2014).

The search for secretion of broad spectrum β-lactamases (ESBL) was done by the double synergy test and the cloxacillin test.

Confirmation of the presence of ESBL and AmpC was observed through the use of the Eurobio commercial kit (France).

2.3. Statistical Analysis

The data was analyzed using SPSS version 16 software for Windows (SPSS, Inc., Chicago, IL, USA). The goodness of fit chi-square test to compare proportions (one-size-fits-all statistics) while the Pearson and McNemar chi-square-tests were used for the bivariate statistics. The significance threshold was set at a probability value of less than 5%.

3. Results

The number of Klebsiella sp strains isolated in the hospitals laboratories during our study was 412, we received 125 strains of Klebsiella intermediate or resistant to C3G; 122 strains were identified and confirmed *Klebsiella pneumoniae* with 106 producers of ESBL.

Table 1. *Characteristics of the Klebsiella.*

Variables	Total n (%)	AmpC n = 0	AmpC + BLSE n = 4	ESBL n = 102	Non producing n = 16	*Klebsiellasp* n = 290	P
Age (years)							
≤ 1	107 (26.0)	0	2	29	3	73	0.28
[1 – 11]	44 (10.7)	0	0	7	1	36	
[11 – 21]	10 (2.4)	0	0	2	0	8	
[21 - 31]	48 (11.7)	0	1	15	1	31	
[31 - 41]	56 (13.6)	0	1	14	1	40	
[41 – 51]	42 (10.2)	0	0	9	2	31	
[51 - 61]	36 (8.7)	0	0	7	2	27	
[61 – 71]	38 (9.2)	0	0	8	6	24	
≥ 71	31 (7.7)	0	0	11	0	20	
Gender							
Female	230 (55.8)	0	4	56	8	162	0.18
Male	182 (44.2)	0	0	46	8	128	
Health structure							
DIAGMED	44 (10.7)	0	0	10	2	32	0.43
HGD	156 (37.9)	0	0	37	5	114	
HGOPED	71 (17.2)	0	0	17	2	52	
HLD	94 (22.8)	0	4	31	7	52	
HMD	14 (3.4)	0	0	1	0	13	
HSJM	33 (8.0)	0	0	6	0	27	
Nature of specimen							
Urine culture	225 (54.6)	0	0	55	10	160	0.45
Blood culture	109 (26.5)	0	2	31	2	74	
Pus	70 (17.0)	0	2	14	4	50	

Variables	Total n (%)	AmpC n = 0	AmpC + BLSE n = 4	ESBL n = 102	Non producing n = 16	*Klebsiellasp* n = 290	P
Cerobro-Spinal fluid	3 (0.7)	0	0	2	0	1	
Vaginal sampling	2 (0.4)	0	0	0	0	2	
Peritoneal fluid	1 (0.2)	0	0	0	0	1	
Gastric tubing	1 (0.2)	0	0	0	0	1	
Tracheal tube secretion	1 (0.2)	0	0	0	0	1	
Service							
Surgery	58 (14.2)	0	0	16	3	39	0.26
Externa	77 (18.6)	0	0	21	2	55	
Gynecology	23 (5.4)	0	2	7	2	12	
Medicine	73 (17.9)	0	0	17	4	52	
Neonatology	63 (15.2)	0	2	20	2	39	
Pediatric	83 (20.3)	0	0	15	2	66	
Intensive care unit	28 (6.9)	0	0	6	1	21	
Emergency	6 (1.5)	0	0	0	0	6	

The sex ratio (F / M) was 1.26; The Douala General Hospital provided the largest number of samples (37.9%) followed by the Laquintinie Hospital with 22.8%. The lowest number came from the Military Hospital (3.4%). Urine culture is the most common test with a frequency of (54.6%), followed by blood culture (26.5%).

The samples came mainly from pediatrics (20.3%) and less came from the Intensive care unit (1.5%). The most represented age group is that of children under one year with a frequency of 26.0% and the least represented is that of 11 to 21 years with a frequency of 2.4%.

106 out of 122 strains produce ESBL (86.9%), 3.30% simultaneously produce ESBL and AmpC. No strain produces only cephalosporinases (AmpC).

Table 2. *Confirmation of the presence of ESBL by a commercial kit.*

Confirmation of the presence of ESBL	Effective	Frequency (%)
Positive	106	100.0
Negative	0	0
Total	106	100.0

It is noted that all of the ESBL producing strains have all been confirmed positive by the commercial kit (EUROBIO ™, France).

There is no statistically significant difference between the antibiograms made with the disc diffusion method and the microchip method for MIC research (P-value = 0.97).

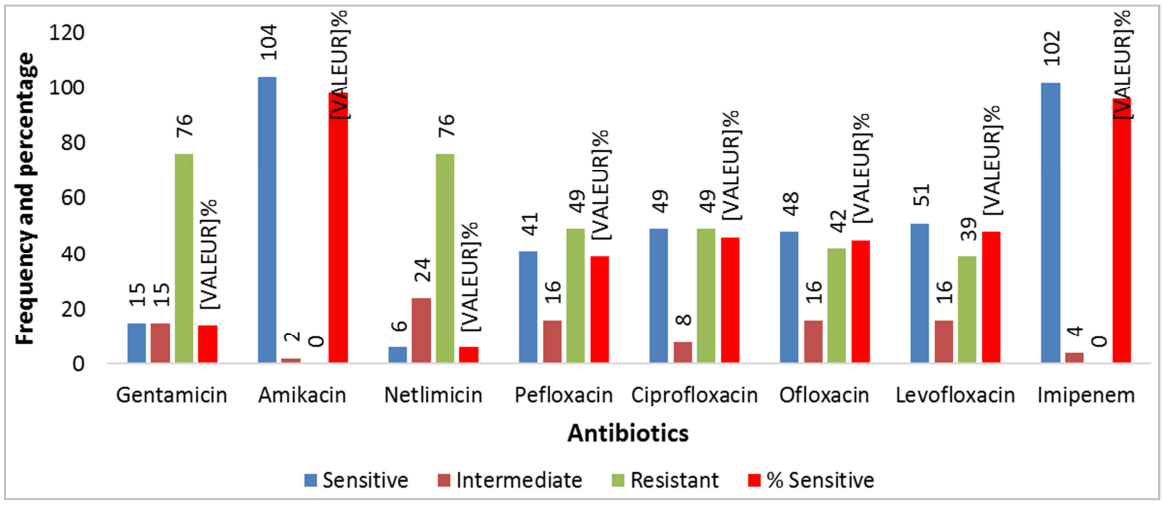

Figure 1. *Antibiotic sensitivity profile of ESBL-producing K. pneumoniae.*

The best sensitivities of antibiotic by ESBL-producing K. pneumoniae are those of amikacin and imipenem with 98% and 96% respectively. The lowest sensitivity is that of netilmicin with 6%.

4. Discussion

4.1. Nature of the Sample

The strains were isolated mainly in the urine and represent 54.6% comparable to the rates of 52.5%, 49.4% reported respectively by Chafa betbeui in Yaoundé in 2013 [10], Gangoué in the same town and published in 2005 [7], slightly higher at Okalla in Douala with 68.7% [3]. This shows that urine is the biological product most infected with enterobacteria.

4.2. Service

The most represented service is pediatrics with 20.3%, associated with neonatology; the rate rises to 35.5% similar to the rate of 35.35% obtained by Chafa -betbeui in Yaoundé

[10]. This is explained by the improper application of hygienic conditions and promiscuity which promote the transmission of germs.

4.3. Age

The age group <11 years is the most represented with 36.7%, including those <1 year with a rate of 26.0%. This representation is in agreement with Flokas [11] who noted an overall rate of 15% in Africa and indicated that it is associated with neonatal mortality. The high rate can also be explained by the still weak immune protection.

This is contrary to Farah in Algeria whose most represented group is ›50 years; this can be explained by the progressive appearance of metabolic diseases and the decline in immunity [12].

4.4. Gender

Female were the most represented 55.8%; in agreement with Farah in Algeria who found 51.25% [12], this can be explained by the proximity between the urinary and anal orifices in women.

4.5. Klebsiella Species

Klebsiella pneumoniae pneumoniae was isolated with a frequency of 82.4%, similar to the rate of 78.7% found by Chafa-betbeui in Yaoundé in 2015 [10].

4.6. ESBL Production

ESBL production was 86.9%, this rate is comparable to the rate of Feizabadi in Iran in the order of 72.1% for *K. pneumoniae* [13], Dadeic-Ljubovic in Bosnia and Herzegovina with 88.8% in enterobacteriaceae [14]. In the same line, Elhani reports that in Tunisia, the production of ESBL by *K. pneumoniae* varies and can reach 87.5% in pediatric intensive care units [15].

Hailaji reports that 20.4% of ESBL is produced by the Klebsielle in Nouakchott-Mauritania [16]. Thus, the prevalence rates of ESBLs vary widely depending on the geographic location and the bacterial species.

In Cameroon, the rates of 12%, 16% were found respectively by Gangoué in 1998 [7] and Magoué-lonchel in Ngaoundéré in 2009 [8]. Moreover, a recent study conducted in Yaoundé in 2012 [6] and published in 2016 gives a rate of 66.3%. Also another study made in 2015 at the General Hospital of Douala and published in 2018 gives a prevalence of 37. 4% [9]. There is thus a considerable variation and emergence of ESBL in Cameroon due to the fact that the majority of patients do not have access to the laboratory and the management of infectious syndromes is probabilistic.

There is therefore a causal link between the increasing use of third generation cephalosporins and the emergence of ESBL.

4.7. AmpC

The production of cephalosporinases (AmpC) was 3.30%, slightly high compared to the high-level cephalosporinase level of 1.01% obtained by Chafa betbeui in Yaoundé in 2013 [10]. The prevalence of AmpC is still low compared to the production of ESBL and still requires monitoring.

4.8. Antibiogram

Klebsiella pneumoniae was sensitive to imipenem in the order of 96%, similar to the rates of 98.7%, 99% and 100% respectively for Okalla in Douala [3], Chafa betbeui in Yaoundé [10]. Also amikacin with a sensitivity of 98%. Close to the rate of 100% reported by Hailaji in Mauritania [16]. So imipenem and amikacin remain the antibiotics of choice against enterobacteria. It is also a challenge to monitor its misuse and probabilistic care.

The resistance to gentamicin is 71.7%, same trend as that obtained by Okalla in Douala at the end of the study which is 72.5% [3], different from the 19.5% obtained by Hailaji in Mauritania [16].

This development may be due to self-medication, the probabilistic treatment that increases resistance to this antibiotic.

5. Conclusion

The urine is the biological product most infected by enterobacteria and the most represented service is pediatrics. The study shows a high frequency of ESBL and the presence of cephlosporinases (AmpC) in the littoral region; therefore the circulation of multi-resistant bacteria remains a major problem which must lead practitioners to make a rational prescription of antibiotics from a correctly performed antibiogram. The presence of champagne cork in the double synergy test is synonymous with the production of ESBL by a bacterium. Imipenem and amikacin remain the best antibiotics against enterobacteria.

Contributions of the Authors

JT, COE, DA participated in the design, analysis and interpretation of the data. CY to data analysis and interpretation, JT to data collection and manuscript design, LK to Statistical analysis. All authors contributed to the review and approved the final version.

Conflicts of Interest

All the authors do not have any possible conflicts of interest.

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