**ABSTRACT**

**Aims:** The main objective of this study was to determine the resistance phenotype of β-lactamines by *Klebsiella* in three hospitals in Yaoundé.

**Study Design:** A cross-sectional descriptive study.
1. INTRODUCTION

Bacterial infections acquired in the hospital as well as in the community settings are a serious public health concern. These infections are mostly caused by Gram-negative bacilli which are nowadays increasingly implicated in the development of antibiotic resistance. The resistance of *Klebsiella* to antibiotics has in recent years experienced an alarming global increase [1].

Since 1980s, the third generation cephalosporins (3GC) belong to the group of antibiotic used for the treatment of severe enterobacterial infection. Quickly the extended-spectrum beta-lactamases (ESBLs), Plasmid transmitted enzymes responsible for resistance to penicillins, cephalosporins and to monobactams began to develop. Due to the broad spectrum activity of the carbapenemes, they have recently become the alternatives to the 3GC [2].

The ESBLs are broad spectrum enzymes conferring resistance to bacteria against most of the β-lactam antibiotics except cephemycins and carbapenemes [2]. The resistance of gram-negative bacilli to carbapenemes, particularly by the production of transmissible carbapenemases is a major public health problem [3]. Most of these Carbapenemases have been identified in *Klebsiella pneumoniae* [4]. The emergence and spread of carbapenemase-producing Enterobacteriaceae in different parts of the world is a major threat especially in nosocomial infections [2]. The isolation of carbapenemase-producing *Klebsiella* spp. is increasing worldwide [4]. In Cameroon, some phenotypic studies presumptively reveal the existence of carbapenemase-producing *Klebsiella* spp. (C Mbakop, University of Yaounde 1, Cameroon, Unpublished results).

*Klebsiella* spp. are involved in many nosocomial infections and many strains are resistant to antibiotics. Studies carried out at the Yaounde Central Hospital found a resistance rate of 18.8% of ESBL -producing *Klebsiella* [5]. However, this observation is unknown in many other hospitals. This study aimed at determining the resistance and the phenotypes of *Klebsiella* spp., isolated from three clinical laboratories in three referrals hospitals in Yaounde.

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**Keywords:** *Klebsiella* spp; resistance phenotypes; ESBL; carbapenemases.
2. MATERIALS AND METHODS

Ninety-nine Klebsiella spp. were isolated from all clinical specimens at three bacteriology laboratories from three referrals hospitals in Yaoundé from May to November 2013. The identification of the Klebsiella isolates was done using standard bacteriological procedures and biochemically using the API 20E gallery (BioMérieux SA, Lyon, France).

Antimicrobial susceptibility testing (AST) was performed on Mueller-Hinton agar according to “Comité d’Antibiogramme de la Société Française de Microbiologie (CASFM)” [6] using antibiotics of the β-lactam family and inhibitors such as clavulanic acid, tazobactam, Ethylene-Diamine-Tetra-Acid (EDTA), cloxacillin and 3-aminoophenyl boronic acid hydrochloride. The antibiotics tested for all isolates were: amoxicillin (25 µg), amoxicillin + clavulanic acid 25/10 ug), ticarcillin (75 µg), ticarcillin + clavulanic acid (75/10 ug), piperacillin (100 ug), piperacillin-tazobactam (100/10 ug) cephalothin (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 ug) and ertapenem (10 ug).

The minimum inhibitory concentration (MIC) was determined using the E-test. The phenotypes were determined from the interpretation of the antimicrobial susceptibility testing as recommended by the CA-SFM. Detection of ESBLs was done using the synergy test by the combination of amoxicillin and clavulanic acid disks, ceftazidime, aztreonam and cefepime.

The detection and classification of carbapenemase were performed by the modified Hodge test and the use of different inhibitors (cloxacillin, boronic acid and EDTA).

3. RESULTS AND DISCUSSION

This study was conducted in the bacteriology laboratories of three public referrals hospitals in Yaoundé: University Teaching Hospital, Yaoundé (UTHY), Yaoundé General Hospital (YGH) and Gynaeco-Obstetric and Pediatric Hospital, Yaoundé (HGOPY). Among the 99 strains of Klebsiella spp. isolated from 99 patients, most of them were from hospitalized patients (n = 85 or 85.85%), mainly from the pediatrics unit, n = 34 (35.35%) and intensive care unit n = 27 (27.27%). These results are similar to those obtained in a study carried out in Cameroon which revealed a predominance of Klebsiella in the pediatric unit (48.4%) and medical intensive care unit (21.3%) (C Mbakop, University of Yaoundé 1, Cameroon, Unpublished results). This could be partly due to misuse of antibiotics or poor application of hygiene in these units. On the other hand it may be due to a high concentration of patients in these units of intensive care which could enhance the transmission of germs between patients [3].

Table 1 shows that all strains were resistant to amoxicillin (aminopenicillin) and ticarcillin (carboxypenicillin).

The wild type was found in all strains. The isolated bacterial strains expressed high resistance to ureidopenicillins (piperacillin, 76%) and first-generation cephalosporins (1GC: cephalothin, 85%).

The action of inhibitors (clavulanic acid, tazobactam) enabled the restoration of penicillins activities such as amoxicillin, piperacillin and ticarcillin (39%, 38%, 22% respectively). A 55.5% (n=55) resistance was observed for cefoxitin. For the 3GCs the resistance was variable: cefotaxime 52% and ceftazidime 54%; for monobactams (aztreonam) 51% and 56% of the strains were resistant 4GCs (cefpime). The most active antibiotics were imipenem (99%) and ertapenem (77%).

Regarding the sensitivity of strains to antibiotics, they expressed a high level of resistance with respect to most of the antibiotics tested. The wild phenotype was confirmed for all of our strains as recommended by CASFM, [6]. Overall, there has been a slight increase in the level of antibiotic resistance as comparison to a 2011 study (C Mbakop, University of Yaoundé 1, Cameroon, Unpublished results) with a p-value = 0.001. The resistance of the 1GC (cephalothin), 2GC (cefoxitin), 3GC (ceftazidime), 4GCs (cefpime), monobactam (aztreonam) and imipenem were 85%, 55%, 54%, 56%, 51% and 1% respectively. They also found 84%, 52%, 45%, 50.3%, 38% and 12% respectively. This increased level of resistance to β-lactam antibiotics could probably be as a result of inappropriate prescribing and indiscriminate use of this antibiotic family [7]. The high prevalence of "resistant" phenotypes acquired primarily within hospital strains (strains 66/85 or 77.64%) shows the possibility of an increased antibiotics selection pressure [8,9,10]. In addition, Escherichia coli and Klebsiella spp. have usually been cited as being the Enterobacteriaceae species presenting the most...
resistant phenotypes [11,12]. The frequency of the "wild type" (27.27%) obtained in our study was low and well below the frequency of the known wild type of Klebsiella spp. (70%) [13,14].

The interpretation of the AST enabled us to highlight the different resistance phenotypes expressed by the isolates (Fig. 1, Table 2, Table 3, Fig. 2).

The phenotypic classification was based on the susceptibility profile of the different antibiotics tested which enabled us to obtain the following phenotypes: expanded spectrum β-lactamase (ESBL) n = 30 (30.3%), low spectrum penicillinase / wild n = 27 (27.27%), penicillinase resistant to inhibitors n = 16 (16.16%), carbapenemase n = 11 (11.11%), high-level penicillinase n = 8 (8.08%), HyperOXY n = 4 (4.04%), high-level cephalosporinase + extended spectrum β-lactamase to n = 2 (2.02%) and high-level cephalosporinase n = 1 (1.01%). The most observed phenotype was the ESBL (30.3%), followed by the wild-type (27.3%). The phenotype of high-level cephalosporinase was the least (1.01).

Nearly a third of the Klebsiella spp. were ESBL producers (30.3%), showing a remarkable increase in the frequency of these strains in Cameroon, as previous studies in the Yaounde Central Hospital and UTHY had revealed frequencies of 18.8% in 2005 [15] and 29% in 2012 respectively (N Kouya, School of Health Sciences / Catholic University of Central Africa, Cameroon, Unpublished results). However, the Alert, Investigation and Surveillance of Nosocomial Infections Network in France indicated in 2008 that 17% of K. pneumoniae responsible for bacteremia were ESBL-producing. The high frequency of ESBL-producing Klebsiella in our context would be the consequence of regular use of β-lactam antibiotics or prolonged hospitalization of patients [16]. Resistance related to the production of carbapenemase by E. coli and Klebsiella spp. have been described [17,5]. Search and classification of carbapenemase in our study were performed by the modified Hodge test and the synergy test. All Carbapenemases producing strains showed a negative modified Hodge test. The results of this test are variable in detecting carbapenemases in Class B, C and D [18]. Among the 11 carbapenemases-producing Klebsiella isolates, 45.45% were Class C and 54.54% class B. These results are different from those of Mbakop et al. in Cameroon with 58 carbapenemase-producing Enterobacteriaceae, 33% were class A, 27% of class B and 31% class C and D. This difference is due to the fact that the previous study (C Mbakop, University of Yaounde 1, Cameroon, Unpublished results) included all Enterobacteriaceae spp. and in addition all inhibitors were not used for the classification of the carbapenemases.

| Antibiotics                        | Susceptibility (%) |
|------------------------------------|--------------------|
|                                    | Sensitive | Intermediate | Resistant |
| Amoxicillin                        | 0         | 0            | 100       |
| Amoxicillin + clavulanic acid      | 39        | 32           | 29        |
| Ticarcillin                        | 0         | 0            | 100       |
| Ticarcillin + clavuanic acid       | 22        | 18           | 60        |
| Piperacillin                       | 24        | 13           | 63        |
| Piperacillin+ tazobactam           | 62        | 29           | 9         |
| Cephalothin                        | 15        | 25           | 60        |
| Cefoxitin                          | 45        | 17           | 38        |
| Cefotaxime                         | 49        | 1            | 51        |
| Cefazidime                         | 46        | 12           | 42        |
| Cefepime                           | 44        | 30           | 26        |
| Aztreonam                          | 49        | 8            | 43        |
| Imipenem                           | 99        | 1            | 0         |
| Ertapenem                          | 77        | 19           | 4         |

NB: Resistant bacterial strain by definition is a non-susceptible strain, that is categorized "resistant" or "intermediate"
The distribution of these carbapenemases with respect to the status permitted us to see that all carbapenemase-producing strains were isolated from hospitalized patients (shown on Table 2). There was an association between ESBL-producing strains and carbapenemase, hospitalized status and hospital units. There was a high prevalence of ESBL-producing strains and/or carbapenemase in patients hospitalized in the pediatric wards and intensive care unit.

The high prevalence of ESBL and carbapenemase-producing strains in hospitalized patient in the pediatric (36.36%) and intensive care units (36.36%) may be due to the fact that ESBL and carbapenemase enzymes are produced mostly by nosocomial strains responsible for some hospital outbreaks [19] and in the units with prolonged hospitalization [20]. All these suggest that frail individuals are the most exposed.

Indeed, it has been observed that the acquisition of ESBL and carbapenemase concerns patients with prolonged hospitalization and exposure to invasive devices [21]. Other risk factors may include malnutrition, hemodialysis, total parenteral nutrition, ICU admission or prior hospitalization. Some studies have reported that the restriction of the use of antibiotics has

![Phenotypic classification of strains](image)

**Fig. 1. Phenotypic classification of strains**

| Hospital units        | ESBL Producing strains | ESBL Non-producing strains | Total | Carbapenemase Producing strains | Carbapenemase Non-producing strains | Total |
|-----------------------|------------------------|----------------------------|-------|---------------------------------|-------------------------------------|-------|
| Pediatric             | 9                      | 26                         | 35    | 4                               | 31                                  | 35    |
| Intensive care        | 8                      | 19                         | 27    | 4                               | 23                                  | 27    |
| Medical               | 3                      | 4                          | 7     | 1                               | 6                                   | 7     |
| Gynaeco-obstetric     | 2                      | 4                          | 6     | 0                               | 6                                   | 6     |
| Surgical              | 2                      | 3                          | 5     | 2                               | 3                                   | 5     |
| Emergency             | 3                      | 2                          | 5     | 0                               | 5                                   | 5     |
| Total                 | 27                     | 45                         | 85    | 11                              | 74                                  | 85    |
contributed to the decrease in the number of ESBL producing strains [22].

Table 3 shows that ESBL phenotypes n = 27 (90%) and carbapenemase n = 9 (81.8%) were the most observed by *Klebsiella pneumoniae pneumoniae*.

The class D carbapenemase (OXA-48) was first described in *K. pneumoniae* and was often associated with other β-lactamases, especially ESBL [23]. In Turkey, several outbreaks of nosocomial infections have been associated with strains producing this type of carbapenemase [20,23]. To our knowledge, no data are available on the clinical impact of cephalosporinases whose spectrum is partially expanded to carbapenemes (Class C).

As shown on Fig. 2, the sensitivity of strains to antibiotics depends on the method used. Out of the 29 strains resistant to cefotaxime by agar diffusion method, only 23 were confirmed by determining the minimum inhibitory concentration (MIC); the other 6 were categorized as "sensitive" or intermediate by the latter.

Table 4 describes the characteristics of some *Klebsiella* strains with respect to the specimens, the hospital unit from which it was isolated, the diameter of the zone of inhibition and MIC values of some beta lactams (ceftazidime, cefotaxime, cefepime and impenem).

### Table 3. Distribution of *Klebsiella* spp. based on ESBL and carbapenemase production

| Bacteria species                   | ESBL            | CARBAPENEMASES |          |          |
|-----------------------------------|-----------------|----------------|----------|----------|
|                                   | Producing       | Non-producing  | Producing| Non-producing|
|                                   | strains         | strains        | strains  | strains |
| *Klebsiella pneumoniae pneumonia* | 27              | 51             | 9        | 69       | 78     |
| *Klebsiella oxytoca*              | 0               | 12             | 2        | 10       | 12     |
| *Klebsiella pneumoniae ozaenae*   | 3               | 2              | 0        | 5        | 5      |
| *Klebsiella pneumoniae rhinoscleromatis* | 0                | 4              | 0        | 4        | 4      |
| **Total**                         | **30**          | **69**         | **11**   | **88**   | **99** |

Fig. 2. Comparison of Minimum Inhibitory Concentration (MIC) and the diameter of the inhibition zone of cefotaxime (CTX)
4. CONCLUSION

The interpretation of the AST has enabled the establishment of a high prevalence of expanded spectrum β-lactamase and consequently leading to an increase in the presence of carbapenemase producing Klebsiella spp. This could lead to therapeutic failure in case of treatment with β-lactamines antibiotics. Therefore this trend needs to be monitored.

ETHICAL APPROVAL

An ethical clearance for this study was obtained from the National Ethic Committee for Human Research in Cameroon.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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