Research Article: Carbapenemase Producing Klebsiella species from Hospitalised Patients at Three Referral Hospitals in Yaounde; Cameroon

Abstract:

**Background:** Carbapenemases are the most potent beta-lactamases capable of hydrolysing all beta-lactam drugs. Data scarcity on laboratory evidence of carbapenemase producers obstructs infection prevention and effective control measures. This study aimed to detect and phenotypically characterize Klebsiella species resistant to carbapenems.

**Method:** A total of eighty-seven (87) Klebsiella spp. isolates were collected from three Yaounde-based tertiary hospitals over a period of twelve months (February 2019 to January 2020). The isolates were identified on API 20E and antimicrobial susceptibility testing was done according to the Kirby Bauer disc diffusion method. Carbapenem resistance was detected based on reduction in inhibition zone diameter of meropenem less than 28mm and resistance phenotypes determined by the combination disc synergy test.

**Results:** Four Klebsiella species were identified namely: Klebsiella pneumoniae 62/87 (71.3%), K. oxytoca 11/87 (12.6%), K. ozaenae 7/87 (8%), K. rhinoscleromatis 7/87 (8%). Klebsiella pneumoniae was most resistant to carbapenems with a considerably high number of multidrug resistant isolates 39/56 (69.6%). Patients from the 0-9 years age group were most represented 29/87 (33.3%). Carbapenemases belonging to Ambler classes A, B and D were detected as follows: KPC 2/87 (2.3%), MBL 2/87(2.3%), OXA-48 4/87 (4.6%). All four Klebsiella species were producers of KPC carbapenemase 12/87 (13.8%) and Extended Spectrum β-lactamase 68/87 (78.2%) enzymes.

**Conclusion:** These results revealed that more than half of Klebsiella species analysed were multidrug resistant and about a quarter in hospitalized patients at the referral hospitals were mostly KPC carbapenemase producers. This raises concerns over the need to control and confine the spread of these isolates of clinical and epidemiological importance for effective infection prevention and control.

**Keywords:** Carbapenems, Carbapenemase, Resistance, Klebsiella pneumoniae carbapenemase, Metallo beta-lactamase, OXA-48
Dissemination of carbapenemase producers is both an epidemiological and clinical problem because resistance genes found on self-conjugative plasmids carrying other resistance determinants, easily spread vertically and via horizontal gene transfer to related Gram-negative bacilli (4) (5) (6) causing cross-resistance. This transforms otherwise antibiotic susceptible strains into non-susceptible strains. (7).

Enterobacteriaceae are Gram-negative bacteria commonly encountered in clinical samples and they may be responsible for approximately 50% of all clinically significant bacteria (8). The emergence of carbapenemase resistant Enterobacteriaceae (CRE) over the past decades in many parts of the world including Japan, United Kingdom, Greece, United States of America, Italy, India and some Central and South American countries, usually involves *Klebsiella pneumoniae* and *Escherichia coli* (9) (10) (11). Rapid clonal dissemination of acquired carbapenemases as a result of class A (KPC), class B metallo-β-lactamases (IMP, VIM, NDM) or class D (OXA-48) serine carbapenemases complicates patient outcome. Sizing up the problem of CPE in Africa and precisely Cameroon is difficult because of the absence of survey reports, published studies, absence of effective infection prevention and control practice in healthcare settings, gaps in training for the detection of CPE and poor diagnostic tools (12) (13). In this context when therapeutic failure occurs, given the limited diagnostic and treatment options, dissemination of undiagnosed and untreated CPE continues to be promoted. This study aimed to detect and phenotypically characterize *Klebsiella* species resistant to carbapenems and to contribute to antimicrobial resistance stewardship.

**Method and materials**

**Study design and study population**

This study was cross-sectional and descriptive carried out over a period of one year (February 2019 to January 2020). The study population was the portion of hospitalized patients of all ages who gave non-repetitive clinical specimens for analysis at the hospital and therein *Klebsiella spp.* was isolated. Eighty-seven (87) *Klebsiella spp.* isolates were obtained.

**Description of study site**

The three referral hospitals namely: the Yaounde University Teaching Hospital (YUTH), the Yaounde General Hospital (YGH) and the Yaounde Gynaeco-Obstetrics and Paediatrics Hospital (YGOPH) situated within the urban setting of Yaounde and offering medical services of diverse speciality to both in and out-patients. The consulted patient population is cosmopolitan in terms of ethnicity, age, and sex. Microbiology analysis was carried out both at the hospitals and at the Centre for the Study and Control of Communicable Diseases, Faculty of Medicine and Biomedical Sciences, The University of Yaounde I.

**Ethical clearance**

This research work was approved by the Comité Institutionnel d’Ethique de la Recherche pour la Santé Humaine, Université Catholique d’Afrique Centrale, Ecole des Sciences de la Santé registration number No.2019/020178/CEIRSH/MI.

**Data collection**

A Structured questionnaire was administered to all consenting participants and clinical information was obtained from hospital registers with the assistance of laboratory and ward staff.

**Isolation of Klebsiella spp.**

Clinical specimens were cultured on eosin methylene blue agar (EMB). Based on colony morphology, suspected 3-5mm lactose positive and mucoid colonies were purified on nutrient agar. Biochemical identification of suspected *Klebsiella spp.* was carried out on API 20E testing system (Biomerieux, Marcy-l’Etoile, France) according to the procedure referenced by the manufacturer (14).

**Antimicrobial susceptibility testing**

Twenty eight antibiotics (from Rapid Labs Ltd, Colchester ESSEX-UK) belonging to four families (β-lactams, aminoglycosides, sulphonamide and quinolones) were tested according to the Kirby Bauer disc diffusion method (15). *Escherichia coli* ATCC 25922 was used for quality control of the antibiotic discs.

**Determination of resistance**

Screening for ESBL, AmpC and carbapenemase producers was simultaneously done with antimicrobial susceptibility testing of third generation cephalosporins, cefoxitin and carbapenems respectively. Each suspected ESBL producing isolate, was confirmed based on the combination disc testing method. Discs containing cefotaxime and cefotaxime clavulanate were applied on an inoculated Mueller Hinton plate. After 18 to 24 hours aerobic incubation at 35±2°C, the inhibition zones around the cefotaxime disc and cefotaxime clavulanate were compared. The isolate was considered an ESBL producer if the inhibition zone diameter was equal to or greater than 5 mm around the cefotaxime clavulanate disc compared with cefotaxime disc. This phenotype is illustrated in figure 1. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 strain were used for quality control.

The detection of a plasmid-mediated AmpC-type cephalosporinase producer was based on cefoxitin zone inhibition diameter less than 19 mm, an inhibition zone diameter equal to or greater than 5 mm around the cefoxitin clavulanate disc compared with cefoxitin disc, alongside phenotypic resistance to ceftazidime and or cefotaxime (fig 1).

Carbapenemase screening was based on the reduction in diameter of inhibition around meropenem discs. The screening breakpoint of meropenem considered was an inhibition zone diameter greater than or equal to 28mm. (16). Confirmation of a carbapenemase producing isolate was based on the combination disk synergy testing method. Synergy between a meropenem disc and meropenem in combination with various inhibitors (boronic acid, EDTA, cloxacillin) and reduction of the inhibition zone diameter around temocillin disc in the absence of synergy confirmed a carbapenemase producer as illustrated in figures 2-4. All the confirmation antibiotic discs used were produced by Liofilchem, Roseto degli Abruzzi-Italy.
Results

Eighty-seven [87] Klebsiella spp. isolates were obtained distributed as follows: K. pneumoniae 62/87 (71.3%), K. oxytoca 11/87(12.6%), K. ozaenae 7/87(8%) and K. rhinoscleromatis 7/87 (8%). Participants were recruited from all wards and age groups ranging from one day old to eighty-seven years old, with a mean age of 31 years. Most participants belonged to the age group 0-9 years with a frequency of 29/87 (33.3%) while the 30-39 years age group was least represented with a frequency of 3/87 (3.4%). The majority of participants were females accounting for a frequency of 50/87 (57.5%) as opposed to males 37/87 (42.5%). Thirty-six [36] isolates were collected from the Yaounde University Teaching Hospital, seventeen [17] from the Yaounde General Hospital and thirty-four [34] from the Yaounde Gynaeco-Obstetrics and Paediatrics Hospital. Klebsiella spp. were isolated from eleven clinical specimens, mostly from urine 32/87. Within the 0-9 years age group, most isolates were recovered from urine 15/29 (51.7%) and blood 13/29 (44.8%). The resistance rates of isolates to the various classes of antibiotics were concordant with current high resistance trends to third generation cephalosporins (70.9%), fourth generation cephalosporins (77.7%), aminoglycosides (44%), sulphonamide (94.5%) and fluoroquinolones (39.8%) and low resistance rates to carbapenems (7.5%). Several isolates 56/87 (64.4%) were multidrug resistant and as expected the bulk of them was K. pneumoniae 39/56 (69.6%). Although overall, K. oxytoca isolates were fewer than K. pneumoniae isolates, at intraspecies level, K. oxytoca recorded more multidrug resistant isolates 8/11 (72.7%). Carbapenemases belonging to all three Ambler classes were detected either as single β-lactamase or in combination with ESBL or AmpC. Class A carbapenemase KPC represented 2/87 (2.3%), class B MBL 2/87(2.3%), and class D OXA-48, 4/87 (4.6%) enzymes were detected in some isolates. All four Klebsiella species were producers of KPC carbapenemases 12/87.

Statistical analysis

Statistical analysis was done with the aid of CS Pro 7.1 and MS Excel softwares. A p-value less than 0.05 was considered statistically significant.
(13.8%) and Extended Spectrum β-lactamases 68/87 (78.2%). One isolate that was resistant to imipenem and sensitive to meropenem was expressed as an ESBL+ porin loss phenotype. There were two isolates that were intermediate to meropenem and susceptible to imipenem. One expressed ESBL+ porin loss and the other ESBL+ AmpC phenotypes. (Table 1)

Table 1: Results of antimicrobial profiles and resistance phenotypes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>K. pneumoniae</th>
<th>K. oxytoca</th>
<th>K. ozaenae</th>
<th>K. rhinoscleromatis</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Bacteria count</td>
<td>62 (71.3%)</td>
<td>11 (12.6%)</td>
<td>7 (8.0%)</td>
<td>7 (8.0%)</td>
<td>87 (100.0%)</td>
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<tr>
<td>Isolate count in most represented age group</td>
<td>22</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>Isolate count YUTH</td>
<td>23</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>37</td>
</tr>
<tr>
<td>Isolate count YGH</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Isolate count YGOPH</td>
<td>29</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Multidrug resistant isolates</td>
<td>39 (62.9%)</td>
<td>8 (72.7%)</td>
<td>5 (71.4%)</td>
<td>4 (57.1%)</td>
<td>56 (64.4%)</td>
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<tr>
<td>Resistance to penicilins</td>
<td>97.6%</td>
<td>92.9%</td>
<td>96.3%</td>
<td>100%</td>
<td>96.7%</td>
</tr>
<tr>
<td>Resistance to third generation cephalosporins</td>
<td>66.6%</td>
<td>61.9%</td>
<td>74.3%</td>
<td>80.9%</td>
<td>70.9%</td>
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<tr>
<td>Resistance to fourth generation cephalosporins</td>
<td>63.6%</td>
<td>71.4%</td>
<td>75.8%</td>
<td>100%</td>
<td>77.7%</td>
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<tr>
<td>Resistance to monobactam</td>
<td>63.6%</td>
<td>71.4%</td>
<td>72.6%</td>
<td>85.7%</td>
<td>73.3%</td>
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<tr>
<td>Resistance to carbapenems</td>
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<td>0%</td>
<td>3.2%</td>
<td>0%</td>
<td>2.0%</td>
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<tr>
<td>Resistance to sulphonamide</td>
<td>90.9%</td>
<td>100%</td>
<td>87.1%</td>
<td>100%</td>
<td>94.5%</td>
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<tr>
<td>Resistance to aminoglycosides</td>
<td>24.6%</td>
<td>53.6%</td>
<td>40.7%</td>
<td>57.15%</td>
<td>44%</td>
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<tr>
<td>Resistance to quinolones</td>
<td>37.9%</td>
<td>42.9%</td>
<td>45.2%</td>
<td>33.4%</td>
<td>39.84%</td>
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<td>ESBL phenotype</td>
<td>63.6%</td>
<td>71.4%</td>
<td>66.1%</td>
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<td>74.3%</td>
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<td>ESBL, AmpC phenotype</td>
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<td>0%</td>
<td>8,1%</td>
<td>0%</td>
<td>4.3%</td>
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<tr>
<td>ESBL, porin loss phenotype</td>
<td>0,0%</td>
<td>0,0%</td>
<td>3,2%</td>
<td>0,0%</td>
<td>0.8%</td>
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<td>None ESBL phenotype</td>
<td>27,3%</td>
<td>28,6%</td>
<td>22,6%</td>
<td>0,0%</td>
<td>14.6%</td>
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<td>0%</td>
<td>0,0%</td>
<td>0%</td>
<td>2.3%</td>
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<td>AmpC, ESBL phenotype</td>
<td>9,1%</td>
<td>0%</td>
<td>8,1%</td>
<td>0,0%</td>
<td>4.3%</td>
</tr>
<tr>
<td>None AmpC phenotype</td>
<td>81,8%</td>
<td>100,0%</td>
<td>90,3%</td>
<td>100,0%</td>
<td>93.0%</td>
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<td>14,3%</td>
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<td>0,0%</td>
<td>5.9%</td>
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<td>MBL phenotype</td>
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<td>0,0%</td>
<td>3,2%</td>
<td>0,0%</td>
<td>0.8%</td>
</tr>
<tr>
<td>OXA-48 phenotype</td>
<td>9,1%</td>
<td>0%</td>
<td>4,8%</td>
<td>0,0%</td>
<td>4.5%</td>
</tr>
<tr>
<td>None carbapenemase phenotype</td>
<td>81,8%</td>
<td>85,7%</td>
<td>75,8%</td>
<td>85,7%</td>
<td>82.3%</td>
</tr>
</tbody>
</table>

Discussion

Our study sought to determine the antimicrobial resistance profile and carbapenemase phenotypes of *Klebsiella* spp. isolated from hospitalized patients at three referral hospitals in Yaounde. Isolates were recovered most from participants within the age group 0-9 years and from two clinical specimens namely urine 15/29 (51.7%) and blood 13/29 (44.8%). This indicates that hospitalized children within this age group from whom *Klebsiella* spp. were isolated suffered most from urinary tract infections (UTIs) and bloodstream infections. Furthermore, among the 29 isolates recovered from this age group, only 4/29 (13.7%) were none-β-lactamase producers while 25/29 isolates produced either ESBL alone 16/29 (55.2%) or in combination with other β-lactamases such as: carbapenemases (7/29) and AmpC (3/29). The consequences for these vulnerable patients with immature immunity can be treatment failure and even death as a result of sepsis or in the case of UTIs, the infection may become chronic and result in kidney scaring, hypertension and renal failure (17).

Multidrug resistant *Klebsiella* spp. have been prominently described (6), (18), (19), (20), (21) (22). Multidrug resistant isolates concomitantly carry resistant genes to several classes of antimicrobials like β-lactams, aminoglycosides and fluoroquinolones. Most of our isolates 56/87 (64.4%) were multidrug resistant and more than half were *K. pneumoniae* (39/56, (69.6%), the species with wide-ranging ecological distribution, high antimicrobial resistance gene diversity, plasticity and a higher plasmid burden (23). The interplay of the genetic disposition of *K. pneumoniae* makes it better organized for mobilizing resistant genes from other drug resistant bacteria in the environment or in animal/human microbial communities. This further explains why *K. pneumoniae* is always the predominant species as was the case in an earlier study (19). Such information is fundamental for shaping effective routine analysis of clinical specimens.

Resistance to carbapenems is a serious problem because they consist of one of the last treatment options for serious infectious diseases and the genes responsible for their resistance are transferable among Enterobacteriaceae. Resistance to carbapenems is either due to an acquired carbapenemase(s) which will enzymatically break down carbapenems or as a result of the production of an ESBL/Class C Cephalosporinase (AmpC)
combined with porin loss (1) (2). Carbapenemases are the most clinically important mechanism of carbapenem resistance and their global trend is on the rise (4), (6) (22), (24). In this study, carbapenemases belonging to all three Ambler classes were detected and most of them were detected in combination with other β-lactamas as ESBL or AmpC. ESBLs render isolates resistant to third, fourth generation cephalosporins and monobactams, and AmpC confer resistance to third generation cephalosporins and cephemycins (16). Three isolates that were intermediate or resistant to either imipenem and meropenem did not produce carbapenemases but rather ESBL + porin loss and/ or AmpC which are known to induce low-level resistance to carbapenems (16). This is an indication of diversity in resistance clones within our research setting especially as all four Klebsiella species were producers of KPC.

Class A and B carbapenemases: KPC 2/87 (2.3%), MBL 2/87(2.3%) respectively, KPC and MBL 11/87 (12.6%) were most detected compared to Class D OXA-48 type enzymes (27) representing 4/87 (4.6%) of carbapenemases. This tendency reflects the situation in parts of Africa where the molecular epidemiology of carbapenemase genes have been described (North Africa, South Africa, West and East African countries), predominantly carbapenemase genes of blaOXA-48 type, blalIMP, blaVIM, and blaNDM in Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae, Citrobacter spp., and Escherichia coli (25), (26). Although risk factor assessment of CPE patient-to-patient transmission was not carried, it has been established that, transmission is common in patients experiencing long-term hospitalisation and without prior screening for CPE colonisation at the time of admission (12). In resource limited countries such as ours, absence of screening for CPE colonisation at patient admission is a setback to efforts at CPE prevention and control.

Conclusion
Evidence from this study on carbapenemase resistance phenotypes and various combinations of co-expressed mechanisms reveal that K. pneumoniae and to an extend K. oxytoca isolates were quite assorted. More than half of Klebsiella species analysed were multidrug resistant and about a quarter of Klebsiella spp. isolates in circulation in referral hospitals in Yaounde were carbapenemase producers. This raises concerns over the need to control and confine the spread of these isolates of clinical and epidemiological importance for antimicrobial stewardship as well as for effective infection prevention and control.

List of abbreviations
AmpC- ampcillinase cephalosporinase
blaIMP- beta-lactamase imipenem
blaNDM- beta-lactamase New Delhi metallo-β-lactamase
blaOXA-48 - beta-lactamase oxacillanase-48 type
blaVIM, - beta-lactamase Verona integron-encoded metallo-β-lactamase
CPE – carbapenem producing Enterobacteriaceae
EDTA- ethylenediaminetetraacetate
ESBL- Extended spectrum beta-lactamase
KPC- Klebsiella pneumoniae carbapenemase
MBL- metallo-β-lactamase
PBA- Phenyl Boronic acid
UTIs- urinary tract infections
YGH - Yaounde General Hospital
YGOPH- Yaounde Gynaeco-Obstetrics and Paediatrics Hospital
YUTH- Yaounde University Teaching Hospital

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