Scientific Research Publishing

Open Journal of Medical Microbiology, 2026, 16(2), 82-100
https://www.scirp.org/journal/ojmm
ISSN Online: 2165-3380
ISSN Print: 2165-3372

Phenotypic Characterization of *Klebsiella* Producing Extended-Spectrum *β*-Lactamases and Carbapenemases in Three Referral Hospitals in the City of Yaounde, Cameroon

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How to cite this paper: Chafa, B.A., Lyonga, E.E.M., Baiye, W.A., Essomba, R.G., Bitoungui, V.N., Ngogang, M.P., Kana, L.F., Killa, C., Teudjieu, A.D., Tembong, M., de Paul Djientcheu, V., Gonsu, H.K. and Adiogo, D. (2026) Phenotypic Characterization of *Klebsiella* Producing Extended-Spectrum *β*-Lactamases and Carbapenemases in Three Referral Hospitals in the City of Yaounde, Cameroon. *Open Journal of Medical Microbiology*, **16**, 82-100.
https://doi.org/10.4236/ojmm.2026.162005

Received: March 31, 2026
Accepted: June 12, 2026
Published: June 15, 2026

Abstract

Background: Antimicrobial resistance has become a major public health threat globally. The resistance mechanisms of *Klebsiella* include the production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases, limiting the effectiveness of standard treatments. **Objectives:** The main objective of this study was to carry out the phenotypic characterization of *β-lactam-resistant Klebsiella* strains isolated from blood, pus and urine in three referral hospitals in the city of Yaounde. **Methods:** This was a cross-sectional study. Data collection was carried out over a one-year period from January to December 2023 in the bacteriology laboratories of three referral hospitals in Yaounde: Yaounde University Teaching Hospital (YUTH), Yaounde General Hospital, Yaounde Gynecological-Obstetric and Pediatric Hospital. Antimicrobial susceptibility testing by the disc diffusion method in accordance with the Antibiogram Committee of the French Society of Microbiology made the identification of the different resistance phenotypes possible. **Results:** Of the 264 clinical specimens analyzed, majority were from female patients, n = 135 (51.1%). The clinical specimens analyzed were urine, suppuration and blood (39.8%, 31.4% and 28.8%

respectively). *Klebsiella pneumoniae pneumoniae* was the most frequently isolated subspecies (62.2%). The antibiotic susceptibility study showed an overall predominance of resistant strains with high rates of resistance to cefalexin, cefixime, ceftriaxone and cefotaxime (75.4%; 74.7%; 73.0% and 72.1% respectively). However, imipenem, meropenem, ceftazidime/avibactam and ertapenem were the most effective molecules against the *Klebsiella* species tested, with resistance rates of 10.6%, 14.3%, 21.7% and 30.4%, respectively. Phenotypic tests revealed the predominance of the following resistant phenotypes among the strains: extended-spectrum β-lactamase (42.4%), low-level/wild-type penicillinase (20.1%), derepressed penicillinase (3.0%), carbapenemases (33.7%) and high-level cephalosporinase (4.9%). Taking into consideration of the "screening carbapenemase-producing strains" algorithm, 89 isolates were suspected of producing carbapenemase, representing 33.7% of our isolates. **Conclusion:** Our findings demonstrate the circulation of strains with worrying resistance phenotypes, highlighting the urgent need for action by the Cameroonian health authorities.

Keywords

Klebsiella spp., Resistance Phenotypes, ESBL, Carbapenemases

1. Introduction

Antimicrobial resistance in Gram-negative bacteria represents a global health crisis, characterized by a rapid reduction in the effectiveness of available antibiotics and a concomitant increase in morbidity and mortality associated with bacterial infections [1]. Species of the genus *Klebsiella*, in particular *Klebsiella pneumoniae*, are major opportunistic pathogens involved in serious infections, including healthcare-associated pneumonia, bacteremia, urinary tract and intra-abdominal infections and are among the priority pathogens due to their multidimensional resistant profiles [2].

The emergence and rapid spread of *Klebsiella* multidrug-resistant organisms can be explained by a remarkable genetic plasticity, favored by the acquisition of resistant genes via horizontal transfer (plasmids, transposons, integrons) [3]. The predominant resistance mechanism is based on the production of β-lactam-hydrolyzing enzymes, including extended-spectrum β-lactamases (ESBLs) of CTX-M, SHV and TEM types, as well as carbapenemases such as KPC, NDM, VIM, IMP and OXA-48. These enzymes confer resistance to third-generation cephalosporins and, in the most severe cases, to carbapenems, which are considered antibiotics of last resort. One of the central characteristics of the resistance in *Klebsiella pneumoniae* is the expression of extended-spectrum β-lactamase (ESBL) enzymes, which hydrolyze and inactivate β-lactams, including third-generation cephalosporins. These enzymes are encoded by genes frequently located on motile plasmids, facilitating their horizontal dissemination between strains and bacterial species [4]. In

addition, the emergence and expansion of plasmid carbapenemases (e.g., KPC, NDM, OXA-48 and variants) confer resistance to carbapenems, drastically limiting therapeutic options. The simultaneous presence of aminoglycoside, fluoroquinolone and polymyxin resistance genes further exacerbates the problematic nature of these multidrug-resistant strains (*multidrug-resistant*, MDR) or even resistant to almost all clinically available antibiotics (*extensively drug-resistant*, XDR) [5].

At the epidemiological level, the most recent global data report an increasing prevalence of resistance in *K. pneumoniae*, with third-generation cephalosporin resistance rates exceeding 55% globally and reaching more than 70% in some parts of Africa, according to data from the World Health Organization (WHO) Global Surveillance System (GLASS). Resistance to carbapenems and fluoroquinolones, once rare, is becoming more and more frequent, severely reducing the clinical effectiveness of first and last resort treatments [2]. International bodies such as the WHO have identified ESBL- and carbapenemase-producing enterobacteriaceae as priority pathogens in need of urgent action in research, development of new antimicrobials, and strategies to combat antimicrobial resistance [6]. The global dissemination of *Klebsiella* Carbapenem-resistant diseases, often associated with high-risk epidemic clones, poses a major threat to patient safety and the efficiency of healthcare systems. This situation underlines the importance of epidemiological surveillance, molecular characterization of resistance mechanisms and the implementation of integrated strategies including reasoned antibiotic therapy, infection prevention and control measures, as well as the development of new antimicrobial molecules or alternative therapeutic approaches [5].

2. Methodology and Results

2.1. Methodology

Between January and December 2023, strains of *Klebsiella* spp. from patient clinical specimens were isolated and identified using the API 20 E biochemical gallery (BioMérieux SA, Lyon, France) at the YUTH's Bacteriology Laboratory. The sampling method used was non-probabilistic convenience. Only one isolate was included per patient. The strains we collected were those isolated on a specific medium for enterobacteriaceae (EMB or MacConkey agar) and identified as *Klebsiella*. The presumed *Klebsiella* spp. strains isolated from the bacteriology laboratories of the YGH and the YGOPH were transported to the bacteriology laboratory of the YUTH for analysis, in compliance with bacterial strain transport conditions. These strains were innoculated into brain heart infusion broth supplemented with 10% glycerol, stored in an isothermal cooler. The strains were thus transported from the laboratories of the YGH and the YGOPH to the bacteriology laboratory of the YUTH. The antibiotics used were those recommended by the Antibiogram Committee of the French Society of Microbiology (EUCAST-CASFM, 2023). The study of the sensitivity of *Klebsiella* strains to antibiotics was determined by the disc diffusion

method in agar medium (Mueller Hinton). The antibiotics tested were chosen according to the recommended panel for enterobacteriaceae. Susceptibility test results were interpreted using EUCAST-CASFM criteria, and interpretive reading of the antibiograms enabled the determination of the various phenotypes expressed by *Klebsiella* spp. The antibiotics studied (Oxoid brand) for all the strains isolated were the following: amoxicillin (20 µg), amoxicillin + clavulanic acid (20/10µg), ticarcillin (75 µg), ticarcillin + clavulanic acid (75/10µg), piperacillin (30 µg), piperacillin-tazobactam (30/6µg), temocillin (30 µg), cefalexin (30 µg), cefoxitin (30 µg), cefixime (5 µg), ceftazidime (10 µg), ceftazidime + avibactam (10/4µg), cefotaxime (5 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), and ertapenem (10 µg). The detection of extended-spectrum β-lactamases was performed during the antibiotic susceptibility tests by highlighting an image of synergy between the inhibitors and C3G and/or C4G and/or aztreonam. Regarding the screening of carbapenemase-producing strains, the EUCAST-CASFM flowchart (2023) was used to detect strains suspected of producing carbapenemase, despite a categorization that may be "sensitive" to carbapenems.

As concerns quality control, the EMB/MacConkey culture media were inoculated with reference strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, then incubated for 24 hours at 37˚C to verify their specificity and fertility. The absence of bacterial growth for *Staphylococcus aureus* and the presence of growth for *Escherichia coli* confirmed that the culture media were selective. The antibiotic disks were tested with reference strains of *Escherichia coli* ATCC 25922, and the agreement between the measured diameters and the expected diameters confirmed the good quality of the antibiotic disks. For ESBL detection, *Klebsiella pneumoniae* ATCC 700603 was used as a positive control, and *E. coli* ATCC 25922 as a negative control, in accordance with EUCAST-CASFM recommendations.

The data obtained were digitized using CSPro 7.1 software. Statistical analysis was performed using R software (version 4.3) and Microsoft Excel. Descriptive statistics were used to summarize socio-demographic and microbiological data as frequencies and percentages. Associations between categorical variables (carbapenemase production status and age group, sex, hospital ward, and clinical specimen type) were assessed using Pearson's chi-squared test or Fisher's exact test when expected cell counts were below 5. The significance threshold was set at $p < 0.05$ for all analyses. Effect sizes were estimated using Cramér's V. For the age-group analysis, the eight original age categories were collapsed into four broader groups (0 - 20, 21 - 40, 41 - 60, and >60 years) to ensure adequate expected cell frequencies, yielding df = 3 for the chi-squared test. Post hoc pairwise comparisons between hospital wards were performed using odds ratios with 95% confidence intervals, with p-values derived from Fisher's exact test. R and Excel were used to extract information from the database and to construct the various graphs and tables.

2.2. Results

2.2.1. Socio-Demographic Characteristics

Table 1 below shows the isolated strain distribution according to the sex of the patients. Of the 264 isolates, 135 (51.1%) were from female patients and 129 (48.9%) were from male patients with sex ratio of 1:1.05. The mean age of the patients was 46.39 years (±21,349). The majority of strains were obtained from patients aged [40 - 50] years (20.5%), followed by those in the [50 - 60] and [60 - 70] age groups (19.3% of strains each) as seen in Table 1 below. This table equally shows the distribution of patients per department. Most isolates were from the emergency department and medicine unit, accounting for 22.7% and 22.0%, respectively. Only ten strains of *Klebsiella* spp. came from the neonatal department (3.8%).

Table 1. Socio-demographic characteristics of the patients.

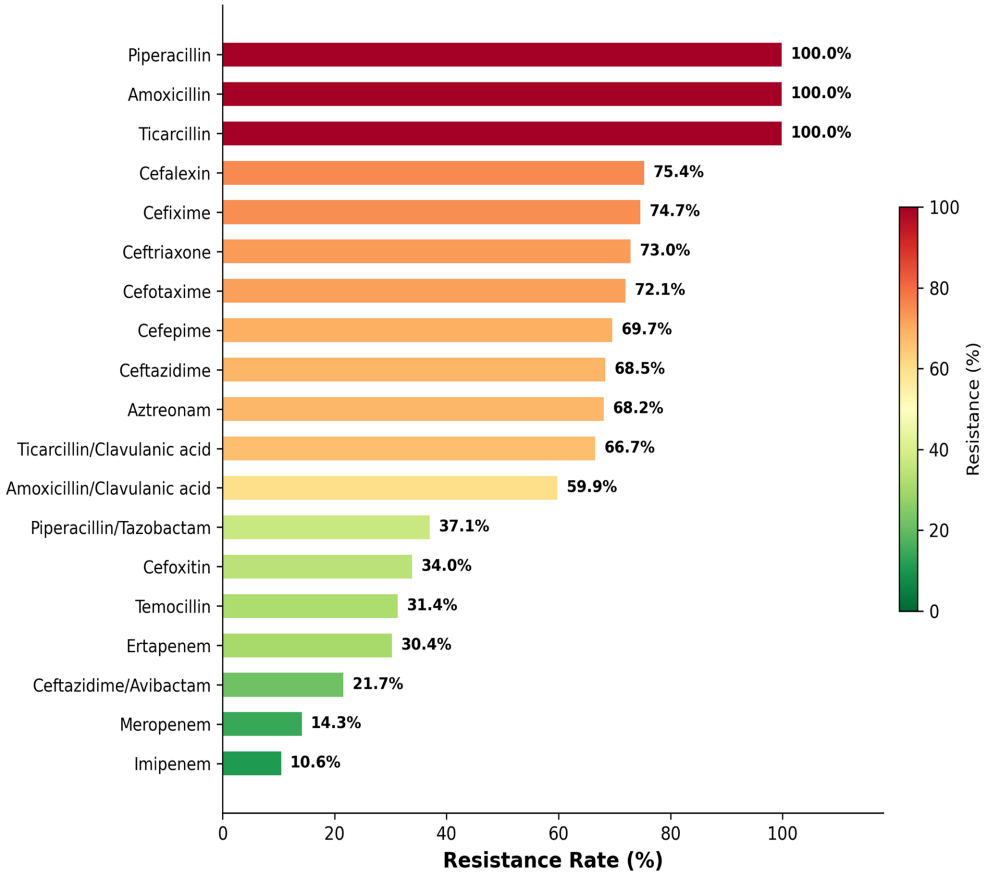
Variables	Frequency (n)	Percentage (%)
Age-group (in years)		
0 - 10	27	10.2
11 - 20	9	3.4
21 - 30	18	6.8
31 - 40	31	11.7
41 - 50	54	20.5
51 - 60	51	19.3
61 - 70	51	19.3
>70	23	
Gender		
Female	135	51.1
Male	129	48.9
Department		
Surgical ward	34	12.9
Outpatient unit	39	14.8
Obstetrics and gynaecology unit	11	4.2
Haemodialysis unit	15	5.7
Medical unit	58	22.0
Neonatology	10	3.8
Paediatrics unit	13	4.9
ICU	24	9.1
Emergency unit	60	22.7

Continued

Hospital		
YUTH	200	75.8
YGH	42	15.9
YGOPH	22	8.3
Clinical specimens		
Urine	105	39.8
Pus	83	31.4
Blood	76	28.8

2.2.2. Overall Antibiotic Resistance Profile

Figure 1 summarizes the resistance rates of the 264 *Klebsiella* spp. isolates against 19 antibiotics spanning seven pharmacological classes. Universal resistance (100%) was observed for three unprotected penicillins: amoxicillin, ticarcillin, and piperacillin. Temocillin, a penicillin with enhanced stability against certain β-lactamases, displayed a considerably lower resistance rate (31.4%).



Color scale: green (low resistance, ≤25%) → yellow (moderate) → red (high resistance, ≥75%).

Figure 1. Antibiotic resistance rates.

Among β-lactam/β-lactamase inhibitor (BL/BLI) combinations, resistance ranged from 37.1% for piperacillin/tazobactam to 66.7% for ticarcillin/clavulanic acid. Cephalosporin resistance was substantial: cefalexin (1st generation) at 75.4%, cefoxitin (2nd generation) at 34.0%, and third-generation cephalosporins ranging from 68.5% (ceftazidime) to 74.7% (cefixime). Cefepime (4th generation) showed 69.7% resistance, and aztreonam (monobactam) exhibited 68.2% resistance.

The carbapenems retained the lowest resistance rates among all antibiotic classes tested: imipenem (10.6%), meropenem (14.3%), and ertapenem (30.4%), yielding a mean carbapenem resistance rate of 18.4%. The novel combination ceftazidime/avibactam demonstrated a resistance rate of 21.7%. The radar plot (**Figure 2**) illustrates the mean resistance rate aggregated per antibiotic class.

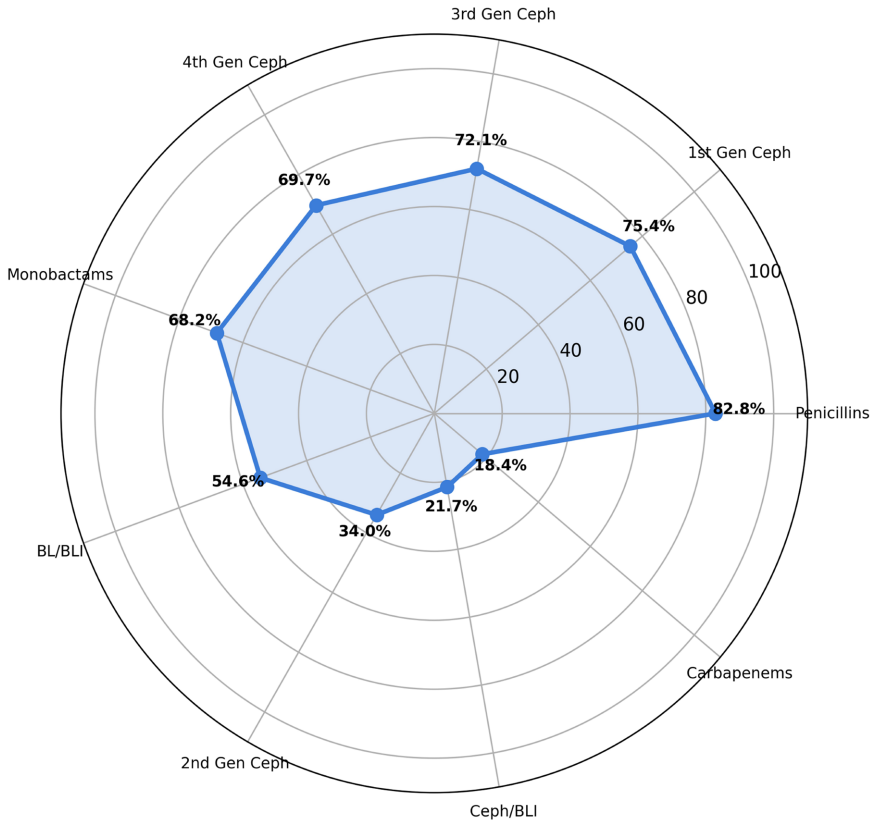
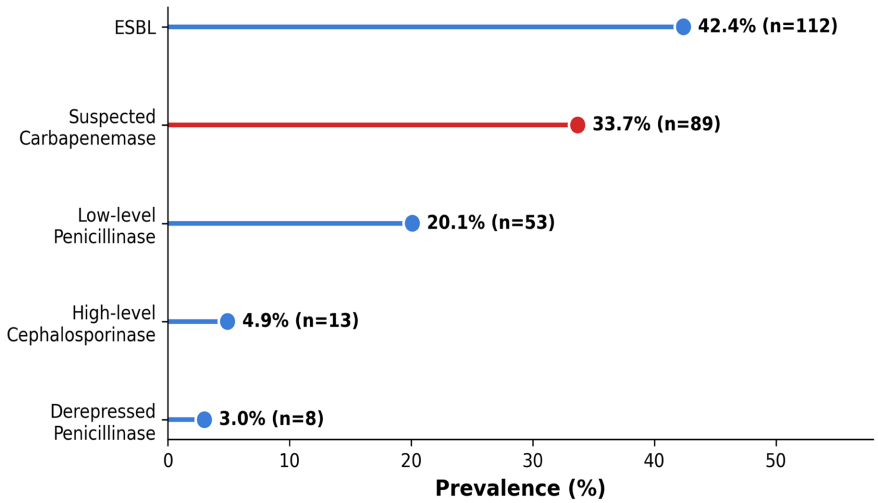


Figure 2. Mean antibiotic resistance rates aggregated per pharmacological class.

2.2.3. Phenotypic Classification of the Strains Studied

The phenotypic classification of the strains based on their susceptibility profiles made it possible to identify the following phenotypes: Extended-spectrum β-lactamase (112 strains or 42.4%), low-level/wild-type penicillinase (53 strains or 20.1%), derepressed penicillinase (8 strains or 3.0%), carbapenemases (89 strains or 33.7%) and high-level cephalosporinase (13 strains or 4.9%). The most observed phenotypes were ESBL (42.4%), followed by the carbapenemase phenotype (33.7%). The derepressed penicillinase phenotype was the least identified (3.0%). Collectively,

ESBL production and suspected carbapenemase production accounted for 76.1% of all resistance mechanisms identified, as shown in Figure 3 below. However, we would like to point out that in some bacterial strains, we found the expression of two phenotypes, such as the ESBL and carbapenemase phenotypes within the same strain.



ESBL = extended-spectrum β-lactamase.

Figure 3. The prevalence of β-lactam resistance mechanisms.

2.2.4. Proportion of Carbapenemase-Producing Strains

A total of 264 *Klebsiella* spp. isolates were subjected to antimicrobial susceptibility testing and subsequently screened for carbapenemase production using the established phenotypic algorithm. Of these, 89 isolates (33.7%) were classified as suspected carbapenemase producers, whereas 175 isolates (66.3%) were classified as non-carbapenemase producers. This finding indicates that approximately one in three clinical *Klebsiella* spp. isolates in the study setting demonstrated phenotypic characteristics consistent with carbapenemase production.

2.2.5. Association between Carbapenemase Production and Socio-Demographic Factors

1) Distribution per age group

The distribution of carbapenemase production status across eight age categories is presented in Figure 4. The highest proportion of suspected carbapenemase producers was observed in the 11 - 20 years age group (55.6%; 5 of 9 patients), while the lowest rate was recorded in the 21 - 30 years age group (27.8%; 10 of 36). The remaining age categories exhibited relatively uniform carbapenemase production rates ranging from 29.0% to 39.2%.

Chi-squared analysis revealed no statistically significant association between carbapenemase production and patient age group ($\chi^2 = 3.620$; df = 3; p = 0.292). Cramér's V was 0.126, indicating a negligible effect size. The elevated rate in the 11 - 20-year-old age group should be interpreted cautiously, given the small sample size (n = 9) in that category.

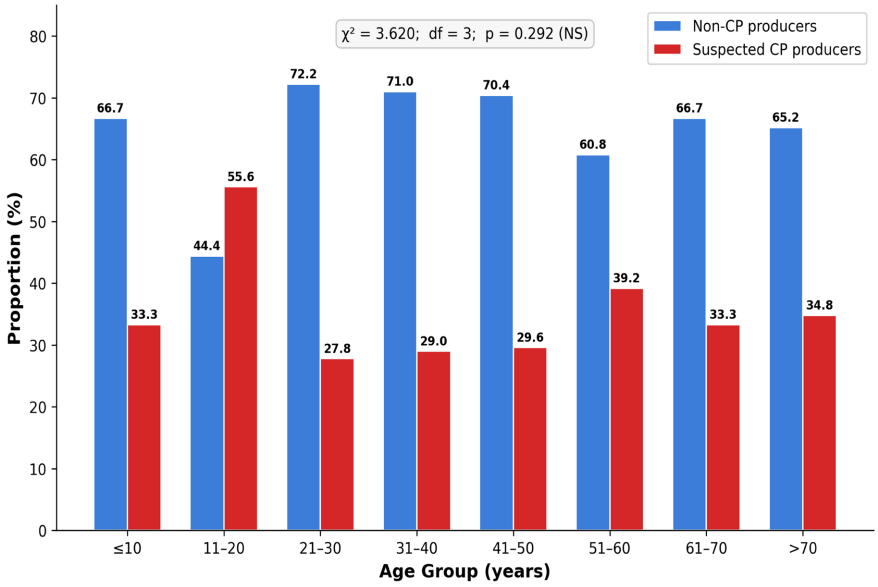


Figure 4. The distribution of carbapenemase production status per age group.

2) Distribution per hospital ward

The distribution of carbapenemase production across nine hospital wards/services is presented in Figure 5. The Medical unit exhibited the highest proportion of suspected carbapenemase producers (48.3%; 28 of 58 isolates), followed by the Intensive Care Unit (ICU; 41.7%; 10 of 24), Pediatrics (38.5%; 5 of 13), and Hemodialysis (33.3%; 4 of 12). The lowest rate was observed among outpatient isolates (17.9%; 7 of 39).

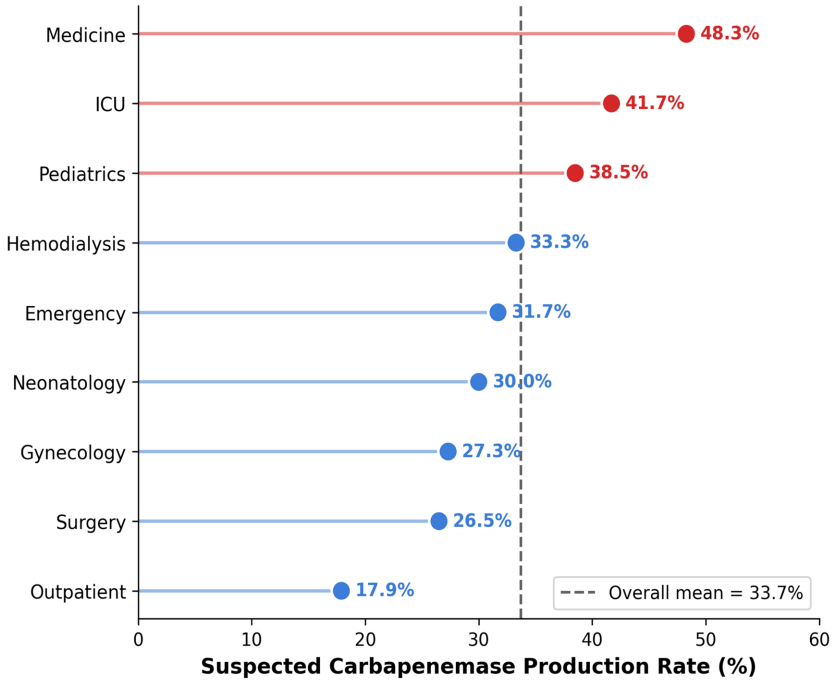


Figure 5. Suspected carbapenemase production rates per hospital ward.

Despite these observed variations, chi-squared analysis did not demonstrate a statistically significant overall association between carbapenemase production and hospital ward ($\chi^2 = 11.829$; df = 8; p = 0.159; Cramér's V = 0.220). However, post hoc odds ratio analysis with Fisher's exact test revealed that isolates from the medical ward had significantly elevated odds of carbapenemase production compared to all other wards combined (OR = 2.25; 95% CI: 1.23 - 4.11; p = 0.011). Conversely, outpatient isolates demonstrated significantly reduced odds (OR = 0.37; 95% CI: 0.16 - 0.89; p = 0.026), suggesting a protective effect of non-hospitalization status.

2.2.6. Comparative Resistance Profile: Carbapenemase Producers vs. Non-Producers

A comparative analysis of antibiotic resistance rates between the two groups is detailed in Table 2. Both groups demonstrated identical universal resistance (100%) to amoxicillin, ticarcillin, and piperacillin. However, suspected carbapenemase producers exhibited significantly elevated resistance rates across the majority of the remaining 16 antibiotics tested.

Table 2. Comparative antibiotic resistance rates (%) between *Klebsiella* spp. non-carbapenemase-producers (n = 175) and suspected carbapenemase-producers (n = 89).

Antibiotic	Non-CP (%)	CP (%)	Δ (%)	p-value
Amoxicillin	100	100	-	NS
Amoxicillin/clavulanic acid	53.7	72.0	**18.3**	**<0.01**
Ticarcillin	100	100	-	NS
Ticarcillin/clavulanic acid	60.0	80.0	**20.0**	**<0.01**
Piperacillin	100	100	-	NS
Piperacillin/tazobactam	25.1	60.7	**35.6**	**<0.001**
Temocillin	21.7	50.6	**28.9**	**<0.001**
Cefalexin	69.7	86.5	**16.8**	**<0.01**
Cefoxitin	22.3	57.3	**35.0**	**<0.001**
Cefixime	68.6	86.5	**17.9**	**<0.01**
Ceftazidime	61.1	83.1	**22.0**	**<0.001**
Ceftazidime/avibactam	10.3	44.0	**33.7**	**<0.001**
Cefotaxime	65.7	84.3	**18.6**	**<0.001**
Ceftriaxone	66.9	85.4	**18.5**	**<0.001**
Cefepime	63.4	82.0	**18.6**	**<0.001**
Aztreonam	61.7	81.0	**19.3**	**<0.001**
Imipenem	5.1	21.3	**16.2**	**<0.001**
Meropenem	6.3	30.3	**24.0**	**<0.001**
Ertapenem	22.9	45.0	**22.1**	**<0.001**

CP = carbapenemase producer; Δ = absolute difference between groups; NS = not significant. Significant p-values are highlighted in bold red.

The most pronounced differences ($\Delta \geq 30\%$) were observed for piperacillin/tazobactam (60.7% vs. 25.1%; $\Delta = 35.6\%$), cefoxitin (57.3% vs. 22.3%; $\Delta = 35.0\%$), and ceftazidime/avibactam (44.0% vs. 10.3%; $\Delta = 33.7\%$). All three carbapenems showed significantly higher resistance in the carbapenemase-producing group: imipenem (21.3% vs. 5.1%), meropenem (30.3% vs. 6.3%), and ertapenem (45.0% vs. 22.9%), all with $p < 0.001$. Statistically significant differences were observed for 14 of 19 antibiotics. The five antibiotics without significant differences were amoxicillin, ticarcillin, and piperacillin (both groups at 100%), as well as imipenem and ertapenem.

3. Discussion

3.1. Socio-Demographic Characteristics

Our study took place in the bacteriology laboratories of three public reference hospitals in the city of Yaounde (YUTH, YGH and GOPHY). The bacteriological analyses took place at the bacteriology laboratory of the YUTH. We included 264 strains of *Klebsiella* spp. isolated from the clinical specimens of 264 patients who had previously undergone a medical consultation. The majority of strains were obtained from patients aged [40 - 50] years (20.5%), followed by those in the [50 - 60] and [60 - 70] age groups (19.3% of strains each). In this study, there was a predominance of *Klebsiella* spp. in the clinical specimens of women. This predominance is also found in Kenkouo's study [7]. This is thought to be due to the high frequency of urinary tract infections in female patients. Of the isolates obtained, *Klebsiella pneumoniae pneumoniae* was the most common subspecies (n = 164 or 62.1%), followed by *K. pneumoniae ozaenae* and *K. pneumoniae rhinoscleromatis*, representing 18.6% (n = 49) and 13.3% (n = 35) respectively. Urine was the most representative clinical specimen, followed by suppurations and blood, accounting for 39.8%, 31.1% and 28.8%, respectively. Mbamyah *et al.* [8] had also found that the lead clinical specimen was urine (68.7%), followed by suppurations (13.5%). This predominance can be explained by the fact that *Klebsiella* spp. is a bacterium of the intestinal commensal flora and can easily be found in the urinary tract due to poor hygiene, especially in women, given their genital anatomy.

Most patients were inpatients (85.2%), while 14.8% were outpatients. According to the study by Ebongue and collaborators in 2015 [9] on the evolution of antibiotic resistance in enterobacteriaceae isolated from clinical specimens at the Douala General Hospital, it appears that the majority of strains came from inpatients, *i.e.*, 58.39%. Our rate is much higher than that found in their study, and this is explained by the fact that we were particularly interested in the *Klebsiellas* and not in all enterobacteriaceae. In addition, this bacterium is most often indexed as a nosocomial germ, which would explain the tendency to encounter it most often in inpatients.

3.2. Antibiotic Resistance Profile

Antibiotic susceptibility results show that the *Klebsiella pneumoniae* studied shows

a very high level of resistance to the antibiotics tested. The universal resistance (100%) observed against unprotected penicillins (amoxicillin, ticarcillin, piperacillin) is concordant with the intrinsic chromosomal β-lactamase (SHV-1) production characteristic of *Klebsiella* spp. and the high prevalence of acquired resistance determinants in this genus [10] [11]. These findings corroborate with published literature and definitively confirm the clinical obsolescence of unprotected penicillins for the empirical treatment of *Klebsiella* spp. Infections [12].

The isolated species were tested against 19 antibiotics belonging to the beta-lactam family. The high resistance rates observed for third-generation cephalosporins (cefotaxime: 72.1%; ceftriaxone: 73.0%; cefixime: 74.7%) underscore the extensive dissemination of ESBL-producing strains within the study population. These rates exceed the global median for ESBL-producing enterobacteriaceae reported by Mbamyah *et al.* [13] and the World Health Organization (WHO) Global Antimicrobial Resistance and Use Surveillance System (GLASS), which documents median rates of approximately 40% - 60% in low- and middle-income countries (LMICs) [14]. The wild resistance phenotype has been confirmed for all of our strains (CASFM, 2023). High resistance rates were observed with cefalexin, cefixime, ceftriaxone and cefotaxime (75.4%; 74.7%; 73.0% and 72.1% respectively). High resistance to cefalexin, cefixime, ceftriaxone and cefotaxime is consistent with the worldwide expansion of CTX-M-type ESBL enzymes [15] [16].

Notably, the carbapenem class maintained the lowest overall resistance rates among all antibiotic classes evaluated, with imipenem demonstrating the highest retained susceptibility (resistance: 10.6%), followed by meropenem (14.3%) and ertapenem (30.4%). The 18.4% mean carbapenem resistance represents a clinically significant departure from the near-universal carbapenem susceptibility reported in earlier surveillance studies [14] [17]. The disproportionately elevated ertapenem resistance relative to imipenem and meropenem is pharmacologically predictable, as ertapenem is more susceptible to hydrolysis by carbapenem-hydrolyzing enzymes, particularly OXA-48-like class D β-lactamases [18] [19]. This differential susceptibility pattern supports the established practice of using ertapenem as a sentinel screening marker for carbapenemase production.

Overall, we observe a slight increase in the level of antibiotic resistance compared to a 2015 study (Betbeui *et al.*, 2015) [20] and a 2024 study (Chafa *et al.*, 2024) [21] with resistance rates to C1G (cefalotin), C2G (cefoxitin), C3G (ceftazidime), C4G (cefepime), monobactam (aztreonam) and imipenem. This observed increase in the level of resistance to β-lactam is probably the consequence of the inappropriate prescription and uncontrolled use of antibiotics in this family [22].

The resistance rate to ceftazidime/avibactam (21.7%) merits particular attention. This combination agent was specifically engineered to overcome resistance mediated by class A serine β-lactamases (including KPC-type carbapenemases) and class D β-lactamases (e.g., OXA-48). The observed resistance may therefore indicate the presence of class B metallo-β-lactamases (MBLs), such as NDM, VIM, or IMP, which are intrinsically refractory to avibactam inhibition [19] [23]. This hypoth-

esis warrants verification through molecular characterization studies, including targeted PCR or whole-genome sequencing.

3.3. Identified Resistant Phenotypes

Based on a precise arrangement of antibiotic discs with or without inhibitors, and the rigorous measurement of inhibition diameters, we were able to perform a phenotypic characterization of 264 strains of *Klebsiella*. It appears from this classification that the distribution of phenotypic frequencies is as follows: extended-spectrum β-lactamase (42.4%), low-level/wild-type penicillinase (20.1%), derepressed penicillinase (3.0%), carbapenemases (33.7%) and high-level cephalosporinase (4.9%). The most observed phenotypes are ESBL (42.4%), followed by the carbapenemase phenotype (33.7%). The high prevalence of "resistant" phenotypes, acquired mainly from the strains (79.9%) isolated from inpatient clinical specimens, is justified by the existence of increased antibiotic selection pressure [24]. The proportion of strains of *Klebsiella* spp. ESBL producers is 42.4%. According to these results, we can observe an increase in the frequency of ESBL-producing strains in Cameroon because a study carried out at the Yaounde Central Hospital in 2005 had revealed a prevalence of 18.8% of *Klebsiella* spp. ESBL producers [25] and the one carried out at the YUTH in 2015 showed a frequency of 30.3% of *Klebsiella* spp. ESBL producers [20]. However, the one conducted in 2023 by Mbamyah *et al.* revealed a percentage of 71.6% [8]. Moreover, in France, the RAISIN states that 17% of *K. pneumoniae* responsible for bacteremia are ESBL producers. In addition, *Klebsiella* spp. is one of the bacteria known to produce ESBL [26]. Our results are quite high as compared to those of other countries. This could be explained by the regular consumption of β-lactams, which have become increasingly familiar to bacteria, or by the prolonged hospital stays of patients.

3.4. Frequency of Carbapenemase Production

The present study revealed that 33.7% of clinical *Klebsiella* spp. isolates were suspected of producing carbapenemases, representing a substantial epidemiological burden. This prevalence is notably elevated compared to several recently published studies from sub-Saharan Africa, where reported carbapenem-resistant enterobacteriaceae (CRE) rates generally range between 5% and 25%, and a study carried out by Betbeui *et al.* in Cameroon (2015), which revealed an 11.1% rate of carbapenemase-producing strains [20] and the one conducted in 2024 by Mbamyah *et al.*, revealed a percentage of 10.7% [8]. The elevated prevalence observed in our setting likely reflects the confluence of multiple contributing factors, including the escalating selective pressure arising from empirical carbapenem prescribing, suboptimal infection prevention and control (IPC) practices, and the facilitation of horizontal gene transfer of resistance determinants within the healthcare environment. This high rate observed in our study could be explained by the fact that *E. coli* and *Klebsiella* spp. are the enterobacteriaceae most reported in literature as being involved in resistance linked to carbapenemase production [24]. The concurrent high

prevalence of ESBL (42.4%) and suspected carbapenemase production (33.7%) in this study population is particularly concerning, as it suggests a progressive evolutionary trajectory of resistance mechanisms. In this paradigm, ESBL production may represent a transitional phenotype preceding the acquisition of carbapenem-hydrolyzing enzymes, consistent with the stepwise resistance model described in literature.

3.5. Socio-Demographic and Ward-Level Associations

The predominance of carbapenemase-producing strains in inpatients of the medical, intensive care and pediatric wards (48.3%, 41.7% and 38.5% respectively) can be explained by the fact that ESBL and carbapenemases are enzymes produced mainly by nosocomial strains responsible for hospital epidemics [27] and in long-term care facilities [28].

The absence of a statistically significant association between carbapenemase production and patient age group ($\chi^2 = 3.620$; p = 0.292; Cramér's V = 0.126) suggests that carbapenemase-producing *Klebsiella* spp. infections affect patients across all age categories without a clear age-related predilection. This finding implies that the primary risk factors for acquisition of carbapenemase-producing organisms are more likely related to healthcare-associated exposures (e.g., duration of hospitalization, antibiotic exposure, invasive device utilization) rather than intrinsic demographic characteristics. The elevated rate in the 11 - 20 years age group (55.6%) should be interpreted with considerable caution, given the small sample size (n = 9), which substantially limits the statistical reliability of this estimate.

The overall chi-squared test did not demonstrate a statistically significant association between carbapenemase production and hospital ward ($\chi^2 = 11.829$; p = 0.159). However, the post hoc odds ratio analysis yielded clinically significant findings: the medical unit demonstrated 2.25-fold higher odds of harboring carbapenemase-producing isolates relative to all other wards combined (OR = 2.25; 95% CI: 1.23 - 4.11; p = 0.011). This association likely reflects the characteristic clinical profile of patients admitted to medical wards, including prolonged lengths of stay, frequent empirical broad-spectrum antibiotic administration, the presence of multiple comorbidities, and increased rates of invasive device utilization, all recognized risk factors for multidrug-resistant organism (MDRO) acquisition.

The relatively high carbapenemase production rates observed in the ICU (41.7%) and Pediatrics (38.5%), although not individually statistically significant, are consistent with the well-established literature identifying these settings as high-risk environments for MDRO colonization and transmission [29] [30]. The significantly lower odds observed among outpatient isolates (OR = 0.37; 95% CI: 0.16 - 0.89; p = 0.026) provide complementary evidence that healthcare-associated exposure plays a pivotal role in the acquisition of carbapenemase-producing strains, consistent with the nosocomial transmission paradigm described for CRE globally

[31] [32].

3.6. Comparative Resistance between Carbapenemase Producers and Non-Producers

The significantly elevated resistance rates observed in suspected carbapenemase producers across 14 of 19 antibiotics tested ($p < 0.001$ for the majority) demonstrate the extensive co-resistance phenotype typically associated with carbapenemase production [19] [31]. The most clinically impactful differences were observed for piperacillin/tazobactam ($\Delta = 35.6\%$), cefoxitin ($\Delta = 35.0\%$), and ceftazidime/avibactam ($\Delta = 33.7\%$). The substantially higher cefoxitin resistance (57.3% vs. 22.3%) in the carbapenemase-producing group is particularly noteworthy, as cefoxitin resistance is characteristically associated with AmpC cephalosporinase production or porin loss mechanisms that frequently co-exist with carbapenemase production on multi-resistance plasmids [29].

These observations support the paradigm of co-resistance and co-selection, whereby mobile genetic elements encoding carbapenemases (particularly large conjugative plasmids of the IncF, IncL/M, and IncN families) frequently carry additional resistance determinants conferring resistance to aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, and other β-lactams. The resulting pan-resistant or extensively drug-resistant (XDR) phenotype critically narrows the therapeutic armamentarium, often limiting effective treatment options to polymyxins, tigecycline, and potentially ceftazidime/avibactam, underscoring the urgent need for comprehensive antimicrobial stewardship and robust infection prevention programs.

3.7. Clinical Implications and Recommendations

The findings of this study carry substantial implications for empirical antibiotic prescribing, infection control policy, and antimicrobial resistance (AMR) surveillance. With approximately one-third of *Klebsiella* spp. isolates exhibiting suspected carbapenemase production, clinicians should exercise heightened vigilance in selecting empirical antibiotic regimens, particularly for patients admitted to high-risk wards such as the medical and the ICU. Routine phenotypic carbapenemase screening should be integrated into clinical microbiology laboratory workflows, complemented where feasible by molecular confirmation methods (e.g., multiplex PCR for blaKPC, blaNDM, blaOXA-48, blaVIM, blaIMP) to guide targeted therapy.

Institutional antimicrobial stewardship programs should prioritize the following strategic interventions: 1) restriction of empirical carbapenem use through prospective audit and feedback mechanisms; 2) promotion of carbapenem-sparing combination regimens for documented CRE infections, guided by antimicrobial susceptibility testing; 3) adoption of rapid diagnostic technologies for carbapenemase detection to minimize time-to-appropriate therapy; and 4) implementation of de-escalation protocols following receipt of definitive culture and

susceptibility results.

Enhanced infection prevention and control measures, including rigorous contact precautions for CRE-colonized or infected patients, active surveillance cultures on admission to high-risk units, environmental decontamination protocols, and antimicrobial stewardship integration, are essential to curtail the nosocomial dissemination of carbapenemase-producing *Klebsiella* spp.

4. Study Limitations

Several limitations of this study should be acknowledged when interpreting the findings. First, carbapenemase production was classified based on phenotypic screening algorithms rather than confirmed by genotypic methods; this may result in both false-positive identifications (overestimation of true carbapenemase production) and false-negative classifications (failure to detect carbapenemases with atypical phenotypic profiles). Second, the cross-sectional study design precludes the assessment of temporal trends and causal relationships between risk factors and carbapenemase acquisition. Third, sample sizes in several subgroups (particularly the 11 - 20 years age category and certain hospital wards) were limited, reducing the statistical power available for detecting clinically meaningful associations. Fourth, the absence of molecular characterization (PCR, sequencing) prevented identification of the specific carbapenemase genes and their associated mobile genetic elements, which would provide critical information regarding the molecular epidemiology and transmissibility of the resistance determinants.

Future studies should address these limitations through multi-center prospective designs incorporating molecular typing, longitudinal follow-up, and risk factor analysis with multivariable regression modeling.

Acknowledgements

The authors express profound gratitude to the bacteriology laboratory personnel of the Yaounde University Teaching Hospital (YUTH), Yaounde General Hospital and Yaounde Gynecological-Obstetric and Pediatric Hospital for their cooperation and technical assistance in isolating, arranging, sorting and reviving bacterial isolates.

Authors' Contributions

B. A. Chafa, H. K. Gonsu, E. E. M. Lyonga, D. Adiogo and W. A. Baiye conceived the study and designed it. B. A. Chafa and H. K. Gonsu carried out the data collection and lab work. B. A. Chafa, L. F. Kana, M. Tembong, A. D. Teudjieu and W. A. Baiye carried out data analysis. H. K. Gonsu, V. N. Bitoungui, R. G. Essomba, M. P. Ngogang and C. Killa supervised the lab work and data collection. The general supervision was carried out by E. E. M. Lyonga, D. Adiogo, V. P. Djientcheu and H. K. Gonsu. E. E. M. Lyonga, B. A. Chafa, H. K. Gonsu, W. A. Baiye, A. D. Teudjieu, L. F. Kana, C. Killa, V. N. Bitoungui, R. G. Essomba, M. P. Ngogang and D. Adiogo drafted the article. All the authors reviewed the article. All the authors read and

agreed to the final manuscript.

Consent

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

Ethical Approval

The study was approved and confirmed under the rules and regulations of research in the Department of Microbiology and Immunology, Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Cameroon. Therefore, at the beginning of the research point, ethical approval and authorization were issued while referring to the Yaounde University Teaching Hospital (YUTH), Yaounde General Hospital and Yaounde Gynecological-Obstetric and Pediatric Hospital. The regulations for research were approved by the ethical committee of the hospitals and the microbiology laboratories as well.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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