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Distribution and Resistance Profile of *Klebsiella pneumoniae* Strains at the Yaoundé Central Hospital: Analysis and Implications

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Abstract

Introduction: The misuse of antibiotics has driven the emergence of antimicrobial resistance, with certain bacterial species, including *Klebsiella pneumoniae*, initially susceptible to most antibiotics, now exhibiting resistance to multiple antimicrobial agents. The objective of this study was to investigate the distribution and antimicrobial resistance profiles of *Klebsiella pneumoniae* strains isolated at the Yaoundé Central Hospital. **Methodology**: The study was conducted over a period of 4 months (March 1st, 2023-July 1st, 2023) and involved 32 strains isolated from bacterial cultures performed on patients, regardless of sex. The different *Klebsiella pneumoniae* strains were isolated using conventional methods. Identification, antibiogram, and detection of resistance enzyme production were generated using the VITEK 2 system. The Carbapenem-resistant K.N.I.V.O. kit was used to detect carbapenemases. Data analysis was performed using EXCEL 2019 software. **Results**: Out of 196 samples collected from various biological products, 32 *Klebsiella pneumoniae* strains were isolated, representing 16.32% (196/32). Urine samples were most frequently affected, accounting for 53.125%. The emergency department was the most represented (40.63%) by these isolates. The mean age was 50 years, with a minimum of 20 years and a maximum of 80 years. The sex ratio was equal to 1. The identified strains were resistant to cefotaxime (78.13%), cefoxitin (62.50%), tobramycin (71.88%), gentamicin (56.25%), ofloxacin (81.25%), and cotrimoxazole (78.13%). 78.25% were ESBL producers. Three strains were resistant to carbapenems, accounting for 9.37%;

one of which exhibited the NDM type. **Conclusion**: This study highlights the evolving bacterial resistance to antibiotics, which requires adequate measures through the strengthening of the Antimicrobial Resistance (AMR) program in Cameroon.

Keywords

Klebsiella pneumoniae, Resistance, Antibiotics, Carbapenemase

1. Introduction

Antibiotic resistance is a major public health concern worldwide. In 2017, the World Health Organization (WHO) identified bacteria with urgent needs for new antibiotics, categorizing Klebsiella as a critical priority [1]. *Klebsiella pneumoniae* is a gram-negative pathogen that poses a significant threat to public health due to its ability to cause a wide range of infections, particularly in healthcare settings. Found in water, soil, and dust, it is also present on human skin, mucous membranes, and especially the upper respiratory tract and digestive tract [2]. It causes infections that are often challenging to treat due to antibiotic resistance.

The emergence of *Klebsiella pneumoniae* strains resistant to antibiotics has become a critical issue, as it limits the effectiveness of therapeutic options. This antibiotic resistance affects certain families of antibiotics, including aminoglycosides, third-generation cephalosporins [3], and more recently, carbapenems [4], which are last-resort antibiotics. Carbapenems are used to treat multi-resistant strains and represent the last resort in antibiotic therapy against extended-spectrum beta-lactamase-producing Enterobacteriaceae. To date, their spectrum of activity is progressively narrowing with the emergence of several resistance mechanisms [5] [6]. Infections with extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* are a significant cause of hospital morbidity and mortality [7].

Since the early 2000s, carbapenem resistance has spread to all regions of the world, raising serious concerns [8] and posing a significant public health problem. According to the European Antibiotic Resistance Surveillance System (EARSS) in 2014, the resistance rate of *Klebsiella pneumoniae* to carbapenems was 31.5% in Romania, 62.3% in Greece, and 0.9% in Croatia [9]. According to the antibiotic resistance surveillance network, out of 5922 multi-resistant bacteria (all species combined) isolated in hospitals, 57% of *Klebsiella pneumoniae* strains were resistant to beta-lactams through the production of extended-spectrum beta-lactamases [8].

Due to the challenges of treatment, we deemed it necessary to conduct this study with the aim of contributing to the control and prevention of Klebsiella pneumoniae antibiotic resistance. In this article, we focus on the distribution and resistance profile of *Klebsiella pneumoniae* strains isolated at the Central Hospital of Yaoundé, with the goal of providing information on the prevalence and resistance patterns of this pathogen in a hospital setting.

2. Methodology

2.1. Population and Study Type

This study was a descriptive cross-sectional study conducted over a period of 4 months, from March 1st, 2023 to July 1st, 2023. It was carried out at the Central Hospital of Yaoundé, on *Klebsiella pneumoniae* strains from various biological samples.

2.2. Ethical Considerations

Ethical clearance was obtained from the National Ethics Committee (No. 3790CEI-Udo/06/2023/M) and authorization for sample collection was granted by the Director of the Central Hospital of Yaoundé.

2.3. Sample Collection

The strains were isolated from various clinical specimens (pus, blood cultures, urinalysis, HVS, catheter tips) from different hospital departments, collected from patients who were prescribed a bacteriological examination. The collection of the different samples was performed in strict accordance with asepsis and antisepsis rules, following procedures that are up-to-date and compliant with standard protocols.

Samples from patients who were undergoing antibiotic therapy and had underlying comorbidities were excluded from the study.

2.4. Identification and Antimicrobial Resistance Profiling

Standard microbiological techniques were used to isolate *Klebsiella pneumoniae* strains. Isolation and subculturing were performed using the quadrant streaking method. Identification, antibiotic resistance profiles, extended-spectrum beta-lactamase (ESBL) production, carbapenemase production, penicillinase production, and cephalosporinase production were determined using the VITEK 2 system. The VITEK 2 is an automated identification and antimicrobial susceptibility testing system that uses card-based technology and features an automated reading system and software. It is also capable of detecting resistance enzyme production, such as beta-lactamase or carbapenemase production.

Furthermore, extended-spectrum beta-lactamase (ESBL) production was also determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBL detection was performed on Muller-Hinton agar. The characteristic "champagne cork" effect indicative of ESBL presence was observed by placing third-generation cephalosporin disks (Cefotaxime, Ceftazidime) 3 cm away from the Ticarcillin + Clavulanic Acid disk. The presence of a synergistic effect between Ticarcillin + Clavulanic Acid and the third-generation cephalosporin, characterized by a "champagne cork" shape, confirmed ESBL production.

The type of carbapenemase was detected using **the Carba K.N.I.V.O. kit**. This kit is a test that utilizes a sandwich immuno-chromatography method.

2.5. Data Analysis.

Data analysis was performed using Excel 2019 software. The analysis was descriptive, involving frequency calculations. Results were expressed in tables and figures.

3. Results

Following the applied method, we were able to collect and identify 32 strains of *Klebsiella pneumoniae*. The results are presented as follows.

3.1. Characteristics of the Isolates

Table 1 shows that the majority of *Klebsiella pneumoniae* strains came from urine samples (53.125%).

Table 1. Distribution of *Klebsiella pneumoniae* strains by sample type.

Samples	Klebsiella Count	Percentage
Blood culture	1	3.125%
Urinalysis	**17**	**53.125%**
HVS	1	3.125%
PUS	10	31.25%
Catheter tip	3	9.375%
Total	32	100%

The majority of *Klebsiella pneumoniae* isolates originated from the Emergency Department (40.63%), as shown in Figure 1.

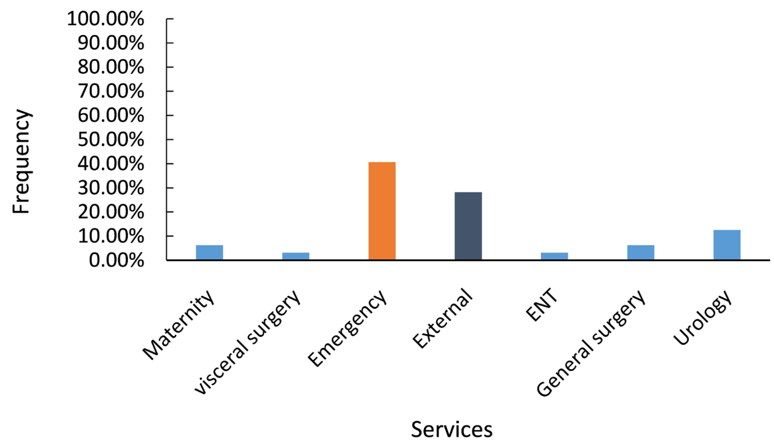

Figure 1. Distribution of *Klebsiella pneumoniae* isolates by department of origin.

Figure 2 shows that the age group most affected by these *Klebsiella pneumoniae* strains was [50 - 60[.

The pie chart in Figure 3 shows an equal distribution of *Klebsiella pneumoniae* isolates by gender.

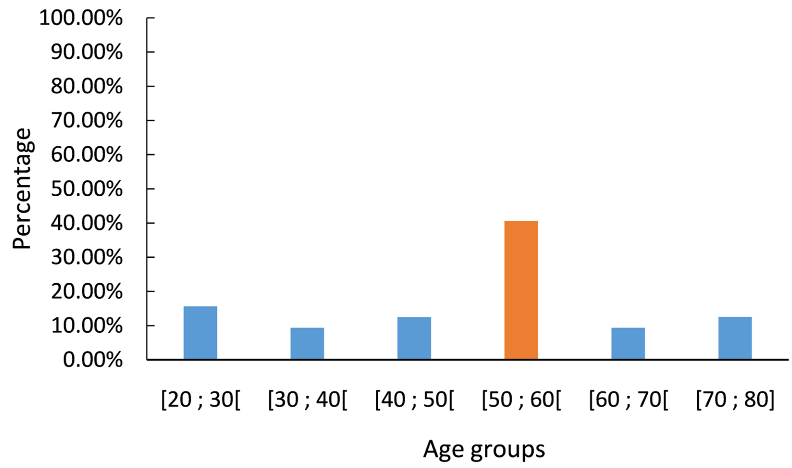

Figure 2. Distribution of *Klebsiella pneumoniae* isolates by age group.

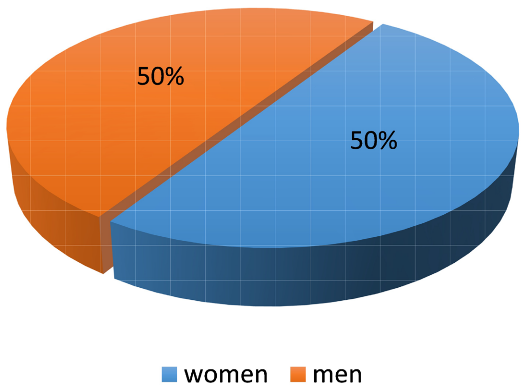

Figure 3. Distribution of *Klebsiella pneumoniae* isolates by patient gender.

3.2. Resistance Profile of Isolates

Pus samples from the Emergency Department, particularly among male patients, exhibited resistance to all tested antibiotics.

Table 2 shows that statistically, pus-derived strains exhibited high resistance rates to ampicillin (100%), ticarcillin (100%), followed by cefotaxime (90%), trimethoprim-sulfamethoxazole (90%), cefoxitin, ofloxacin, and gentamicin (80%), whereas imipenem and ertapenem were the most effective antibiotics (90.63% susceptibility).

Table 3 displays the resistance profile of Klebsiella strains according to department. Strains from the Emergency Department were 100% resistant to ticarcillin and 92.30% resistant to ampicillin. Following these antibiotics, resistance was also observed to trimethoprim-sulfamethoxazole (84.61%), cefotaxime, tobramycin, and ofloxacin (76.92% each).

Table 2. Resistance distribution by sample type.

Antibiotics Effective	Urinaysis n = 17	PUS n = 10	HVS n = 1	Catheter tip n = 3	Blood culture n = 1
IMI	0 (0%)	**1 (10%)**	0 (0%)	0 (0%)	0 (0%)
ERTA	0 (0%)	**2 (20%)**	0 (0%)	0 (0%)	0 (0%)
CTX	12 (70.58%)	**9 (90%)**	0 (0%)	3 (100%)	1 (100%)
FOX	9 (52.94%)	**8 (80%)**	0 (0%)	2 (66.66%)	1 (100%)
CAZ	9 (52.94%)	**6 (60%)**	0 (0%)	1 (33.33%)	0 (0%)
PTZ	*9 (52.94%)*	**5 (50%)**	0 (0%)	2 (66.66%)	1 (100%)
AMP	15 (88.23%)	**10 (100%)**	1 (100%)	3 (100%)	1 (100%)
TIC	15 (88.23%)	**10 (100%)**	1 (100%)	3 (100%)	1 (100%)
AK	6 (35.29%)	**2 (20%)**	0 (0%)	0 (0%)	0 (0%)
TOB	12 (70.58%)	**8 (80%)**	0 (0%)	2 (66.66%)	1 (100%)
CN	8 (47.05%)	**8 (80%)**	0 (0%)	2 (66.66%)	0 (0%)
CIP	6 (35.29%)	**5 (50%)**	0 (0%)	0 (0%)	0 (0%)
OFX	15 (88.23%)	**8 (80%)**	0 (0%)	2 (66.66%)	1 (100%)
SXT	13 (76.47%)	**9 (90%)**	0 (0%)	2 (66.66%)	1 (100%)
F	3 (17.64%)	2 (20%)	0 (0%)	1 (33.33%)	0 (0%)

Table 3. Frequency of resistance by department.

Antibiotics	Maternity n = 2	Vis surg n = 1	External n = 9	Emergency n = 13	ENT n = 1	Gen surg n = 2	Urology n = 4
IMI	0 (0%)	0 (0%)	0 (0%)	**1 (7.69%)**	0 (0%)	0 (0%)	0 (0%)
ERTA	0 (0%)	0 (0%)	0 (0%)	**2 (15.38%)**	0 (0%)	0 (0%)	0 (0%)
CTX	0 (0%)	1 (100%)	6 (66.66%)	**10 (76.92%)**	1 (100%)	2 (100%)	4 (100%)
FOX	1 (50%)	1 (100%)	5 (55.55%)	**7 (53.84%)**	1 (100%)	2 (100%)	3 (75%)
CAZ	1 (50%)	0 (0%)	4 (44.44%)	**7 (53.84%)**	0 (0%)	2 (100%)	2 (50%)
PTZ	1 (50%)	0 (0%)	4 (44.44%)	**8 (61.53%)**	0 (0%)	2 (100%)	2 (50%)
AK	1 (50%)	1 (100%)	5 (55.55%)	**7 (53.84%)**	1 (100%)	2 (100%)	3 (75%)
AMP	2 (100%)	1 (100%)	9 (100%)	**12 (92.30%)**	1 (100%)	2 (100%)	3 (75%)
TIC	2 (100%)	1 (100%)	7 (77.77%)	**13 (100%)**	1 (100%)	2 (100%)	4 (100%)
TOB	0 (0%)	1 (100%)	6 (66.66%)	**10 (76.92%)**	1 (100%)	2 (100%)	3 (75%)
CIP	0 (0%)	1 (100%)	3 (33.33%)	**5 (38.46%)**	1 (100%)	1 (50%)	0 (0%)
OFX	1 (50%)	0 (0%)	7 (77.77%)	**10 (76.92%)**	1 (100%)	1 (50%)	4 (100%)
SXT	1 (50%)	1 (100%)	5 (55.55%)	**11 (84.61%)**	1 (100%)	2 (100%)	4 (100%)
CN	0 (0%)	1 (100%)	3 (33.33%)	**9 (69.23%)**	0 (0%)	2 (100%)	3 (75%)
F	0 (0%)	0 (0%)	1 (11.11%)	3 (23.07%)	0 (0%)	0 (0%)	2 (50%)

Figure 4 shows that in our study, men exhibited higher resistance to antibiotics, particularly to ampicillin (93.75%), ticarcillin (93.75%), ofloxacin (93.75%), trimethoprim-sulfamethoxazole (93.75%), cefotaxime (87.5%), and cefoxitin (81.27%).

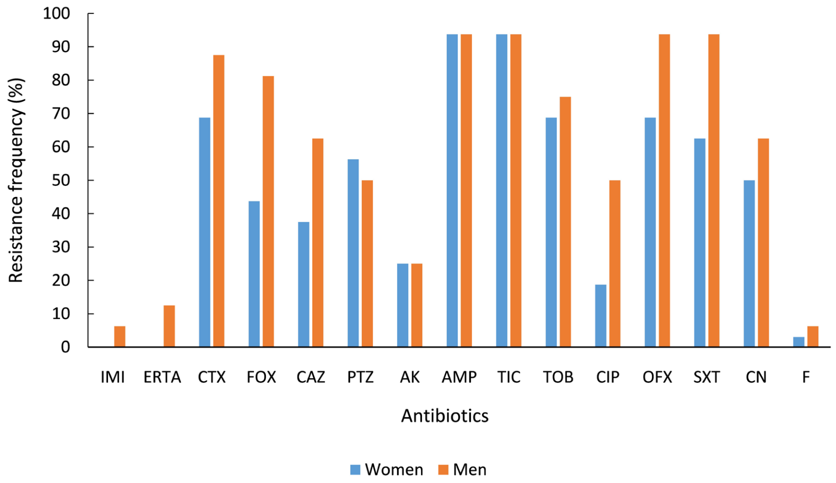

Figure 4. Antibiotic resistance by gender.

3.3. Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* by Bacterial Characteristic

Figure 5 reveals that the most produced resistance enzymes were penicillinases (100%), followed by ESBLs (78.25%).

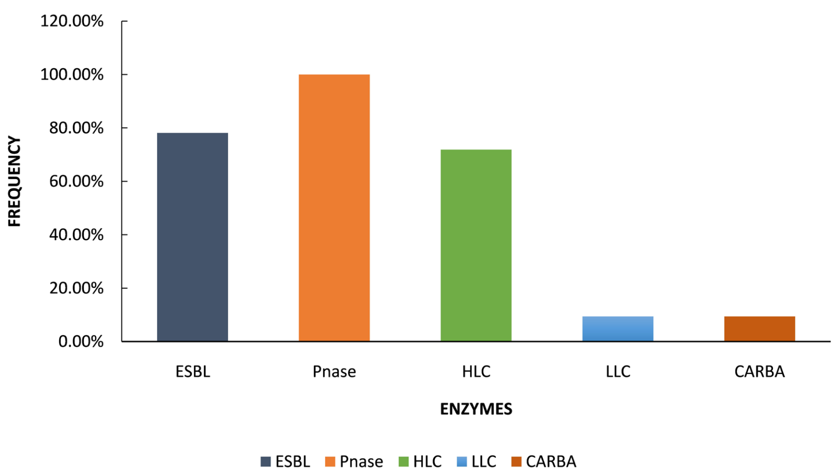

Figure 5. Frequency of resistance enzymes.

Among the identified enzymes, males were found to be the most affected, as presented in Table 4.

Emergency department and urine samples are most affected by resistance enzyme production (Figure 6).

Table 4. Enzyme distribution by gender.

Resistance Phenotypes	Number of women	% Women	Number of men	% Men
ESBL	11	68.75%	14	**87.5%**
Pnase	16	100%	16	**100%**
HLC	11	68.75%	12	**75%**
LLC	0	0%	3	**18.75%**
CARBA	1	6.25%	2	**12.5%**

Légende: ESBL (Extended Spectrum Betalactamase); Pnase (Penicillinase); HLC (High Level Cephalosporinase); LLC (Low Level Cephalosporinase) CARBA (Carbapenemase).

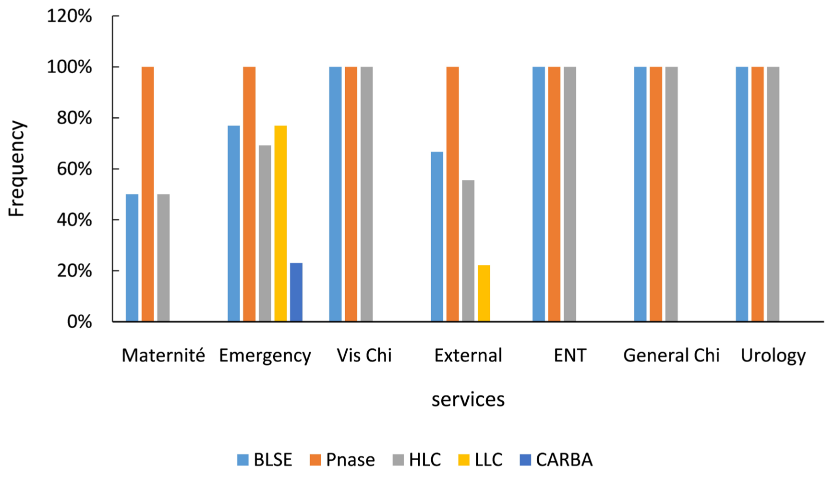

Figure 6. Frequency of resistance enzymes by department.

Blood culture samples show the highest expression of resistance enzymes, as illustrated in Figure 7.

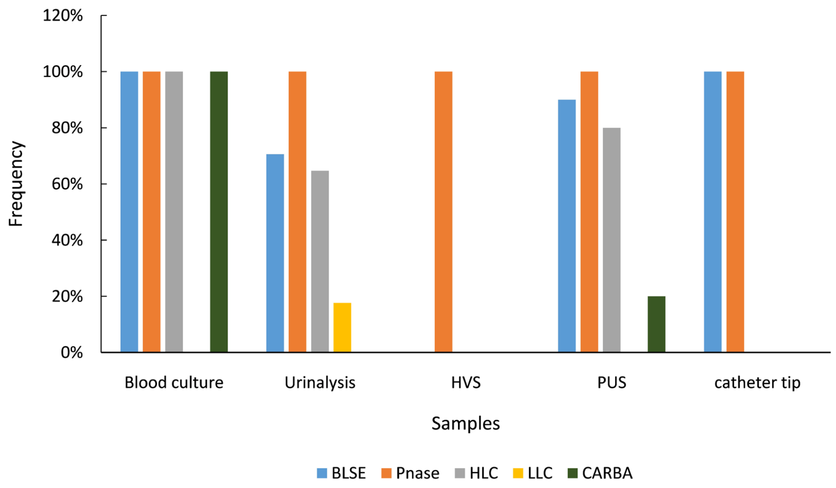

Figure 7. Resistance enzymes by sample type.

NDM was the only carbapenemase detected, isolated from a 59-year-old female patient's blood culture in the emergency department

4. Discussion

Klebsiella pneumoniae isolates were predominantly recovered from hospitalized patients, with a range of infections, including urinary tract infections, bacteremia, and pus infections. Our findings are consistent with a 2014 Lebanese study by Obeid Charrouf *et al.* [10], which reported a higher prevalence of *Klebsiella pneumoniae* in urine and pus samples. This mirrors global trends, where urine and pus are commonly identified as high-yield samples for pathogenic bacteria.

We observed significant antibiotic resistance. However, increased resistance to penicillins is expected due to natural penicillinase production. A French study by F. Guillard *et al.* reported a lower ofloxacin resistance rate (16.4%) compared to our findings, possibly due to geographical differences [11]. Our results exceeded the ESBL production rate found by Fissou Henry in Chad (56.52%), likely due to widespread and unchecked cephalosporin use [12]. A Togolese study also showed urine samples had the highest ESBL production (57.94%) [13]. In Asia, Clement Yaw *et al.* identified cefotaxime (79.2%), ceftazidime (75.7%), ciprofloxacin (59.8%), and amikacin (40.8%) as antibiotics with high resistance rates [11], contrasting with our findings where ofloxacin (81.25%), cefotaxime (78.13%), tobramycin (71.88%), and cefoxitin (62.50%) showed highest resistance. These disparities may be attributed to geographical and antibiotic testing differences. Notably, NDM carbapenemase was the only detected enzyme. NDM dissemination poses a public health concern transcending borders [14].

Antimicrobial resistance is a growing public health concern. High resistance rates to commonly used antibiotics in Cameroonian hospitals, such as Yaoundé Central Hospital, highlight the complexity of patient treatment via antibiotic therapy. The presence of New Delhi metallo-β-lactamase (NDM) carbapenemase exacerbates this issue. Alan P. Johnson *et al.* (2013) noted that NDM dissemination involves complex epidemiology, including transmission of diverse NDM-positive bacterial species and inter-strain, inter-species, and inter-genus transfer of blaNDM-containing plasmids [14]. This mechanism underscores the evolving resistance of bacterial species via NDM carbapenemase. Our study identified NDM-producing bacteria in a hospitalized patient, raising concerns about potential dissemination within the ward and uncontrolled spread of clones. Faryal Yumus *et al.* (2019) emphasized that NDM-mediated resistance is alarming, as carbapenems are considered last-resort antibiotics against multidrug-resistant (MDR) bacteria, particularly in intensive care units and high-risk services [15]. The escalating and persistent threat of antimicrobial resistance in Cameroonian healthcare facilities, particularly at Yaoundé Central Hospital, underscores the need for vigilant monitoring and control of resistance enzymes. Cameroon's Antimicrobial Resistance (AMR) program includes surveillance of resistance mechanisms. This study aims to contribute to this program by highlighting resistance patterns in healthcare settings and improving patient care. Our findings emphasize the importance of monitoring the spread of resistant strains, enhancing healthcare personnel education on nosocomial infection risks, and patient education on hygiene practices.

This study has some limitations. Conducted in a single institution, its findings may not be representative of the NDM situation in other hospitals or regions of the country. However, our sample included both inpatients and outpatients, providing a basis for potential extrapolation in larger-scale studies.

5. Conclusion

Klebsiella pneumoniae strains isolated from various samples exhibited increased resistance to antibiotics, producing ESBL, penicillinase, cephalosporinase, and carbapenemase. Continuous surveillance of antibiotic resistance in *Klebsiella pneumoniae* is crucial for guiding treatment strategies and preventing the spread of multidrug-resistant strains. Strict control measures must be implemented to minimize the impact of this pathogenic bacterium in healthcare settings.

Conflicts of Interest

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

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