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Phenotypic Characterization of Extended Spectrum Beta-Lactamase, Class C Cephalosporinase and Carbapenemase-Producing *Klebsiella* Species Isolated from Patients Consulted at Four Yaounde-Based Hospitals

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Abstract

Background: *Klebsiella* spp. are bacteria of medical importance for their role in opportunistic infections which are often difficult to treat because of resistance to one or several antimicrobials. The aim of this study was to determine antimicrobial resistance due to Extended Spectrum Beta-lactamase (ESBL), Class C cephalosporinase (AmpC) and carbapenemase enzymes in *Klebsiella spp.* isolated from patients consulted at four hospitals. **Methodology:** The study was cross-sectional and descriptive. A total of 4190 non-repetitive patients' specimens from 13 types of clinical specimens were analysed from February to November 2020. Two hundred and twenty-five (225) *Klebsiella spp* isolates were identified using API 20E and antimicrobial susceptibility testing done according to the Kirby Bauer disc diffusion method. ESBL and AmpC phenotypes were determined by the combination disc method and carbapenemases by double disc synergy method, referenced by EUCAST guidelines for the resistance testing. **Results:** The frequency of the species was

Klebsiella pneumoniae (69%, 155/255), *K. oxytoca* (14%, 31/255), *K. ozaenae* (12%, 27/225) and *K. rhinoscleromatis* (5%, 11/225). Isolates were most resistant to sulphomethoxazole trimethoprim (84%, 189/225), cepaholosporins (80%, 180/225), and least resistant to carbapenems (10.7%, 24/225). Two *K. oxytoca* and one *K. pneumoniae* were resistant to all antibiotics tested. *Klebsiella pneumoniae* had the most multidrug resistant isolates (59.4%, 134/225). Most isolates (83.6%, 188/225) expressed at least one enzyme, while 63.6% (143/225) of the isolates expressed at least two enzymes. Some isolates were ESBL (71.6%, 161/225), carbapenemase (10.7%, 24/225) and AmpC (6.6%, 15/225) producers. Three carbapenemases (*Klebsiella pneumoniae* carbapenemase-KPC, Metallo-Beta Lactamase-MBL and OXA-48) were detected. **Conclusion:** These results revealed that resistance of *Klebsiella* spp. to cephalosporins is high and this may be exacerbated by co-expression of AmpC and carbapenemases aggravating associated patient morbidity and mortality. Monitoring of antimicrobial resistance of local strains is necessary for informed decisions on empirical treatment.

Keywords

Klebsiella Spp., Multidrug Resistance, ESBL, AmpC, Carbapenemase

1. Introduction

Klebsiella species are bacteria responsible for community and hospital acquired infections [1]. These bacteria are commensals or environmental bacteria which are otherwise not pathogens, commonly found in water, soil, vegetation and in the hospital environment [2] [3]. However they can cause opportunistic infections in immunocompromised persons such as bronchopneumonia, urinary tract infections, liver abscesses, wound and bloodstream infections [4] [5] [6]. Within the hospital environment, the risk of infection is exacerbated by intensive antibiotic use which increases antibiotic resistance selection pressure [2], the use of invasive devices for treatment [7] and gaps in hygiene practices [6].

The emergence and global spread of *Klebsiella* species harbouring *Klebsiella pneumoniae* carbapenemase (KPC), Metallo-β-lactamases (MBL) and even ESBL, is a public health problem as these enzymes confer resistance to several β-lactam antibiotics. This threatens the effective control and treatment of *Klebsiella* species related invasive infections [8], such that patient outcome is characterized by high morbidity and mortality [9]. Antimicrobial resistance (AMR) complicates treatment achieved through the early use of empirical antibiotics in Cameroon and parts of the world, where the bulk of health problems are caused by infectious diseases. Effective treatment requires antimicrobial susceptibility testing to choose the most suitable antimicrobial or a combination of antimicrobials from several families in the event of multidrug resistance [10].

Klebsiella pneumoniae and to an extent *K. oxytoca* are the species that pose the most problems in terms of AMR. Enzymes (ESBL, carbapenemases), horizontal gene transfer (HGT) and other resistance mechanisms are responsible for extensive AMR determinants in pathogens [6]. The burden of AMR due to *Klebsiella spp.* and the putative resistance phenotypes responsible may not be fully appreciated in Cameroon because of the paucity of data [11]. AMR surveillance has gaps because the capacity for early detection of AMR and surveillance of infections caused by priority pathogens is not sufficient. Only a few laboratories have the capacity to identify pathogens, and detect resistance profiles because of an inadequacy of technical platforms and training [12]. Previous studies from Cameroon on bacteria resistance to certain families of antibiotics commonly used to treat infections caused by Gram negative bacteria such as β-lactams, derivatives (cephalosporins and carbapenems) and quinolones, underscored a high prevalence of drug resistance among Enterobacteriaceae [13] [14] [15]. Antimicrobial susceptibility testing revealed a generally high resistance to first, second and third generation cephalosporins and low resistance to carbapenems [16]. Phenotypic and genotypic characterization of resistance revealed a high prevalence of ESBL (CTX-M, SHV-2, AmpC) [14] [17] and the presence of plasmid mediated quinolone resistant genes (*qnrB* and *qnrS*) [15]. In Cameroon, clinicians resort to empiric treatment regimens in the absence of antibiogram results which may not be available because of limited resources. Constant monitoring and identification of resistance phenotypes is therefore necessary for tracking drug resistance, infection control and choosing empiric treatment. Therefore this study aimed to determine the extent of antimicrobial resistance due to Extended Spectrum Beta-lactamase (ESBL), Class C cephalosporinase (AmpC) and carbapenemase enzymes in *Klebsiella spp.* from patients consulted at four hospitals in Yaounde, Cameroon.

2. Materials and Method

- Study type and study setting

The study was cross-sectional and descriptive and carried out over a period of 10 months (February 2020 to November 2020). Isolates were collected from the Yaounde University Teaching Hospital, Yaounde Central Hospital, Yaounde Gynaeco-Obstetric and Paediatric Hospital and the Yaounde General Hospital. Microbiology analysis was carried out at the Centre for the Study and Control of Communicable Diseases, the University of Yaounde I.

- Study population

The study population was that part of the consenting consulted population who supplied clinical specimens for analysis at the hospitals and from which *Klebsiella* spp. was isolated.

- Statistical analysis

Statistical analysis was done using Epi info 7.1 and Microsoft Excel. The frequencies of antimicrobial resistance profile to 21 antimicrobial, resistance phenotypes detected, age groups, and type of clinical specimen were determined and comparisons between *Klebsiella* species, and patient category were made. A *p*-value less than 0.05 was considered statistically significant.

- Identification of isolates

Isolates were cultured on eosin methylene blue agar (EMB) purified on nutrient agar and identified using API 20E (Biomerieux, Marcy-l'Etoile France) according to the procedure referenced by the manufacturer [18].

- Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing of isolates to twenty-one antibiotics (Rapid Labs Ltd, Colchester ESSEX-UK) from four families was done according to the Kirby Bauer disc diffusion method [19]. *Escherichia coli* ATCC 25922 was used for quality control of the antibiotic discs.

- Detection of ESBL, AmpC and carbapenemases

The screening of ESBL, AmpC and carbapenemase resistant phenotypes was done simultaneously with AST of third generation cephalosporins, cefoxitin and carbapenems. Screening for ESBL producers was based on the hydrolysis of third generation cephalosporins (cefotaxime and or ceftazidime). It was visualized as a reduction in diameter of inhibition corresponding to <21 mm and <22 mm for cefotaxime and ceftazidime respectively. Screening for AmpC producers was based on the hydrolysis of cefoxitin with a reduction in diameter of inhibition around the antibiotic disc corresponding to <19 mm combined with phenotypic resistance to ceftazidime and or cefotaxime.

Screening for carbapenemase producers was based on the hydrolysis of meropenem with a reduction in diameter of inhibition around the antibiotic disc corresponding to <28 mm.

Confirmation of ESBL and AmpC phenotypes was done based on the combination disc diffusion test (CDT). For each suspected ESBL producing isolate, discs containing cefotaxime alone and cefotaxime in combination with clavulanate were applied on an inoculated Mueller Hinton plate. After aerobic incubation at 35˚C - 37˚C, the inhibition zone around the cefotaxime disc was compared to the inhibition zone around the cefotaxime clavulanate disc. The test was considered positive for the presence of ESBL if the inhibition zone diameter was equal to or greater than 5 mm around the cefotaxime clavulanate disc compared to the cefotaxime disc. Similarly, confirmation of an AmpC producer was based on an increase in zone diameter of cefoxitin clavulanate greater than or equal to 5 mm compared to the cefoxitin disc alone. Confirmation of carbapenemases was based on the combination disc synergy test. Synergy between a meropenem disc and meropenem in combination with various inhibitors (boronic acid, EDTA, cloxacillin) and the inhibition zone diameter around temocillin disc were reported and interpreted according to the algorithm in "S Figure 1". Synergy corresponds to an increase in zone diameter (mm) of the meropenem disc towards the meropenem inhibitor combination discs. The detection and confirmation of resistance mechanisms were carried out as per referenced guidelines by the European Union Committee on Antimicrobial Susceptibility Testing-EUCAST [20]. The discs were produced by Liofilchem, Roseto degli Abruszzi-Italy and *Klebsiella pneumoniae* ATCC 700603 was used for quality control of ESBL production.

3. Results

The ages of the participants ranged from 0 to 95 years, with a mean of 32.6 years. The 0 - 9 years age group was most represented and accounted for 24.4% (55/225) of isolates. The majority of participants were hospitalized patients 57% (129/225). *Klebsiella* species were isolated from thirteen [13] clinical specimens. Urine was the specimen that was the most frequently analysed accounting for 42.7% (96/225) of the isolates. The frequency of the species isolated was *Klebsiella pneumoniae* (69%), *K. oxytoca* (14%), *K. ozaenae* (12%) and *K. rhinoscleromatis* (5%).

Isolates were most resistant to sulphamethoxazole trimethoprim (84.0%). Resistance to cephalosporins was very high in the case of first generation (cefalotin 84.9%) and second generation cephalosporins (cefuroxim 82.2%). The resistance of isolates to third generation cephalosporins was ceftriaxone 76.4%, ceftazidime-75.1% and cefotaxime-54.7%, and resistance was equally as high to the lone fourth generation cephalosporin tested (cefepime-70.2%) and the lone monobactam tested (aztreonam-68.4%). Carbapenems were the most sensitive drugs with isolates expressing the lowest resistance rates of 10.7% and 10.2% to

Table 1. Antimicrobial susceptibility profile of isolates to all classes of antibiotics tested.

Antibiotic Antibiotics Family		Intermediate		Resistant		Sensitive	
		Frequency	%	Frequency	%	Frequency	%
Beta lactams	Amoxicillin clavulanate	1	0.4%	210	93.3%	14	6.2%
	Piperacillin tazobactam	22	9.8%	88	39.1%	115	51.1%
	Cefalotin	6	2.7%	191	84.9%	28	12.4%
	Cefuroxime	2	0.9%	185	82.2%	38	16.9%
	Ceftazidime	21	9.3%	169	75.1%	35	15.6%
	Cefotaxime	14	6.2%	123	54.7%	88	39.1%
	Cefepime	13	5.8%	158	70.2%	54	24%
	Aztreonam	12	5.3%	154	68.4%	59	26.2%
	Imipenem	9	4.0%	23	10.2%	202	89.8%
	Meropenem	23	10.2%	24	10.7%	201	89.3%
Sulphonamide	Sulfamethaxazole	2	0.9%	189	84.0%	34	15.1%
Aminoglycosides	Gentamicin	5	2.2%	139	61.8%	81	36%
	Amikacin	4	1.8%	33	14.7%	188	83.6%
	Tobramycin	23	10.2%	128	56.9%	74	32.9%
	Netilmicin	13	5.8%	86	38.2%	126	56%
Quinolones	Ciprofloxacin	20	8.9%	90	40%	115	51.1%
	Norfloxacin	10	4.4%	78	34.7%	137	60.9%
	Levofloxacin	12	5.3%	76	33.8%	137	60.9%
	Ofloxacin	12	5.3%	90	40%	123	54.7%
	Gatifloxacin	13	5.8%	98	43.6%	114	50.7%
	Moxifloxacin	14	6.2%	100	44.4%	111	49.3%

meropenem and imipenem respectively. Four aminoglycosides were tested. Although the antibiotic resistance rate was generally high within the class (gentamicin-61.8%, tobramycin-56.9% and netilmicin 38.2%), resistance to amikacin was relatively low with a rate of 14.7%. Among the six quinolones tested, resistance to levofloxacin (33.8%) was least. These results have been highlighted in Table 1.

There was a statistically significant difference in antibiotic resistance to nine antibiotics between hospitalized and outpatients. Surprisingly, resistance to meropenem was higher in isolates from outpatients than hospitalized patients. These results can be seen in Table 2 below.

Table 2. Comparison of the resistance profile amid hospitalized and outpatients.

Beta lactams	Patient category				*p*-value
	Hospitalized patient		Out-patient		
	Frequency	%	Frequency	%	
Amoxicillin clavulanate	123	95.3%	87	90.6%	0.263
Piperacillin tazobactam	52	40.3%	36	37.5%	0.679
Cefalotin	119	92.2%	72	75%	**0.002**
Cefuroxime	114	88.4%	71	74%	**0.011**
Ceftazidime	106	82.2%	63	65.6%	**0.012**
Cefotaxime	73	56.6%	50	52.1%	0.737
Cefepime	97	75.2%	61	63.5%	0.166
Aztreonam	4	3.1%	5	5.2%	0.393
Imipenem	9	7%	14	14.6%	0.115
Meropenem	8	6.2%	16	16.7%	**0.042**
Sulphonamide					
Sulfamethoxazole trimethoprim	116	89.9%	73	76%	**0.018**
Aminoglycosides					
Gentamicin	90	69.8%	49	51%	**0.005**
Amikacin	21	16.3%	12	12.5%	0.707
Tobramycin	80	62%	48	50%	**0.021**
Netilmicin	58	45%	28	20.2	**0.008**
Quinolones					
Ciprofloxacin	59	45.7%	31	32.3%	0.124
Norfloxacin	52	40.3%	26	27.1%	0.085
Levofloxacin	49	38%	27	28.1%	0.193
Ofloxacin	59	45.7%	31	32.3%	0.07
Gatifloxacin	65	50.4%	33	34.4%	**0.037**
Moxifloxacin	65	50.4%	35	36.5%	0.092

The resistance rate to imipenem between species was statistically significant between the four *Klebsiella* species as seen in Table 3 below. Three isolates from hospitalized patients were resistant to all 21 antibiotics tested, that is one *K. pneumoniae* and two *K. oxytoca*. The isolates were from three different clinical specimens namely: cerebrospinal fluid, wound and catheter drain.

The isolates tested expressed several resistance enzymes namely: Extended spectrum β-lactamases, AmpC and carbapenemases. These results are presented in Table 4.

Table 3. Comparison of antibiotic resistance profile amid bacteria species.

	Klebsiella oxytoca		*Klebsiella ozaenae*		*Klebsiella pneumoniae*		*Klebsiella rhinoscleromatis*		*p*-value
	Count	%	Count	%	Count	%	Count	%	
Beta lactams									
Amoxicillin clavulanate	32	100%	21	80.8%	145	93.5%	12	100%	**0.024**
Piperacillin tazobactam	14	43.8%	9	34.6%	63	40.6%	2	16.7%	0.728
Cefalotin	30	93.8%	19	73.1%	130	83.9%	12	100%	0.217
Cefuroxime	28	87.5%	22	84.6%	124	80%	11	91.7%	0.076
Ceftazidime	23	71.9%	16	61.5%	119	76.8%	11	91.7%	0.139
Cefotaxime	18	56.3%	10	38.5%	90	58.1%	5	41.7%	0.315
Cefepime	20	62.5%	16	61.5%	112	72.3%	10	83.3%	0.302
Aztreonam	19	59.4%	15	57.7%	111	71.6%	9	75%	0.531
Imipenem	6	18.8%	1	3.8%	16	10.3%	0	0%	**0.032**
Meropenem	7	21.9%	2	7.7%	15	9.7%	0	0%	0.09
Sulphonamide									
Sulfamethaxazole trimethoprim	27	84.4%	23	88.5%	128	82.6%	11	91.7%	0.752
Aminoglycosides									
Gentamicin	20	62.5%	15	57.7%	95	61.3%	9	75%	0.757
Amikacin	6	18.8%	4	15.4%	23	14.8%	0	0%	0.722
Tobramycin	16	50%	18	69.2%	85	54.8%	9	75%	0.157
Netilmicin	13	40.6%	14	53.8%	55	35.5%	4	33.3%	0.595
Quinolones									
Ciprofloxacin	13	40.6%	12	46.2%	62	40%	3	25%	0.638
Norfloxacin	13	40.6%	11	42.3%	52	33.5%	2	16.7%	0.778
Levofloxacin	13	40.6%	11	42.3%	48	31%	4	33.3%	0.669
Ofloxacin	15	46.9%	12	46.2%	59	38.1%	4	33.3%	0.825
Gatifloxacin	15	46.9%	12	46.2%	68	43.9%	3	25%	0.497
Moxifloxacin	16	50%	13	50%	66	42.6%	5	41.7%	0.692

Table 4. General profile of resistance phenotype.

Classes of phenotypes	Phenotypes	Frequency	%
Extended spectrum beta-lactamase with variations (ESBL) (N = 155)	ESBL	143	63.6%
	ESBL, AmpC	11	4.9%
	ESBL, porin loss	7	3.1%
	None	70	28.4%
Class C cephalosporinases (N = 14)	AmpC	3	1.3%
	AmpC, ESBL	11	4.9%
	None	211	93.7%
Carbapenemases (N = 23)	KPC	13	5.8%
	MBL	7	3.1%
	OXA-48	3	1.1%
	None	202	89.7%

There was a high frequency of isolates that expressed resistance phenotypes (85.3%-192/225) compared with 14.7% (33/225) of the isolates that did not express any resistance phenotype. A majority of the isolates were ESBL producers (63.6%), while ESBL and AmpCs were produced by 75.1% (169/225) of isolates. Class C cephalosporinases alone were expressed by 1.3% (3/225) of isolates while in combination with other enzymes they were expressed by 4.9% (11/225) of isolates. The most frequently produced carbapenemases were KPC and MBL enzymes (8.9%). These results are highlighted on Table 4.

Klebsiella pneumoniae was the only species which expressed all of the phenotypes. The distribution of the different phenotypes among the *Klebsiella* spp was not statistically significant as shown on Table 5.

Table 5. Comparison of resistance phenotypes per species.

Classes of phenotypes	Phenotypes	*Klebsiella oxytoca*	*Klebsiella ozaenae*	*Klebsiella pneumoniae*	*Klebsiella rhinoscleromatis*	*p*-value
Extended spectrum beta-lactamase with variations (ESBL) (N = 161)	ESBL	50%	53.8%	66.5%	83.3%	0.470
	ESBL, AmpC	6.3%	7.7%	4.5%	0%	
	ESBL, porin loss	3.1%	0%	3.9%	0%	
	None	40.6%	38.5%	25.2%	16.7%	
Class C cephalosporinases (AmpC) with variations (N = 15)	AmpC	6.3%	0.0%	0.6%	0%	0.468
	AmpC, ESBL	6.3%	7.7%	4.5%	0%	
	AmpC, KPC and MBL	0%	0%	0.6%	0%	
	None	87.5%	92.3%	94.2%	100%	
Carbapenamases with variations (N = 60)	KPC	9.4%	7.7%	2.6%	0%	0.579
	KPC, MBL	18.8%	7.7%	12.9%	8.3%	
	AmpC, KPC, and MBL	0%	0 %	0.6%	0%	
	MBL	0%	0%	2.6%	0%	
	OXA-48	9.4%	0%	9%	0%	

4. Discussion

Our findings revealed that the predominant species isolated was *Klebsiella pneumoniae* (69%). Current evidence suggests that *K. pneumoniae* is the most prevalent *Klebsiella* species due to its wide-ranging ecological distribution, considerably more varied DNA composition that facilitates adaptation to diverse environments, greater AMR gene diversity and plasticity and a higher plasmid burden than other Gram-negative opportunistic bacteria [2]. Therefore, *K. pneumoniae* is better organized for mobilizing resistant genes from other drug resistant bacteria in the environment or in animal/human microbial communities rendering otherwise non-resistant strains multidrug resistant. Isolates were most recovered from urine 42.7% (96/225) confirming that urinary tract infections (UTIs) are among the most common infections both in hospital and community infections in Cameroon [21]. Unfortunately, the treatment of such common infections has been complicated by auto medication, widespread inappropriate and disproportionate use of antimicrobials resulting in the emergence of multidrug resistant isolates [22].

Resistance rates to first generation cephalosporins (cefalotin 84.9%), second generation cephalosporins (cefuroxim 82.2%), third generation cephalosporins (ceftriaxone-76.4%. ceftazidime-75.1% and cefotaxime-54.7%), fourth generation cephalosporin (cefepime-70.2%) and a monobactam (aztreonam-68.4%) was higher than those earlier reported within our study setting [16] (cefalotin 60%, ceftazidime 51%, cefotaxime 51%, cefepime 26% and aztreonam 45%). This indicates that there has been more than a 20% increase in resistance to all generations of cephalosporins since 2015. Resistance to cephalosporins is mainly as a result of the production of Extended Spectrum β-Lactamases (ESBL). They hydrolyse penicillins, first, second, third, fourth generation, monobactams but not cephamycins (cefoxitin), beta-lactam inhibitors and carbapenems. Though there may be large variations in geographical distribution of ESBL genes due to differences in antibiotic use, hygiene and co-expression of other virulence factors such as efflux pumps, siderophores, polysaccharide capsule proteins and fimbriae [1], generally there is a global rise in resistance to cephalosporins. This is most likely because of clonal expansion of resistant strains and an increase in dissemination of ESBL genes through horizontal gene transfer by plasmids and inappropriate antibiotic use that increases the selection pressure on resistant strains. In our study, 71.6% of isolates were ESBL producers (63.6% ESBL alone, 4.9% ESBL and AmpC, 3.1% ESBL with porin loss).

Ambler Class C cephalosporinases (AmpC) are naturally produced by some Enterobacteriaceae like *E. coli*, *Shigella* spp. and *Enterobacter spp.* but not *Klebsiella* spp. They are known to have acquired mobile AmpC genes through HGT mechanisms. AmpCs hydrolyze penicillins, third generation cephalosporins, monobactams but not fourth generation cephalosporins and carbapenems and they are poorly inhibited by ESBL inhibitors like clavulanate [20]. Their overall frequency has remained comparatively far below that of ESBL in most studies [1]

[14] [23]. In this study 6.2% (14/225) of isolates were AmpC producers (1.3% AmpC alone, 4.9% AmpC and ESBL). It is not uncommon for AmpC producers to co-express other enzymes. This was mostly the case among hospitalized patients and within the species *K. pneumoniae* and *K. oxytoca*. Prolonged hospitalization and intensive use of antibiotics within the hospital seem to increase the selection pressure of multidrug resistant isolates. Carbapenems constitute one of the last treatment options for serious infections. Resistance to carbapenems is a serious call for concern because carbapenemases confer resistance to all beta-lactam drugs engendering associated high patient morbidity, mortality rates, increased hospital stay and high treatment cost. Furthermore, the carbapenemase genes are easily transferable among Enterobacteriaceae and easily disseminate to other geographical areas. Since the first case of carbapenemase (KPC) epidemics in America in the mid-1990s, other outbreaks have since occurred as a result of other carbapenemases such as VIM, OXA-48 and NDM enzymes which have been disseminated globally [2]. Whilst, the associated morbidity and mortality as a result of carbapenemase producing bacteria is high, carbapenems are still the most efficient drugs with the lowest levels of drug resistance. In this study, the isolates' resistance rate is quite high 10.2% (23/225) compared to previous reports from our study setting [15] [16] [17]. Findings by Betbeui *et al.*, 2015, indicate that five years ago, there was no resistance to meropenem (0%) within our study setting. Even though our values are higher than the values by Lyonga-Mbamyah *et al.*, 2020 (resistance to imipenem 9/440 (2.1%)), their study included all Enterobacteriaceae, thus their values may have been diluted because some genera do not harbour carbapenemase resistant genes to the same extent as *Klebsiella*. In the absence of other published data from our research setting it is not clear whether the rise in carbapenem resistance is as a result of an outbreak or practises related to wrong drug usage. In Nigeria [7], an incidence of 7.7% was reported, in Tanzania [21], 32.24% was reported, in the United Kingdom [1], 0% was reported. In India [24], it was reported that the trend of carbapenems resistance rose from 7.4% to 84.1% between 2004 and 2013. These results are alarming because resistance to carbapenems is on the rise mostly in low-income countries where there is little or no research on alternative drug options against multi-resistant bacteria. There was a significant difference in resistance to meropenem between hospitalized and community patients with higher resistance rates observed in outpatients. More than half of the isolates were multidrug resistant. This indicates that clinically important clones of *Klebsiella* spp. are not only circulating in hospitals affecting critically ill patients but they are also circulating in our environment complicating treatment for community patients who may resort to auto-medication or empirical treatment without antibiogram results.

5. Conclusion

The frequency of *Klebsiella* spp. isolates was high. It represented about a quarter of all isolates identified in biological specimens at the collection sites. Isolates were most resistant to cephalosporins and sulphamethazaxole trimethoprim. Above three quarters of isolates were resistant to first, second and fourth generation cephalosporins mostly caused by ESBL. The latter resistance may be exacerbated due to co-expression of AmpC and carbapenemase by isolates. The isolates were least resistant to imipenem with slightly over 10% resistance rate. Though the resistance rate to carbapenems is on the rise, it still remains the most effective drug class. The majority of isolates were ESBL producers, while about a handful of the isolates were carbapenemase and AmpC producers. One isolate expressed all three resistance phenotypes (ESBL, AmpC, and carbapenemase). Three carbapenemases (KPC, MBL and OXA-48) accounted for the carbapenemases detected and most expressed the KPC phenotypes. Carbapenemases confer resistance to all beta-lactamases. The resistance burden is further strengthened in isolates that acquired carbapenemase and other enzymes aggravating associated patient morbidity and mortality. Therefore, it is necessary to continue monitoring the antimicrobial resistance of local strains for better informed decisions on empirical treatment guides and better patient care.

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Ethical Approval and Consent to Participate

This research work received ethical approval from the *Comité Institutionnel d'Ethique de la Recherche pour la Santé Humaine*, *Université Catholique d'Afrique Centrale*, *Ecole des Sciences de la Santé* registered No.2019/020178/ CEIRSH/MI and authorizations for research from all four hospitals. Only participants who consented were included in this study.

Authors' Contributions

Emilia Enjema Lyonga Mbamyah conceived the study and designed it together with Mangum Patience Kumcho and Hortense Kamga Gonsu. Emilia Enjema Lyonga Mbamyah, Patience Mangum Kumcho, Florence Anjabie Enyeji, Dieudonne Sedena, Aime-Caesar Teukam, Modestine Djuissi, Agnes Bedie Eyoh, Anicette Chafa Betbeui**,** William Baiye conducted the laboratory aspect of the study with contributions from Martha Tongo Mesembe and George Mondinde Ikomey. The general supervision was carried out by Emilia Enjema Lyonga Mbamyah. Emilia Enjema Lyonga Mbamyah drafted the article with contributions from Mangum Patience Kumcho and Martha Tongo Mesembe. All the authors reviewed the article. All the authors read and agreed to the final manuscript.

Conflicts of Interest

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

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Abbreviations

AmpC: Ampicillinase
AMR: Antimicrobial resistance
API: Analytical profile index
AST: Antimicrobial susceptibility testing
ATCC: American Type Culture Collection
CDT: Combination disc diffusion test
CSCCD: Centre for the Study and Control of Communicable Diseases
DHA-1: Dhahran-1 plasmid
DNA: Deoxyribonucleic acid
EMB: Eosin methylene blue
ESBL: Extended spectrum *β*-lactamase
EUCAST: European Union Committee on Antimicrobial Susceptibility Testing
HGT: Horizontal gene transfer
KPC: *Klebsiella pneumoniae* carbapenemase
MBL: Metallo-Beta lactamase
NDM: New Delhi Metallo-Beta lactamase
OXA-48: Oxacillinase-like 48
qnrB: Quinolone resistant gene B subunit
qnrS: Quinolone resistant gene S subunit
SHV: Sulphydryl variable
UTI: Urinary tract infection
VIM: Verona integron-encoded microorganism
WHO: World Health Organization