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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Research Article

Keywords: *Klebsiella* spp., multidrug resistance, ESBL, AmpC, Carbapenemase

Posted Date: August 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-793227/v1>

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Abstract

Background: *Klebsiella* spp. are bacteria of medical importance for their role in opportunistic infections. These infections are often difficult to treat because of acquired resistance to one or several families of antimicrobials. The present study aimed at detecting Extended Spectrum Beta-lactamase (ESBL), Class C cephalosporinase (AmpC) and carbapenemase resistant phenotypes of *Klebsiella* spp. isolated from patients consulted at four Yaounde-based hospitals.

Results: 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 penicillins (90%), sulphonamides (84%), cephalosporins (80%), and least resistant to carbapenems (10.2%). Three isolates namely: two *K. oxytoca* and one *K. pneumoniae* were resistant to all twenty-eight (28) antibiotics tested. *Klebsiella pneumoniae* was the species with the most multidrug resistant isolates (59.4%). Most isolates (83.6%) expressed at least one resistance phenotype, while 63.6% of the isolates expressed all three phenotypes. Many of the isolates were ESBL producers (71.6%), while fewer isolates were carbapenemase (26.7%) and AmpC (6.6%) producers. Three carbapenemases (*Klebsiella pneumoniae* carbapenemase-KPC, Metallo-Beta Lactamase-MBL and OXA-48) were detected from 26.7% of the isolates and the combination KPC and MBL were the most detected phenotypes (12.9%).

Conclusion: These results reveal that resistance of *Klebsiella* spp. to cephalosporins is high and this may be exacerbated as a result of the co-expression of AmpC and carbapenemases. About a quarter of the isolates had acquired carbapenemases that confer resistance to all beta-lactamases and carbapenems which constitute last line drugs. The resistance burden is further strengthened in isolates that acquired more than one carbapenemase aggravating associated patient morbidity and mortality. Therefore, it is necessary to continue monitoring antimicrobial resistance of local strains for better informed decisions on empirical treatment guide and better patient care.

Background

Klebsiella species are bacteria responsible for community and hospital acquired infections (1). The emergence and global spread of *Klebsiella* species harbouring *Klebsiella pneumoniae* carbapenemase (KPC), Metallo- β -lactamases (MBL) and even ESBL, confer resistance to several antibiotics. This threatens effective control and treatment of ensuing invasive infections (2), as such patient outcome is characterized by high mortality (3). Antimicrobial resistance (AMR) complicates treatment achieved through early use of empirical antibiotics in parts of the world, where the bulk of health problems are caused by infectious disease, posing big challenges for public health.

Klebsiella pneumoniae and to an extent *K. oxytoca* are the species that pose the most problem in terms of AMR. Enzymes (ESBL, carbapenemases), horizontal gene transfer (HGT) and other resistance mechanisms are responsible for extensive AMR determinants in pathogens (4). The burden of AMR due to *Klebsiella* spp. and the putative resistance phenotypes responsible may not be fully appreciated in Cameroon because of paucity of data (5). AMR surveillance has gaps because the capacity for early detection of AMR and surveillance of infections caused by priority pathogens is not sufficient. Only few laboratories have the capacity to identify pathogens, detect resistance profiles because of an inadequacy of technical platforms and training (6). 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, underscore a high prevalence of drug resistance among some WHO high priority Enterobacteriaceae (7) (8) (9). Antimicrobial susceptibility testing revealed a generally high resistance to first, second and third generation cephalosporins and low resistance to carbapenems. Phenotypic and genotypic characterization of resistance revealed a high prevalence of ESBL (CTX-M, SHV-2, AmpC) (10) (8) and the presence of plasmid mediated quinolone resistant genes (*qnrB* and *qnrS*) (9). Constant monitoring and identification of resistance phenotypes is necessary for the purpose of infection control and choosing empiric treatment.

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%).

Resistance of isolates to amoxicillin was highest with a frequency of 99.1% (223/225) and similarly very high resistance rates were recorded for amoxicillin clavulanate (93.3%), ticarcillin (95.1%), ticarcillin clavulanate (83.1%) and piperacillin (86.2%). The action of the inhibitor tazobactam improved the activity of piperacillin with a resulting moderate resistance rate of 39.1%. Resistance to cephalosporins was very high against first generation (cefalotin 84.9%) and second generation cephalosporins (cefuroxime 82.2%). The resistance of isolates to third generation cephalosporins was high (ceftriaxone 76.4%, ceftazidime 75.1% and cefotaxime 54.7%) and 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 the lowest resistance frequencies of 10.7% and 10.2% for meropenem and imipenem respectively. The only sulphonamide tested was sulphamethoxazole trimethoprim (84.0%), which had a resistance frequency comparable to that of penicillins (ticarcillin and piperacillin).

Four aminoglycosides were tested and their resistance profiles were diverse. Although the antibiotic resistance rate was generally high within the class (gentamycin 61.8%, tobramycin 56.9% and netilmicin 38.2%), amikacin was the exception with a low resistance rate of 14.7%.

The resistance to quinolones was fairly high compared to the penicillins, cephalosporins and monobactam. Isolates were most resistant to pipermedic acid (53.8%) and least resistant to levofloxacin (33.8%). Tables 1 and 2 in the supplementary file highlight these results.

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

Antibiotic Antibiotics		Intermediate		Resistant		Sensitive		
Family		Frequency	%	Frequency	%	Frequency	%	
Beta lactams	Amoxicillin	0	0%	223	2	0.9%		
	Amoxicillin clavulanate	1	0.4%	210	14	6.2%		
	Ticarcillin	4	1.8%	214	7	3.1%		
	Ticarcillin clavulanate	15	6.7%	187	23	10.2%		
	Piperacillin	15	6.7%	194	16	7.1%		
	Piperacillin tazobactam	22	9.8%	88	39.1%	115	51.1%	
	Cefalotin	6	2.7%	191	28	12.4%		
	Cefuroxime	2	0.9%	185	38	16.9%		
	Ceftazidime	21	9.3%	169	35	15.6%		
	Ceftriaxone	7	3.1%	172	46	20.4%		
	Cefotaxime	14	6.2%	123	54.7%	88	39.1%	
	Cefepime	13	5.8%	158	54	24%		
	Aztreonam	12	5.3%	154	68.4%	59	26.2%	
	Sulphonamide	Sulfamethaxazole	2	0.9%	189	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	NalidixicAcid	14	6.2%	90	40%	121	53.8%	
	PipemidicAcid	12	5.3%	121	53.8%	92	40.9%	
	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%	
	Mixofloxacin	14	6.2%	100	44.4%	111	49.3%	

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

Beta lactams		Patient category			
Hospitalized patient		Out-patient		p-value	
Frequency	%	Frequency	%		
Amoxicillin	128	99.2%	95	99%	0.833
Ticarcillin	126	97.7%	88	91.7%	0.045
Piperacillin	118	91.5%	76	79.2%	0.007
Cefalotin	119	92.2%	72	75%	0.002
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
Sulfamethoxazole Trimethoprim	116	89.9%	73	76%	0.018
Aminoglycosides					
Tobramycin	80	62%	48	50%	0.021
Quinolones					
Nalidixic acid	55	42.6%	35	36.5%	0.6
Pipemidic acid	75	58.1%	46	47.9%	0.281
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
Mixofloxacin	65	50.4%	35	36.5%	0.092

Table 3
Comparism of antibiotic resistance profile amid bacteria species

	<i>Klebsiella oxytoca</i>		<i>Klebsiella ozaenae</i>		<i>Klebsiella pneumoniae</i>		<i>Klebsiella rhinoscleromatis</i>		<i>p</i> -value
	Count	%	Count	%	Count	%	Count	%	
Beta lactams									
Amoxicillin	32	100%	25	96.2%	154	99.4%	12	100%	0.379
Ticarcillin	32	100%	22	84.6%	149	96.1%	11	91.7%	0.058
Ticarcillin clavulanate	29	90.6%	19	73.1%	128	82.6%	11	91.7%	0.646
Piperacillin	29	90.6%	21	80.8%	133	85.8%	11	91.7%	0.771
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
Ceftriaxone	22	68.8%	16	61.5%	123	79.4%	11	91.7%	0.136
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
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									
Nalidixic acid	16	50%	12	46.2%	59	38.1%	3	25%	0.304
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
Mixofloxacin	16	50%	13	50%	66	42.6%	5	41.7%	0.692

Three isolates from hospitalized patients were resistant to all 28 antibiotics tested (super bugs). One *K. pneumoniae* and two *K. oxytoca*. The isolates were from three different clinical specimens namely: cerebrospinal fluid, wounds and catheter drain. The isolates tested expressed several resistance enzymes namely: Extended spectrum β -lactamases, AmpC and carbapenamases. These results are presented in Table 4

Table 4
General profile of resistance phenotype

Classes of phenotypes	Phenotypes	Frequency	%
Extended spectrum beta-lactamase with variations (ESBL) (N = 161)	ESBL	143	63.6%
	ESBL, AmpC	11	4.9%
	ESBL, porin loss	7	3.1%
	None	64	28.4%
Class C cephalosporinases with variations (N = 15)	AmpC	3	1.3%
	AmpC, ESBL	11	4.9%
	AmpC, KPC and MBL	1	0.4%
	None	210	93.3%
Carbapenamases with variations (N = 60)	KPC	9	4%
	KPC, MBL	29	12.9%
	AmpC, KPC and MBL	1	0.4%
	MBL	4	1.8%
	OXA-48	17	7.6%
	None	165	73.3%

There was a high frequency of isolates which expressed resistance phenotypes or mechanisms (83.6% -188/225) compared with 16.4% (37/225) of the isolates that did not express any resistance phenotype.

A majority of the isolates were ESBL producers (63.6%), while ESBL in combination with porin loss and AmpC were produced by 71.6% (161/225) of isolates. Class C cephalosporinases alone were produced by 1.3% (3/225) of isolates while in combination with other enzymes they were produced by 6.7% (15/225). The isolates produced several carbapenamases either as sole enzyme or in multiple enzyme combinations resulting in a total of 26.7 % of the isolates being carbapenamase producers. The most frequently produced carbapenamases were the combination of KPC and MBL enzymes (12.9%). These results are highlighted on Tables 4 and 5.

Table 5
Comparison of resistance phenotypes per species

Classes of phenotypes	Phenotypes	<i>Klebsiella oxytoca</i>	<i>Klebsiella ozaenae</i>	<i>Klebsiella rhinoscleromatis</i>	p-value
Extended spectrum beta-lactamase with variations (ESBL) (N = 161)	ESBL	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%
	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	7.7%	2.6%	0%	0.579
	KPC, MBL	7.7%	12.9%	8.3%	
	AmpC, KPC, and MBL	0%	0 %	0%	
	MBL	0%	0%	2.6%	0%
	OXA-48	9.4%	0%	9%	0%
	None	62.6%	84.6%	72.3%	91.7%

Discussion:

Our findings revealed that the predominant species 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 opportunists (14). 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 (15). In a bid to treat common infections, widespread inappropriate and disproportionate use of antimicrobials has led to the emergence of multidrug resistant isolates of *Klebsiella* spp. making such infections more difficult to treat (16).

As expected, resistance of isolates to amoxicillin was highest 99.1% (223/225) likewise, amoxicillin clavulanate (93.3%), ticarcillin (95.1%), ticarcillin clavulanate (83.1%) and piperacillin (86.2%). It has been demonstrated that *K. pneumoniae* has intrinsic resistance to penicillin G and ampicillin due to the presence of a Class A SHV-1 penicillinase. However, the resistance of isolates to penicillin inhibitors like clavulanate and tazobactam that normally restore the activity of penicillins was also very high in our study as observed in amoxicillin clavulanate (93.3%), ticarcillin clavulanate (83.1%) and piperacillin (86.2%). These results contrast results within the same setting (17) which reported resistance rates of 39% and 22% respectively to amoxicillin clavulanate and ticarcillin clavulanate and 38% while piperacillin tazobactam 39.1% was comparable to the present study. Such high rates of resistance to penicillins and penicillin inhibitor combinations can be further explained by enhanced resistance mechanisms co-expressed by a good number of the isolates such as ESBL (71.6%) on the one hand that hydrolyze penicillins while AmpC and carbapenemases hydrolyze ESBL inhibitors like clavulanate.

Resistance rates to first generation cephalosporins (cefalotin 84.9%), second generation cephalosporins (cefuroxime 82.2%), third generation cephalosporins (ceftriaxone 76.4%, ceftazidime 75.1% and cefotaxime 54.7%) and likewise fourth generation cephalosporin (cefepime 70.2%) and a monobactam (aztreonam 68.4%) was higher than those earlier reported (17) (cefalotin 60%, ceftazidime 51%, cefotaxime 51%, cefepime 26% and aztreonam 45%). This indicates that within our study setting, there has been more than 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 hydrolyze 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), what cuts across the studies cited above is that there is a global rise in resistance to cephalosporins. This is most likely because of clonal expansion and an increase in dissemination of ESBL genes through horizontal gene transfer mechanisms by plasmids or *de novo* mutations. 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., *P. aeruginosa* and *Enterobacter* spp. but not *Klebsiella* spp. which are known to have acquired mobile AmpC genes (CMY-2 and DHA-1) through HGT mechanisms. AmpCs hydrolyze penicillins, third generation cephalosporins, monobactams but not fourth generation cephalosporins, carbapenems and they are poorly inhibited by ESBL inhibitors like clavulanate (13). Their overall frequency has remained comparatively far below that of ESBL in most studies (1), (8), (18). In this study (15/225) 6.6% of isolates were AmpC producers (1.3% AmpC alone, 4.9% AmpC and ESBL, 0.4% AmpC + MBL + KPC). 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 seems to increase selection pressure of multidrug resistant isolates, implicitly such infections are more difficult to treat. 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 rendering ensuing infections associated with high morbidity and mortality rates. Furthermore, the carbapenemase genes are easily transferable among Enterobacteriaceae. 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 (14). 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 rate is quite high 26.7% (60/225) compared to previous reports from our study setting (9), (10), (17). Our values are higher than values by Lyonga-Mbamyah *et al.*, 2020. Their study included all Enterobacteriaceae, thus their values were diluted because some species do not harbour carbapenemase resistant genes as much as some *Klebsiella* spp. Findings by Betbeui *et al.*, 2015 were carried out only on *Klebsiella* spp., and they indicate that five years ago, there was a high resistance rate to carbapenems within our study setting. In Nigeria (19), an incidence of 7.7% was reported, in Tanzania (15), 32.24% was reported, in the United Kingdom (1), 0% was reported and in India (20), it was reported that the trend of carbapenems resistance rose from 7.4 to 84.1% between 2004 and 2013. In the present study, 23 isolates of 60 carbapenem resistant isolates co-expressed AmpC, ESBL and resistance to quinolones and there was no significant difference between hospitalized and community patients. This implies that carbapenem resistant *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.

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 penicillins, cephalosporins and sulphamethoxazole 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 quarter of the isolates were carbapenemase producers and only a few were AmpC producers. One isolate expressed all three resistance phenotypes (ESBL, AmpC, carbapenemase). Three carbapenemases (KPC, MBL and OXA-48) accounted for a quarter of carbapenemase resistance and the combination of KPC and MBL were the most represented phenotypes. Carbapenemases confer resistance to all beta-lactamases. The resistance burden is further strengthened in isolates that acquired

more than one carbapenemase aggravating associated patient morbidity and mortality. Therefore, it is necessary to continue monitoring antimicrobial resistance of local strains for better informed decisions on empirical treatment guide and better patient care.

Methods

- Aim of study

The study aimed to detect Extended Spectrum β -lactamase (ESBL), AmpC and carbapenemase phenotypes of antibiotic resistant *Klebsiella spp.* isolated from patients consulted at four hospitals in Yaounde.

- Study type and study setting

The study was cross-sectional and descriptive carried out over a period of nine 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 from whom *Klebsiella spp.* was isolated from their biological specimens.

- Identification of isolates

Isolates were sub-cultured on eosin methylene blue agar (EMB) purified on nutrient agar and identified using API 20E (Biomérieux, Marcy-l'Etoile France) according to the procedure referenced by the manufacturer (11).

- Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing of twenty eight antibiotics (Rapid Labs Ltd, Colchester ESSEX-UK) from four families was done according to the Kirby Bauer disc diffusion method (12). *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 resistance phenotypes was done simultaneously with AST of third generation cephalosporins, ceftazidime and carbapenems respectively. Confirmation of ESBL and AmpC phenotypes was done based on the combination disc diffusion test (CDT) and carbapenemases based on the combination disc synergy test. The detection and confirmation of resistance mechanisms were carried out as per referenced guidelines by the European Union Committee on Antimicrobial Susceptibility Testing (13). The discs were produced by Liofilchem, Roseto degli Abruzzi-Italy and *Klebsiella pneumoniae* ATCC 700603 was used for quality control of ESBL.

- Statistical analysis

Statistical analysis was done using CS Pro software. The general antimicrobial resistance profile to 28 antimicrobial was tabulated and the resistance phenotypes detected were compared among *Klebsiella* species, age groups, type of clinical specimen and patient category. A *p*-value less than 0.05 was considered statistically significant.

Declarations

Ethical consideration

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.

Consent for publication

Not applicable

Availability of data and materials

Data generated or analysed during this study are included in the supplementary information files, the remainder of the data are available from the corresponding author on reasonable request.

Competing interests

The authors and co-authors have no financial and non-financial competing interests.

Funding

This work was carried out with the aid of a grant from UNESCO and the International Development Research Center (IDRC), Ottawa, Canada through the Organization for Women in Science from the Developing World (OWSD). The views expressed herein do not necessarily represent those of UNESCO, IDRC or its Board of Governors.

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.

Acknowledgements

This work was carried out with the aid of a grant from UNESCO and the International Development Research Center (IDRC), Ottawa, Canada. The views expressed herein do not necessarily represent those of UNESCO, IDRC or its Board of Governors.

The authors wish to thank the hospital personnel at the Bacteriology Unit of the Yaounde University Teaching, Yaounde Central Hospital, Yaounde General Hospital and Yaounde Gynaeco-Obstetric and Paediatric Hospital for the services offered during the period of specimen collection and the staff of the Centre for the Study and Control of Communicable Diseases (CSCCD), Faculty of Medicine and Biomedical Sciences, the University of Yaounde1.

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