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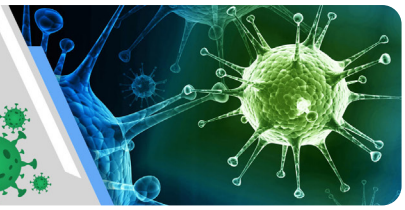

Molecular Characterization of Clinical Strains of Extended-Spectrum Beta-Lactamases-Producing *Klebsiella Pneumoniae* Isolated in A Tertiary Hospital in Dakar-Senegal

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Abstract

Klebsiella pneumoniae (K. pneumoniae) isolates are often multidrug-resistant (MDR) and clones producing extended-spectrum beta-lactamases (ESBL) are increasingly reported all over the world. This study aimed to determine drug-susceptibility profiles and characterize ESBL genes in clinical strains of *K. pneumoniae* in an university teaching hospital of Dakar. Sixty-six ESBL-producing *K. pneumoniae* strains were selected for this study and subjected to the Kirby-Bauer disk diffusion method and standard polymerase chain reaction (PCR) for the screening of major ESBL genes (bla_{CTX-M}, $bla_{CTX-M-9}$, $bla_{CTX-M-15}$, $bla_{CTX-M-25}$, bla_{OXA-1}, bla_{TEM}, bla_{SHV}).

All isolates were resistant to ampicillin, ticarcillin, amoxicillin/clavulanic acid combination, cefalotin, cefotaxime, ceftazidime and cefepime, while 60% (40/66) of them were resistant to fosfomycin. Amikacin, ertapenem and imipenem remained effective with respective sensitivity rate of 77.3% (51/66), 75.8% (50/66) and 100% (66/66). bla_{CTX-M} (64/66; 97%) with its variant $bla_{CTX-M-15}$ (63/66; 95.5%) were the most prevalent ESBL genes; followed by bla_{TEM} (58/66; 87.9%), bla_{OXA-1} (47/66; 71.2%) and bla_{SHV} (31/66; 47%). Sixty-four isolates out of 66 (97%) of isolates carried at least 2 ESBL genes and the most prevalent ESBL gene combinations were ($bla_{CTX-M-15}$ + bla_{OXA-1} + bla_{TEM}) (24/66; 36.4%) and ($bla_{CTX-M-15}$ + bla_{TEM} + bla_{OXA-1} + bla_{SHV}) (18/66; 27.3%).

Cephalosporins, quinolones and aminoglycosides (except amikacin) would no longer be recommended as first-line therapy in *Enterobacteriaceae* infections in Dakar. CTX-M-15-type ESBL seems to be predominant ESBL in *K. pneumoniae* isolates in Dakar. Amikacin would be an alternative molecule for the treatment of MDR ESBL-producing-*K. pneumoniae* infections. Whole-genome sequencing of these isolates could provide additional and in-depth informations.

Keywords: *Klebsiella pneumoniae*; Extended Spectrum beta-lactamase; tertiary hospital; Dakar-Senegal

Introduction

By 2030, around 24 million people, especially in low-income countries, will live in extreme poverty due to antimicrobial resistance (AMR) and around 28.3 million European people can fall into poverty. Furthermore, by 2050,

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around 3.8% of the world annual gross domestic product (GDP) will be lost due to AMR, while low-income countries will lose up to 5.6% of their GDP and AMR could lead 10 million people to death [1-3]. *K. pneumoniae*, an ESKAPE pathogen group member, is one of the main and widespread bacteria showing uncommon drug resistance patterns in the world [4-6].

K. pneumoniae, a human gastrointestinal tract commensal *Enterobacteriaceae* is an opportunistic bacterium, also implicated in about 20% of enterobacterial infections and frequently implicated in hospital-acquired infections [7-9]. In community, *K. pneumoniae* is often involved in serious extra-intestinal infections such as urinary tract infections (UTI) and pneumonia, while in hospitals it generally causes septicemia through secondary infections of wounds, catheters and probes [4, 10]. Moreover, carriage of *K. pneumoniae* among hospitalized patients can be up to 77% in the stool, 19% in the pharynx and 42% on the hands [11].

The most common resistance mechanism in MDR *K. pneumoniae* is the production of extended spectrum beta-lactamases (ESBL) and carbapenemases [4, 10]. ESBLs hydrolyze penicillins, cephalosporins (except cephamycins) and monobactams. However, they are inactive on beta-lactamase inhibitors (BLI) and carbapenems [12, 13]. At least 13 ESBL families have been reported worldwide including CTX-M, SHV-type ESBL, TEM-type ESBL, OXA-type ESBL, IRT, CMT, GES, PER, VEB, BEL, TLA, SFO and OXY. However, the most active and prevalent ESBL families are CTX-M, SHV-type ESBL, TEM-type ESBL and OXA-type ESBL. SHV-type ESBL and TEM-type ESBL are known as “old” ESBLs while CTX-M are known as “new” ESBLs. Furthermore, OXA-1 which is not originally an ESBL-type oxacillinases, can be added to ESBLs because of its ability to hydrolyze cefepime [14, 15].

ESBL genes are frequently located on the bacterial chromosome or on mobile genetic supports (plasmids, integrons and transposons). These mobile genetic carriers are strongly involved in intra- and interspecific bacterial genes exchanges and are therefore powerful catalysts for the global spread of multidrug resistant bacteria. ESBL-producing strains are often resistant to aminoglycosides and quinolones as mostly supported by the same mobile genetic elements [16-20].

The screening and study of ESBL-producing *K. pneumoniae* strains is therefore critical in order to improve therapeutic management of patients, prevent and contain epidemics and collect useful epidemiological data that will help public health authorities to plan short-, medium- and long-term activities. To our knowledge, few studies focused on circulating clinical strains of ESBL-producing *K. pneumoniae* in Senegal and in West Africa globally. This study therefore aimed to describe antibiotics resistance profiles and to characterize ESBL genes in clinical *K. pneumoniae* strains isolated in Aristide le Dantec University Teaching Hospital (HALD), one of the major tertiary facilities in Dakar, Senegal. Additionally, we compared community-acquired (CA) to hospital-acquired (HA) isolates, and uropathogenic *K. pneumoniae* (UPKP) to No-Uropathogenic *K. pneumoniae* isolates (No-UPKP).

Materials and methods

Bacterial isolates

This study focused on 66 ESBL-producing *K. pneumoniae* strains isolated from different biological samples, including urines, pus, sputum, bronchial fluid and vaginal secretions in routine diagnosis activities of HALD bacteriology laboratory from January 01st, 2018 to December 31, 2020. Isolation and identification were done on Eosin Methylene Blue (EMB) agar (Merck KGaA, Darmstadt, Germany) and Api 20E for *Enterobacteriaceae* (bioMérieux, Lyon / France), respectively. Majority of isolates was from urine specimen (n = 42, 63.63%). 54 (81.8%) isolates were community-acquired whereas 12 (18.2%) were hospital-acquired strains. Bacterial isolates were stored at -80 °C in brain heart infusion broth with 15% glycerol until the study begins.

Antibiotic susceptibility testing

We used Kirby-Bauer disc diffusion method for the antibiotic susceptibility testing and results were interpreted according to the guidelines of the antibiogram committee of the French society of microbiology (CA-SFM, 2022). Briefly, bacterial suspensions were prepared at 0.5 McFarland and inoculated onto Mueller-Hinton agar for an overnight incubation at 37°C. These following antibiotic disks were tested: ampicillin (AMP, 10 µg), ticarcillin (TIC, 75 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), cefalotin (CEF, 30 µg), cefoxitin (FOX, 30 µg), cefotaxime (CTA, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (CEP, 30 µg), aztreonam (AZT, 30 µg), imipenem (IMP, 10 µg), ertapenem (ERT, 10 µg), nalidixic acid (NAL, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GEN, 10 µg), amikacin (AMI, 30 µg), fosfomycin (FOS, 50 µg), tetracycline (TET, 30 µg) and sulfamethoxazole-trimethoprim (TRS, 1.25 µg / 23.75 µg). *Escherichia coli* ATCC 25922 was used for quality control. ESBL production was detected by double-disk synergy test using AMC disc surrounded at a radius of 30 mm by CEP, CAZ, CTA and AZT (figure 1).

DNA Extraction

We used mechanical thermal lysis method to extract bacterial DNA. Briefly, a well-separated bacterial colony was dispersed in a tube containing 1ml of sterile distilled water, vortexed, boiled for 15 minutes at 100°C and

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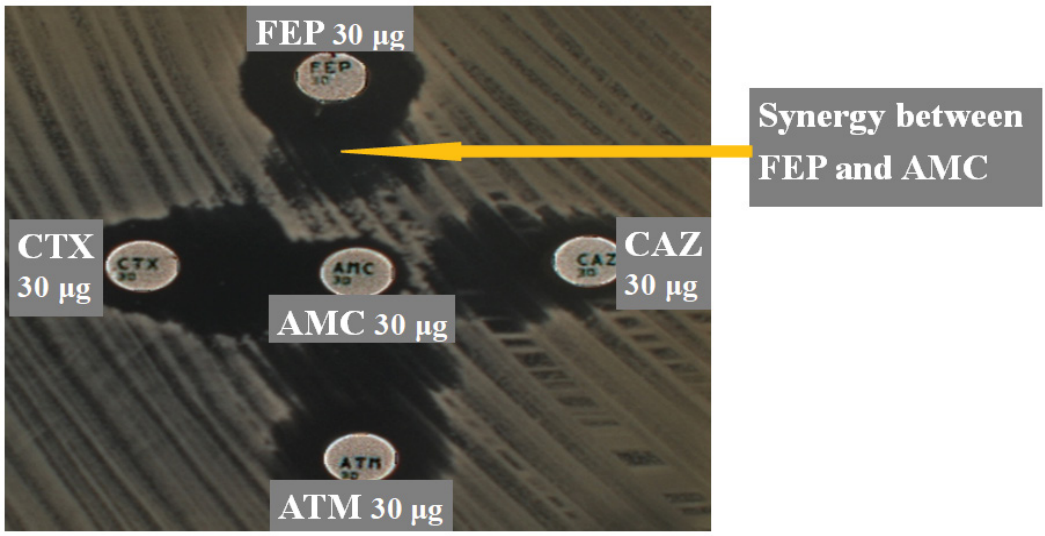

Figure 1: ESBL detection by double-disk synergy test. AMC, Amoxicillin-clavulanic acid; CTX, cefotaxim; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam

centrifuged at 13,200 rpm for 10 min. The supernatant was carefully recovered, aliquoted and stored at -20°C until used. Invitrogen™ Qubit™ 3 Fluorometer, (Thermo Fisher Scientific Inc, Strasbourg / France) was used for the DNA quantification.

ESBL genes amplification

Each DNA sample was subjected to a simplex end-point PCR (on Thermocycler 2720, Applied Biosystems, Lincoln Centre Drive, Foster City, California 94404, USA). Specific primers (table 1) were used to amplify ESBL genes (bla_{CTX-M}, $bla_{CTX-M-9}$, $bla_{CTX-M-15}$, $bla_{CTX-M-25}$, bla_{OXA-1}, bla_{TEM}, bla_{SHV}). Each reaction included positive and negative controls. Reaction volume of each PCR reaction was 20 µl (2.5 µl of DNA + 17.5 µl Master Mix FIREPol, Tartu / Estonia) and amplification of ESBL genes was according to the program below: initial denaturation at 95°C for 3 min, 35 PCR cycles (denaturation: 94°C, 30 sec; annealing; extension: 72°C, 60 sec) and a final elongation at 72°C for 7 min. Each amplicon (10 µl) was separated on 2% agarose gel in 1X TAE buffer for 35 min at 135 volts and the amplified fragment detected using a GelDoc imager (BioRad, Hercules, California / USA).

Statistical analysis

Statistical analyses were performed with Microsoft-Excel. The statistical test used is the Chi-square at 5% and 10% risk thresholds. p-values are obtained from the proportion comparison test and the level of significance for all statistical tests was set at $p < 0.05$ for the strong evidence of difference and $0.05 < p < 0.1$ for moderate evidence of difference [33, 34, 37].

Results

Antibiotic susceptibility testing

Based on resistance profiles observed, all 66 strains of this study are MDR. Antibiotic resistance profiles are compiled in the table 2. All strains (100%) were resistant to ampicillin (AMP), ticarcillin (TIC), Amoxicillin-clavulanic acid (AMC), cefalotin (CEF), cefotaxim (CTA), ceftazidime (CAZ) and cefepime (CEP). Strains were almost resistant to sulfamethoxazole-trimethoprim (TRS) and tetracycline (TET), respectively 65/66 (98.5%) and 57/66 (86.4%) and are highly resistant to most used antibiotics such as ciprofloxacin (CIP) and gentamicin (GEN), respectively 51 (77.3%) and 45 (68.2%). Forty strains out of 66 (60.6%) were resistant to fosfomycin (FOS). Amikacin, ertapenem and imipenem remained effective with respective sensitivity rate of 77.3% (51/66), 75.8% (50/66) and 100% (66/66) (table 2). CA were significantly more resistant to FOX ($p = 0.07$) and to sulfamethoxazole-trimethoprim ($p = 0.03$) than HA isolates. Based on resistance profiles, no significant difference was observed comparing UPKP to No-UPKP (table 2)

Twenty-two strains out of 66 (31.8%) shared identical resistance profiles. These isolates with identical drug resistance profiles were generally uropathogenic and community-acquired (Table 3). These identical resistance patterns ranged from 13 to 16 antibiotics.

ESBL genes

Of the 7 ESBL genes screened during this study, bla_{CTX-M} (64/66; 97%) and its variant $bla_{CTX-M-15}$ (63/66; 95.5%) were the most prevalent, followed by bla_{TEM} (58/66; 87.9%),

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Table 1. Oligonucleotide primers sequence used for PCR to detect ESBL genes

Target genes	Sequences genes	Sizes (bp)	Annealing Temp (°C)	References
bla_{CTX-M}	F: 5' - ATGTGCAGYACCAGTAARGTKATGGC - 3'	592	55	[35]
	R: 5' - TGGGTRAARTARGTSACCAGAAYSAGCGG - 3'			
$bla_{CTX-M-9}$	F: 5' - GTGACAAAGAGAGTGCAACGG - 3'	856	55	[35]
	R: 5' - ATGATTCTCGCCGCTGAAGCC - 3'			
$bla_{CTX-M-15}$	F: 5' - CACACGTGGAATTTAGGGACT - 3'	995	50	[35]
	R: 5' - GCCGTCTAAGGCGATAAACA - 3'			
$bla_{CTX-M-25}$	F: 5' - GCACGATGACATTCGGG - 3'	327	52	[35]
	R: 5' - AACCCACGATGTGGGTAGC - 3'			
bla_{OXA-1}	F: 5' - ATGAAAAACACAATACATATC - 3'	830	56	[36]
	R: 5' - AATTTAGTGTGTTTAGAATGG - 3'			
bla_{TEM}	F: 5' - TTGGGTGCACGAGTGGGTTA - 3'	506	55	[35]
	R: 5' - TAATTGTTGCCGGGAAGCTA - 3'			
bla_{SHV}	F: 5' - TCGGGCCGCGTAGGCATGAT - 3'	628	52	[35]
	R: 5' - AGCAGGGCGACAATCCCGCG - 3'			

Table 2: Antibiotics resistance rate of total strains, CA and HA, UPKP and no-UPKP.

Antibiotics		**Total strains**	**Pathogenicity**			**Origin**		
Class	**codes**	N (%)	**UPKP**	**No-UPKP**	**p**	**CA**	**HA**	**p**
			N (%)	N (%)		N (%)	N (%)	
Beta-lactams	AMP	66 (100)	42 (100)	24 (100)	1	54 (100)	12 (100)	1
	TIC	66 (100)	42 (100)	24 (100)	1	54 (100)	12 (100)	1
	AMC	66 (100)	42 (100)	24 (100)	1	54 (100)	12 (100)	1
	CEF	66 (100)	42 (100)	24 (100)	1	54 (100)	12 (100)	1
	FOX	26 (39.4)	16 (38.1)	10 (41.7)	0.8	24 (44.4)	2 (16.7)	0.07*
	CTA	66 (100)	42 (100)	24 (100)	1	54 (100)	24 (100)	1
	CAZ	66 (100)	42 (100)	24 (100)	1	54 (100)	24 (100)	1
	CEP	66 (100)	42 (100)	24 (100)	1	54 (100)	24 (100)	1
	AZT	57 (86.4)	38 (90.5)	19 (79.2)	0.19	47 (87)	10 (83.3)	0.73
	IMP	0	0	0	-	0	0	-
	ERT	10 (15.2)	6 (14.3)	4 (16.7)	0.79	9 (16.7)	1 (8.3)	0.47
Quinolones and Fluoroquinolones	NAL	39 (59.1)	25 (59.5)	14 (58.3)	0.92	32 (59.3)	7 (58.3)	0.95
	CIP	51 (77.3)	34 (81)	17 (70.8)	0.34	43 (79.2)	8 (66.7)	0.33
Aminoglycosides	GEN	45 (68.2)	27 (64.3)	18 (75)	0.36	35 (64.8)	10 (83.3)	0.21
	AMI	15 (22.7)	9 (21.4)	6 (25)	0.73	11 (20.4)	4 (33.3)	0.33
Phosphonic acid	FOS	40 (60.6)	25 (59.5)	15 (62.5)	0.82	34 (63)	6 (50)	0.4
Cyclines	TET	57 (86.4)	36 (85.7)	21 (87.5)	0.83	46 (85.2)	11 (91.7)	0.55
Antifolates	TRS	65 (98.5)	41 (97.6)	24 (100)	0.44	54 (100)	11 (91.7)	0.03**

UPKP, Uropathogenic *K. pneumoniae*; No-UPKP, *K. pneumoniae* isolated from pus, sputum, bronchial fluid and vaginal secretions; CA, Community-acquired; HA, Hospital-acquired; AMP, ampicillin; TIC, ticarcillin; AMC, Amoxicillin-clavulanic acid; CEF, cefalotin; FOX, cefoxitin; CTA, cefotaxim; CAZ, ceftazidime; CEP, cefepime; AZT, aztreonam; IMP, imipenem; ERT, Ertapenem; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; AMI, amikacin; FOS, fosfomycin; TET, tetracycline; TRS, sulphamethoxazole-trimethoprim; **, strong evidence of difference ($p < 0.05$); *, moderate evidence of difference ($0.05 < p < 0.1$).

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bla_{OXA-1} (47/66; 71.2%) and bla_{SHV} (31/66; 47%). None strain carried $bla_{CTX-M-9}$ or $bla_{CTX-M-25}$ (table 4 and figure 2). All HA (12/12; 100%) carried $bla_{CTX-M-15}$. We noticed that UPKP carried significantly more bla_{OXA-1} than No-UPKP (p = 0.08) (table 4). For the prevalence of $bla_{CTX-M-15}$, bla_{TEM} and bla_{SHV}, no significant difference was noted when comparing UPKP to No-UPKP and CA to HA (table 4).

In terms of accumulation of ESBL genes, (65/66; 98.5%) of isolates carried at least 2 ESBL genes out of the 7 sought and (01/66; 1.5%) strain did not carry any of the 7 genes sought. The combination (bla_{TEM} + bla_{SHV}) genes was detected in only one strain (01/66; 1.5%). The most prevalent ESBL gene combinations were ($bla_{CTX-M-15}$ + bla_{OXA-1} + bla_{TEM}) (24/66; 36.4%) and ($bla_{CTX-M-15}$ + bla_{TEM} + bla_{OXA-1} + bla_{SHV}) (18/66; 27.3%) (table 5).

Discussion

Antibiotic susceptibility testing

High resistance rates (68.2% to 100%) reported during our study for AMP, TIC, AMC, CEF, CTA, CAZ, CEP, TRS, TET, CIP and GEN are very suggestive as CIP, GEN, 3rd and 4th generation cephalosporins are widely prescribed as empirical therapies to treat community-acquired and hospital-acquired bacterial infections. It is therefore necessary to expand research on the susceptibility profiles of *Enterobacteriaceae* to these first-line antibiotics. This could lead to modification or adjustment of empirical antibiotic therapies in Senegal. [28, 29, 31, 32] mentioned prevalence of resistance to CAZ (50% and 67%); CEP (38% and 75%); CIP (83%, 67% and 65%); GEN (42%, 38% and 8%); AMI (12% and 0%); TRS (58% et 50%) and TET (42%) in ESBL-producing K. pneumoniae isolates. By comparing theirs results with those obtained during our present study, we noted that our isolates were generally more resistant. Right now, we do not have the exact reason why clinical *K. pneumoniae* from Dakar seem to be more resistant than those isolated in other countries. However, self-medication and the easy access to antibiotics without medical prescription could be among main causes. 60.6% of the strains in our study

Table 3. Identical drug resistance profiles in tested isolates.

Drugs	Total Strains N (%)	Pathogenicity		Origin	
		UPKP N (%)	No-UPKP N (%)	CA N (%)	HA (%)
AMP, TIC, AMC, CEF, FOX, CTA, CAZ, CEP, AZT, ERT, NAL, CIP, GEN, FOS, TET, TRS	3 (4.5)	3 (7.1)	0	3 (5.6)	0
AMP, TIC, AMC, CEF, FOX, CTA, CAZ, CEP, AZT, NAL, CIP, GEN, AMI, FOS, TET, TRS	2 (3)	1 (2.4)	1 (4.2)	2 (3.7)	0
AMP, TIC, AMC, CEF, FOX, CTA, CAZ, CEP, AZT, NAL, CIP, GEN, AMI, TET, TRS	3 (4.5)	2 (4.8)	1 (4.2)	2 (3.7)	1 (8.3)
AMP, TIC, AMC, CEF, CTA, CAZ, CEP, AZT, NAL, CIP, GEN, AMI, TET, TRS	2 (3)	2 (4.8)	0	1 (1.9)	1 (8.3)
AMP, TIC, AMC, CEF, CTA, CAZ, CEP, AZT, NAL, CIP, GEN, FOS, TET, TRS	4 (6.1)	2 (4.8)	2 (8.3)	3 (5.6)	1 (8.3)
AMP, TIC, AMC, CEF, CTA, CAZ, CEP, AZT, NAL, CIP, FOS, TET, TRS	3 (4.5)	2 (4.8)	1 (4.2)	3 (5.6)	0
AMP, TIC, AMC, CEF, CTA, CAZ, CEP, AZT, NAL, CIP, GEN, TET, TRS	4	4	0	3	1

UPKP, Uropathogenic *K. pneumoniae*; No-UPKP, *K. pneumoniae* isolated from pus, sputum, bronchial fluid and vaginal secretions; CA, Community-acquired; HA, Hospital-acquired; AMP, ampicillin; TIC, ticarcillin; AMC, Amoxicillin-clavulanic acid; CEF, cefalotin; FOX, cefoxitin; CTA, cefotaxim; CAZ, ceftazidime; CEP, cefepime; AZT, aztreonam; IMP, imipenem; ERT, Ertapenem; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; AMI, amikacin; FOS, fosfomycin; TET, tetracycline; TRS, sulphamethoxazole-trimethoprim; N, total isolates number

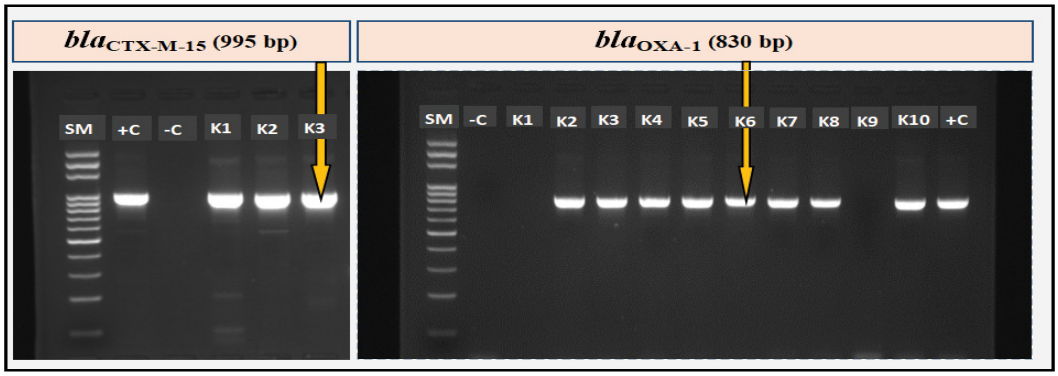

Figure 2: Pictures of PCR running gels: $bla_{CTX-M-15}$ on the left and bla_{OXA-1} on the right. SM, size marker; +C, positive control; -C, negative control; K1-K10, representative of tested *K. pneumoniae* isolates.

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Table 4: Prevalences of ESBL genes in total strains, CA and HA, UPKP and No- UPKP.

ESBL		Total strains	Pathogenicity			Origin		
Family	Genes	N (%)	UPKP	No- UPKP	p	CA	HA	p
			N (%)	N (%)		N (%)	N (%)	
Cefotaximase-Munich	bla_{CTX-M}	64 (97)	41 (97.6)	23 (95.8)	0.85	50 (96.2)	12 (100)	0.55
	$bla_{CTX-M-9}$	0	0	0	-	0	0	-
	$bla_{CTX-M-15}$	63 (95.5)	41 (97.6)	22 (91.7)	0.26	51 (94.4)	12 (100)	0.4
	$bla_{CTX-M-25}$	0	0	0	-	0	0	-
Oxacillinase	bla_{OXA-1}	47 (71.2)	33 (78.5)	14 (58.3)	0.08*	40 (74.1)	7 (58.3)	0.27
Temoneira	bla_{TEM}	58 (87.9)	36 (85.7)	22 (91.7)	0.48	48 (88.9)	10 (83.3)	0.59
Sulfhydryl variable	bla_{SHV}	31 (47)	19 (45.2)	12 (50)	0.71	25 (46.3)	6 (50)	0.82

UPKP, Uropathogenic *K. pneumoniae*; No-UPKP, *K. pneumoniae* isolated from pus, sputum, bronchial fluid and vaginal secretions; CA, community-acquired; HA, hospital-acquired; %, percentage; N, number of isolates; **, strong evidence of difference ($p < 0.05$); *, moderate evidence of difference ($0.05 < p < 0.1$).

Table 5: Prevalence of ESBL genes combinations in total strains, CA and HA, UPKP and No-UPKP.

Combination of ESBL genes	Total strains	Pathogenicity			Origin		
	N (%)	UPKP	No-UPKP	p	CA	HA	p
		N (%)	N (%)		N (%)	N (%)	
$bla_{CTX-M-15} + bla_{TEM} + bla_{OXA-1} + bla_{SHV}$	18 (27.3)	12 (28.6)	6 (25)	0.75	15 (27.8)	3 (25)	0.84
$bla_{CTX-M-15} + bla_{OXA-1} + bla_{TEM}$	24 (36.4)	17 (40.8)	7 (29.2)	0.35	20 (37)	4 (33.3)	0.81
$bla_{CTX-M-15} + bla_{TEM} + bla_{SHV}$	7 (10.6)	3 (7.1)	4 (16.7)	0.22	5 (9.3)	2 (16.7)	0.45
$bla_{CTX-M-15} + bla_{OXA-1} + bla_{SHV}$	3 (4.5)	3 (7.1)	0	0.18	3 (5.6)	0	0.4
$bla_{CTX-M-15} + bla_{TEM}$	7 (10.6)	4 (9.5)	3 (12.5)	0.7	6 (11.1)	1 (8.3)	0.77
$bla_{CTX-M-15} + bla_{SHV}$	2 (3)	1 (2.4)	1 (4.2)	0.68	1 (1.9)	1 (8.3)	0.24
$bla_{CTX-M-15} + bla_{OXA-1}$	1 (1.5)	1 (2.4)	0	0.44	1 (1.9)	0	0.63
$bla_{TEM} + bla_{OXA-1}$	1 (1.5)	0	1 (4.2)	0.18	1 (1.9)	0	0.63
$bla_{TEM} + bla_{SHV}$	1 (1.5)	0	1 (4.2)	0.18	1 (1.9)	0	0.63

UPKP, Uropathogenic *K. pneumoniae*; No-UPKP, *K. pneumoniae* isolated from pus, sputum, bronchial fluid and vaginal secretions; CA, community-acquired; HA, hospital-acquired %, percentage; N, number of isolates; **, strong evidence of difference ($p < 0.05$); *, moderate evidence of difference ($0.05 < p < 0.1$).

were resistant to fosfomycin. This is alarming as until now, fosfomycin was an alternative and capital molecule to treat UTIs caused by MDR *Enterobacteriaceae* without resorting to carbapenems. Clinicians must thererfore properly control fosfomycin prescription and researchers must conduct large-scale studies on the resistance profiles of *Enterobacteriaceae* to this antibiotic. It is also necessary to investigate resistance mechanisms involved in fosfomycin resistance in Dakar. It would be worrying in case resistance to fosfomycin was mediated by genes located on mobile genetic elements, as these mobile genetic elements are major carriers of transmission of inter- and intraspecific drug resistance. These studies could help to quickly contain *Enterobacteriaceae* resistance to fosfomycin and to continue to use it as an alternative treatment to carbapenems.

To "protect" carbapenems (by delaying their use), it is therefore important to study the susceptibility profiles of our 66 *K. pneumoniae* strains to alternative antibiotics such as tigecycline, temocillin, ceftazidime-avibactam and ceftolozane-tazobactam. Only 15.2% of the strains in this study were resistant to Amikacin. While waiting for larger-scale studies on the efficacy of amikacin on MDR *K. pneumoniae* in Senegal, amikacin would constitute an alternative molecule for the treatment of MDR *K. pneumoniae* infections. Nevertheless, clinicians should carefully control the prescription of amikacin to delay the emergence of amikacin-resistant bacterial clones. In this study, all CA strains were resistant to TRS, and CA were significantly more resistant to TRS than HA ($p = 0.03$). This might be due to the fact that in our area, TRS is a widely consumed drug in community. TRS selection pressure may have caused high carriage of TRS-resistant *K. pneumoniae* in the community. In addition, it is necessary to investigate the resistance mechanisms, involved genes and their genetic supports.

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ESBL genes

Since the appearance of CTX-M-type ESBLs in 1990 [21], they have been reported all over the world and have become the major ESBL, relegating SHV, TEM and OXA-types ESBLs. The results obtained during our study follow this trend. Indeed, $bla_{CTX\text{-}M\text{-}15}$, gene encoding CTX-M-15, which confers a high level of resistance to cefotaxime, ceftriaxone, ceftazidime and aztreonam [22], was present in 95.5% of isolates. $bla_{CTX\text{-}M\text{-}15}$ was present in 97% of clinical isolates in Spain [23]; in 91% of hospital-acquired isolates in Portugal and in 84% of isolates in Ethiopia [24, 25]. [26] had previously mentioned a high prevalence of $bla_{CTX\text{-}M\text{-}15}$ (96.9%) in clinical *K. pneumoniae* strains isolated in another university teaching hospital in Dakar. Paradoxically, [27] and [28] had reported respectively an absence of $bla_{CTX\text{-}M\text{-}15}$ in 70 strains of *K. pneumoniae* in Uganda and a low prevalence of 30%. $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}25}$ were not detected during our study. This seems to confirm claims that CTX-M-9 and CTX-M-25 are minor variants of CTX-M. [26] had also mentioned absence of $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}25}$ in Dakar.

During this study, prevalence of "old" beta-lactamases (TEM and SHV-type) genes were respectively 87.9% and 47%. Prevalence of bla_{TEM} of 78.1% in Senegal [26], 40% in China [28], and 3.8% in Uganda had been recently reported. Moreover, [28] had mentioned a predominance of bla_{SHV} of 60%. Some variants of bla_{TEM} and bla_{SHV} encode penicillinases while others code for ESBLs. We need Therefore to sequence these bla_{TEM} and bla_{SHV} genes to find accurate variants present in our isolates as well as their real impact on the ESBL phenotypes observed in isolates.

The presence of $bla_{OXA\text{-}1}$ (known to strongly hydrolyze cefepime) in 71.2% of isolates strains seems to be one main reason for the resistance of all isolates to cefepime. Our isolates seem to carry more $bla_{OXA\text{-}1}$ than isolates reported during other similar studies. Indeed, the highest prevalence of $bla_{OXA\text{-}1}$ in Sudan, Ghana and Dakar was 30% [26, 29, 30]. The logical continuation of this study will be the whole genome sequencing (WGS). This will provide additional informations and deepen this study. Indeed, WGS will detect other ESBL genes different from those sought in our study. WGS will also specify TEM and SHV variants responsible for ESBL production in the isolate that carried only (bla_{TEM} + bla_{SHV}) combination. The fact that the strains of our study cumulated on average 4 out of 7 ESBL genes sought could be the cause of their resistance to all penicillins and cephalosporins classes. This high accumulation of ESBL genes per strain combined with resistance to multiple antibiotic families is suggestive of the carriage of mobile genetic elements (plasmids, transposons and integrons). WGS could confirm and deepen our assertion. Finally, we will also check bacterial clones within this studied bacterial population. This will provide more epidemiological data and will contribute to initiate preventive and curative measures, especially in hospital settings.

Conclusion

We note that amikacin and carbapenems would still be effective in the treatment of MDR ESBL-producing *K. pneumoniae* infections, and $bla_{CTX\text{-}M\text{-}15}$ seems to be predominant ESBL gene in clinical *K. pneumoniae* strains isolated in Dakar. Furthermore, uropathogenic strains carried significantly more $bla_{OXA\text{-}1}$ than No-uropathogenic strains. We recommend to avoid monotherapy and to prohibit C3G, C4G and fluoroquinolones as empirical treatment of UTI in Dakar-Senegal. More than half of our strains were resistant to fosfomycin. We therefore recommend an in-depth and larger-scale study in order to assess the true extent of *Enterobacteriaceae* fosfomycin resistance in Dakar. These actions may help preserve the efficacy of this precious molecule. Whole-genome sequencing could provide additional and in-depth informations. Such studies will help to initiate or improve the public health policy of limiting MDR bacteria spread, especially in hospital settings.

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Ethical research approval

Our study has received the Ethical Research approval of the Research Ethics Committee (CER) of Cheikh Anta Diop University (UCAD) under the reference CER/UCAD/AD/MSN/051/2020.

Conflict of interests

The authors have not declared any conflict of interests.

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