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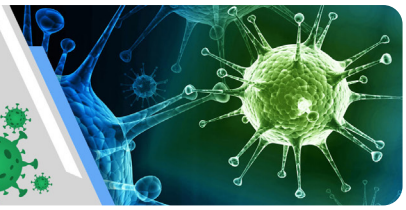

Antibiotic Resistance and Virulence Factors of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella Pneumoniae* Involved in Healthcare-Associated Infections in Dakar, Senegal

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Abstract

Background: Virulent and resistant *Klebsiella pneumoniae* strains are considered as one of the most significant causes of healthcare-associated infections (HAIs). The aim of this study was to investigate the phenotypic and genotypic factors of antibiotic resistance and virulence factors of ESBL *K. pneumoniae* strains isolated from healthcare-associated infections (HAIs) in Dakar, Senegal.

Methods: Twenty-eight strains of *K. pneumoniae* isolated from HAIs were collected from 2018 to 2021 in 2 main hospitals in Dakar. Antibiotic susceptibility and molecular characterization were studied using disk diffusion by the Kirby-Bauer method and PCR, respectively. Virulence factors were also determined by PCR.

Results: These ESBL *K. pneumoniae* isolates showed high resistance to antibiotics such as β-lactams, aminoglycosides, cyclins, fluoroquinolones and trimethoprim-sulfamethoxazole. Among these strains, ten (10) were resistant to carbapenem and cefoxitin (17.8%, n=5), chloramphenicol (25%, n=7) and fosfomycin (28.5%, n=8) considered as the most active antibiotics against ESBL-KP isolates. Eighteen (18) strains were considered as MDR and ten (10) strains as XDR.

For the genes associated to phenotypic resistance, β-lactams resistance was conferred through bla_{SHV} (24/28), bla_{TEM} (20/28) and mainly by bla_{CTX-M}. All strains carried the $bla_{CTX-M15}$ gene. *OXA-48* (6/28) gene was found responsible for carbapenem resistance and other genes like IMP, VIM, NDM, OXA-23 were not detected. Plasmids-mediated resistance genes *qnrB* (16/28), *qnrS* (11/28) and *aac(6')-Ib* (21/28), were mostly responsible for resistance to fluoroquinolones and aminoglycosides. Also 3 of 6 virulence genes searched that are the most associated to the pathogenicity of *Klebsiella pneumoniae* were found on these strains with *uge* (19/28), *mrKD* (21/28) and *fyuA* (13/28).

Conclusion: The ESBL *K. pneumoniae* strains isolated in this study showed a high prevalence of antibiotic resistance and virulence genes The combination of these factors poses a potential risk for infections that could be highly virulent and difficult to treat. These findings demonstrated the importance of closely monitoring the resistance patterns of *K. pneumoniae* in hospitals seetings and emphasize the need to monitor effective antibiotic treatments for *K. pneumoniae* infections. Aditionnally, the scarcity of available data on HAIs, especially the prevalence of Multidrug resistance bacteria and virulence factors associated with these HAIs in Senegal, further emphasizes the significance of implementing surveillance programs to better know their prevalence, impact on patient health and on length of hospital stays.

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Introduction

Antimicrobial resistance is a worldwide major public health problem, leading to elevated rates of illness and mortality, especially in immunocompromised patients [1]. Numerous reports and studies released by international organizations have outlined the far-reaching consequences of this issue on both healthcare and economic levels [2, 3].

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic and rod shape bacterium belonging to the *Enterobacteriaceae* family [4]. *Klebsiella pneumoniae* is also among the ESKAPE pathogens which adopt different mechanisms to "escape" from different antimicrobials actions [5] and is frequently associated with a wide range of healthcare associated infections (HAIs) such as pneumonia, bacteremia, urinary tract infection (UTI), soft tissue and burn infections [6]. Infections due to *K. pneumoniae* are difficult to treat because of combination of both expression of virulence factors and emergence of antibiotic resistances [4]. Some hypervirulent serotypes are particularly associated with various clinical syndromes characterized by community-acquired *K. pneumoniae* bacteremia with primary liver abscess, metastatic meningitis and endophthalmitis [7].

Klebsiella pneumoniae strains isolated from HAIs have exhibited a notable degree of antibiotic resistance and epidemiological investigations have identified multiple genes within these *K. pneumoniae* strains that confer resistance to commonly prescribed antibiotics including beta-lactams, fluoroquinolones, aminoglycosides, cyclines, macrolides, lincosamides, folate inhibitors, and phenicol [8]. In low and middle income countries, multidrug resistant *K. pneumoniae* is one important driver of unfavorable outcome in infections, primarily because suitable treatment options are often either unavailable or unaffordable [9].

Resistance of *K. pneumoniae* to antibiotics can be attributed to various mechanisms including modification of antibiotic target sites, alteration of metabolic pathways, activation of efflux pump systems, change in membrane permeability and release of antibiotic-inactivating enzymes [10]. Antibiotic resistance is a complex and multifactorial mechanism resulting from antibiotic exposure in hospital settings. Consequently, the selective pressure created leads to the development of numerous genetic mechanisms of resistance [11]. The acquired resistance over the years has led to the emergence of strains that are classified as Multi Drug-Resistant (MDR), Extensively Drug-Resistant (XDR) and Pan Drug-Resistant (PDR) strains [12].

Pathogenicity of *K. pneumoniae* can be attributed to the presence of virulence genes which encode for different types of virulence factors. These factors such as adhesin for attachment to host cells, capsules that are antiphagocytic, siderophores that aid the bacterium in its competition with the host for iron and other various endotoxins [13]. Virulence-associated genes searched includes those encoding regulators of mucoid phenotype A (*rmp*A), mucoviscosity associated gene (*mag*A), uridine diphosphate galacturonate 4-epimerase gene (*uge*), type 3 fimbrae (*mrKD*), yersiniabactin receptor (*fyuA*) and the iron uptake system gene (*kfu*) which are responsible for colonization, invasion and pathogenicity, and have a predominant role in pathogenicity of *K. pneumoniae* strains isolated from HAIs [13, 14].

In Senegal, it is difficult to assess accurate incidence of healthcare associated infections due to limited research funds and a scarcity of published data. The few studies conducted in Senegal from 2005 to 2019 have shown a prevalence of healthcare associated infections ranging from 6.8% to 13.6% [15-19]. These studies have also identified highly resistant bacteria, including *K. pneumoniae* the most found one. However, none of them have investigated the molecular mechanisms of resistance. Therefore, the aim of this study is to investigate presence of key main virulence factors and the antibiotic resistance profiles of Extended-Spectrum Beta-lactamases (ESBL) strains of *K. pneumoniae* involved in HAIs in Dakar, Senegal, and the relationship between phenotypic and genetic patterns of antibiotic resistance. This is the first study investigating the molecular diversity of HAIs strains in Senegal.

Materials and Methods

Bacterial strains

In this retrospective study, we focused on 28 strains of *K. pneumoniae* isolated during routine laboratory activities from 2018 to 2021 of 2 major hospitals in Dakar, i.e. Hospital Aristide Le Dantec and the Center Albert Royer of Fann. Strains were isolated from various clinical specimens and were considered as hospital acquired if the infection occurs at least 48 hours after the hospitalization. As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

The epidemiological data of collected strains were obtained from the registers at the bacteriology laboratory of each hospital. All laboratory techniques and procedures of isolation, identification, and storage of the included isolates in the current study were performed according to standards microbiological protocols included Gram staining, cultural characteristics on agar media and biochemical testing such as API 20E (Biomérieux, Marcy-l'Étoile, France) or Vitek2® system (BioMérieux® -France).

The identification of *K. pneumoniae* isolates was then confirmed using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF/MS)

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(Vitek MS; BioMérieux, Inc., Marcy-l'Etoile, France) in Pasteur Institute of Dakar.

Antibiotic susceptibility testing

Nineteen (19) antibiotics disks (Bio-Rad) were selected from the standard list proposed by CA-SFM/EUCAST version 2022 and interpreted according to the given guidelines. The cyclins (tetracycline, minocyclin and doxycyclin) were interpreted from the CLSI guidelines, version 2020. Stored cultures of *K. pneumoniae* strains were subcultured in Bromocresol purple (BCP) incubated at 37° for 24 h. Inoculum were adjusted to 1.5×10^8 CFU, corresponding to 0.5 McFarland and streaked on Mueller Hinton agar (Oxoid, UK) surface with sterile swab. Antimicrobial susceptibility was determined by strain growth zone diameter using the Kirby-Bauer method and interpretation done according to CA-SFM/EUCAST (version 2022) and CLSI guidelines (version 2021).

The presence of an extended-spectrum beta-lactamase (ESBL) was detected on the antibiogram by the synergy test based on the visualization of a "champagne cork" image between third- or fourth-generation cephalosporins and amoxicillin-clavulanic acid or piperacillin-tazobactam discs. The following 22 antimicrobials disks from Biorad were used: AMP: Ampicillin (10 µg), AMC: amoxicillin-clavulanic acid (20+10 µg), TIC: ticarcillin (75µg), TIM: ticarcillin-clavulanic acid (75+10 µg), FOX: cefoxitin (30 µg), CTX: cefotaxim (30 µg), CAZ: ceftazidime (30 µg), FEP: cefepime (30 µg), AZT: aztreonam (30 µg), IMP: imipenem (10 µg), ERT: ertapenem (10 µg), FOS: fosfomycin (10 µg), CIP: ciprofloxacin (5 µg), NOR: norfloxacin (10 µg), GN: gentamicin (10 µg), TMN: tobramycin (10 µg), AK: amikacin (30 µg), TET: tetracycline (30 µg), MIN: minocyclin (30 µg), DO: doxycyclin (30 µg), CHL: chloramphenicol (30 µg) and SXT: trimethoprim-sulfamethoxazole (1.25/23.75 µg).

Reference strain *E. coli* ATCC 25922 were used for quality control. Bacterial strains that were resistant to a minimum of at least 3 different classes of antibiotics were considered as MDR and those only susceptible to only one or two class of antibiotics and resistant to all sub classes in all classes of antibiotics were considered as XDR and PDR, respectively as previously described [12].

Detection of virulence and antibiotics resistance-associated genes by PCR

DNA extraction by thermolysis

Three colonies of each isolate on nutrient agar plate were picked and suspended in 200µl of distilled water. After vortexing, the cell suspension was boiled for 10 minutes and 150µl of the supernatant was collected after spinning for 10 minutes at 13,000 rpm in a microcentrifuge.

Determination of antimicrobial resistance genes

PCR was performed on Thermocycler 2720 (Applied Biosystems, Lincoln 113 Centre Drive, Foster City, California 94404 USA) to detect 26 antimicrobials resistance-associated genes include: β-lactamase genes (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}2}$ group, $bla_{CTX\text{-}M\text{-}8}$ group, $bla_{CTX\text{-}M\text{-}9}$ group, $bla_{CTX\text{-}M\text{-}25}$ group and $bla_{CTX\text{-}M\text{-}15}$), carbapenem resistance-associated genes (*IMP*, *NDM*, *OXA48*, *OXA23*, *VIM* and *KPC*), quinolone resistance-associated genes (*gyrA, gyrB, parC, parE, qnrA, qnrB, qnrC, qnrD and qnrS*) and aminoglycoside resistance-associated genes (*aac(6')-Ib*, *Aad*A1).

Each reaction included positive and negative controls. The PCRs were carried out in 20 µl reaction volume (2.5 µl DNA + 17.5 Master Mix FIREPol® + 0.5µl of each primer). All primers used and PCR thermo cycling conditions in this study are listed in Table 5 and 6 respectively (Supplementary). PCR products were loaded on a 1.5% (w/v) agarose gel stained with ethidium bromide (0.5 mg/mL), electrophoretic migrations were done at 120 volts for 45 min in 1X TAE buffer and amplified fragments visualized using a GelDoc imager (BioRad).

Detection of virulence genes

Distribution of 6 genes (*rmp*A, *mrK*D, *kfu*, *mag*A, *uge*, *fyu*A) associated with the virulence of *K. pneumoniae* were investigated. PCR were carried out with their specific primers and all PCR thermo cycling conditions are listed respectively in Table 7 and Table 8 (Supplementary).

Data were entered and analyzed using Excel.

Results

Total isolates

Twenty-eight (28) *K. pneumoniae* strains isolated from patients with HAIs were collected. Strains were isolated from 2018 to 2021 from different clinical samples: wound (n=14), urine (n=7), bacteriemia (n=7).

Antimicrobial susceptibility testing

Among the 22 antibiotics tested, antimicrobial susceptibility tests demonstrated that all the 28 strains were resistant to amoxicillin + clavulanic acid, ticarcillin + clavulanic acid, cefotaxim, ceftazidime, cefepime, aztreonam and cyclins (Table 1). Very high resistance rates (70-96.8%) were found for piperacillin + tazobactam, ciprofloxacin, norfloxacin, gentamicin, tobramycin, amikacin, trimethoprim/sulfamethoxazole. Ertapenem moderate resistance rates were found (32.1%) while a lowest level rate of resistance was found for imipenem (17.8%), cefoxitin (17.8%), chloramphenicol (25%) and fosfomycin (28.5%) (Table 1). Our work has shown ten carbapenem resistant strains in our cohort. Eighteen strains (64.3%) were considered as MDR as

Table 1: Antibiotic Resistance of *K. pneumoniae* strains isolated from healthcare-associated

Group	Antibiotics	Resistance Rate N (%)
β-lactams	Amoxicillin + clavulanic acid	28 (100)
	Ticarcillin + clavulanic acid	28 (100)
	Piperacillin + tazobactam	22 (78.5)
	Cefoxitin	5 (17.8)
	Cefotaxim	28 (100)
	Ceftazidime	28 (100)
	Cefepime	28 (100)
Monobactams	Aztreonam	28 (100)
Carbapenems	Ertapenem	9 (32.1)
	Meropenem	6 (21.4)
	Imipenem	6 (21.4)
Fluoroquinolones	Ciprofloxacin	27 (96.4)
	Norfloxacin	23 (82.1)
Aminogycosides	Gentamicin	21 (75)
	Tobramycin	23 (82.1)
	Amikacin	20 (71.4)
Cyclins	Tetracycline	28 (100)
	Minocyclin	28 (100)
	Doxycyclin	28 (100)
Phosphonic acids	Fosfomycin	8 (28.5)
Phenicols	Chloramphenicol	7 (25)
Folate pathway inhibitor	trimethoprim/sulfamethoxazole	26 (92.8)

they were resistant to 1 antibiotic in 3 different classes and 10 strains (35.7) were XDR Table 4.

Gene diversity of antimicrobial resistance genes

The results of the current study showed that all of the strains harbored at least one ESBL gene. Specifically, 22 strains (78.5%) positives for bla_{TEM}, 21 (75%) for bla_{SHV}, and 21 strains (75%) for bla_{OXA-1}. All the strains were positives for bla_{CTX-M} with the variant $bla_{CTX-M15}$ identified in all of the 28 strains. For carbapenems, among the 10 resistant strains (35%), six (6) were found to carried *OXA-48* gene and were phenotypically resistant to all antibiotics belonging to this class of antibiotics. The *aac(6')-Ib* gene that confer resistance to aminoglycosides was found in 21 strains (75%). Regarding fluoroquinolone resistance, gene *qnrB* were detected for 16 strains (57.1%), *qnrS* for 11 strains (39.3%), *gyrB* for 3 strains (10.7%) and *parE*, *qnrA*, *qnrC* and *qnrD* for 1 strain respectively (3.6%). (Table 2 and Table 4).

Molecular detection of virulence genes

Three virulence genes were detected in the 28 strains: *uge* (19, 67.8%), *mrK*D (21, 75%) and *fyu*A (13, 46.4%) (Table 3). Among the different virulence genes, we found *mrK*D gene

Table 2: Genotypic resistance profile of 28 *K. pneumoniae* strains isolated from health-care associated infections

Group	Resistance Genes	N (%)
β-lactams	bla_{CTX-M1}	28 (100)
	$bla_{CTX-M15}$	28 (100)
	bla_{TEM}	20 (71.4)
	bla_{SHV}	24 (85.7)
	bla_{OXA-1}	21 (75)
Carbapenems	*OXA-48*	6 (21.4)
Fluorquinolons	*gyrB*	3 (10.7)
	parE	1 (3.6)
	qnrA	1 (3.6)
	qnrB	16 (57.1)
	qnrC	1 (3.6)
	qnrD	1 (3.6)
	qnrS	11 (39.3)
Aminogycosides	*aac(6')-Ib*	21 (75)

Table 3: Distribution of virulence genes amongst the *K. pneumoniae* from health-care associated infections.

Virulence factors	Genes	N (%)
Metabolic enzyme	uge	19 (67.8)
Type 3 fimbrae	mrKD	21 (75)
Yersiniabactin receptor	fyuA	13 (46.4)

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Table 4: Characterization of *K. pneumoniae* isolated from healthcare-associated infections

Isolate	Resistance profile	Resistance type	Antibiotic resistance gene	Virulence gene
1KP1	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, QnrB, aac(6')-Ib*	*mrkD, uge*
1KP2	AMC, TIM, TZP, FOX, CTX, CAZ, FEP, ATM, ERT, MEM, IPM, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	XDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, OXA-48, QnrB, aac(6')-Ib*	*mrkD, uge*
1KP3	AMC, TIM, TZP, FOX, CTX, CAZ, FEP, ATM, ERT, CIP, NOR, FOS, GM, TMN, AKN, TET, MN, DO, SXT	XDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, QnrB, aac(6')-Ib*	*mrkD, uge*
1KP4	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, FOS, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, QnrB, QnrD, aac(6')-Ib*	*mrkD, uge, fyuA*
1KP5	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, ERT, MEM, IMP, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, OXA-48, QnrB, aac(6')-Ib*	*mrkD, uge, fyuA*
1KP9	AMC, TIM, TZP, FOX, CTX, CAZ, FEP, ATM, ERT, MEM, IMP, CIP, NOR, FOS, GM, TMN, AKN, TET, MN, DO, SXT	XDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, OXA-48, QnrB, aac(6')-Ib*	*mrkD*
1KP10	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, FOS, GM, TMN, AKN, TET, MN, DO, SXT	XDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, QnrB, aac(6')-Ib*	*fyuA*
1KP11	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, ERT, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, QnrB, aac(6')-Ib*	
1KP12	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, ERT, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaSHV, blaOXA-1, blaCTX-M15, QnrB, aac(6')-Ib*	*mrkD, uge, fyuA*
1KP13	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaSHV, blaOXA-1, blaCTX-M15, QnrS, aac(6')-Ib*	*mrkD, uge*
1KP14	AMC, TIM, CTX, CAZ, FEP, ATM, CIP, NOR, TET, MN, DO, SXT	MDR	*blaSHV, blaCTX-M1, QnrS*	
1KP15	AMC, TIM, CTX, CAZ, FEP, ATM, FOS, TET, MN, DO, SXT	MDR	*blaTEM, blaCTX-M15, QnrS*	
1KP16	AMC, TIM, TZP, FOX, CTX, CAZ, FEP, ATM, ERT, IMP, MEM, CIP, GM, TMN, TET, MN, DO, CHL, SXT	XDR	*blaCTX-M15, OXA-48, QnrS*	*mrkD, uge, fyuA*
2KP1	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaSHV, blaOXA-1, blaCTX-M15, QnrS, aac(6')-Ib*	
2KP3	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, GM, TMN, AKN, TET, MN, DO	MDR	*blaOXA-1, blaCTX-M15, GyrB, QnrS, aac(6')-Ib*	*mrkD, uge*
2KP4	AMC, TIM, CTX, CAZ, FEP, ATM, CIP, NOR, GM, TMN, TET, MN, DO	MDR	*blaTEM, blaSHV, blaCTX-M15, QnrB*	
2KP5	AMC, TIM, TZP, FOX, CTX, CAZ, FEP, ATM, ERT, MEM, IMP, CIP, NOR, GM, TMN, AKN, TET, MN, DO, CHL, SXT	XDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, OXA-48, QnrS, aac(6')-Ib*	*mrkD, uge, fyuA*
2KP7	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, ERT, CIP, NOR, GM, TMN, AKN, MN, CHL, SXT	XDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, OXA-48, QnrS, aac(6')-Ib*	*mrkD, uge, fyuA*
2KP8	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M15, QnrB, aac(6')-Ib*	*mrkD, uge, fyuA*
2KP9	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, ERT, CIP, NOR, FOS, GM, TMN, AKN, TET, MN, DO, CHL, SXT	XDR	*blaOXA-1, blaCTX-M15, parE, QnrA, aac(6')-Ib*	*uge*
2KP11	AMC, TIM, CTX, CAZ, FEP, ATM, CIP, TET, MN, DO, SXT	MDR	*blaTEM, blaSHV, blaCTX-M1, blaCTX-M15, QnrB*	*mrkD, uge, fyuA*
2KP12	AMC, TIM, CTX, CAZ, FEP, ATM, CIP, TET, MN, DO, SXT	MDR	*blaTEM, blaSHV, blaCTX-M1, blaCTX-M15, QnrB*	*mrkD, uge, fyuA*
2KP13	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, FOS, GM, TMN, AKN, TET, MN, DO, SXT	XDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M1, blaCTX-M15, GyrB, QnrS, aac(6')-Ib*	*mrkD, fyuA*
2KP14	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaOXA-1, blaTEM, blaSHV, blaCTX-M1, blaCTX-M15, QnrB, aac(6')-Ib*	*mrkD, uge, fyuA*
2KP15	AMC, TIM, CTX, CAZ, FEP, ATM, TET, MN, DO, CHL, SXT	MDR	*blaTEM, blaCTX-M1, blaCTX-M15, QnrC, QnrS*	*mrkD, uge, fyuA*
2KP16	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, TMN, AKN, TET, MN, DO, SXT	MDR	*blaOXA-1, blaCTX-M1, blaCTX-M15, QnrS, aac(6')-Ib*	*mrkD*
2KP18	AMC, TIM, TZP, CTX, CAZ, FEP, ATM, CIP, NOR, FOS, GM, TMN, AKN, TET, MN, DO, SXT	MDR	*blaTEM, blaSHV, blaOXA-1, blaCTX-M1, blaCTX-M15, QnrB, aac(6')-Ib*	*mrkD, uge*
2KP20	AMC, TIM, TZP, FOX, CTX, CAZ, FEP, ATM, CIP, NOR, FOS, GM, TMN, AKN, TET, MN, DO, SXT	XDR	*blaTEM, blaOXA-1, blaCTX-M1, blaCTX-M15, GyrB, QnrB, aac(6')-Ib*	*mrkD, uge*

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Table 5: Sequencing of primers used in PCR for 32 antimicrobials resistance-associated genes of *K. pneumoniae*

Gene	Oligo sequence (5'-3')	Product size (bp)	Reference
β-lactams genes			
blaTEM	F ATGAGTATTCAACATTTCCG R CCAATGCTTATTCAGTGAGG	858	Dossouvi et al. 2022[34]
blaSHV	F TTATCTCCCTGTTAGCCACC R GATTTGCTGATTTCGCTCGG	800	Dossouvi et al. 2022[34]
CTX-M	F ATGTGCAGYACCAGTAARGTKATGGC R GGGTRAARTARGTSACCAGAAYSAGCGG	592	Dossouvi et al. 2022[34]
CTX-M-2	F ATGATGACTCAGAGCATTCGCCGC R TCAGAAACCGTGGGTTACGATTTT	876	Dossouvi et al. 2022[34]
CTX-M-9	F GTGACAAAGAGAGTGCAACGG R ATGATTCTCGCCGCTGAAGCC	327	Dossouvi et al. 2022[34]
CTX-M-1	F GGTTAAAAAATCACTGCGTC R TTACAAACCGTYGGTGACGA	873	Dossouvi et al. 2022[34]
CTX-M-15	F CACACGTGGAATTTAGGGACT R GCCGTCTAAGGCGATAAACA	995	Dossouvi et al. 2022[34]
CTX-M-8	F TGATGAGACATCGCGTTAAG R TAACCGTCGGTGACGATTTT	666	Dossouvi et al. 2022[34]
CTX-M-25	F GCACGATGACATTCGGG R AACCCACGATGTGGGTAGC	327	Dossouvi et al. 2022[34]
Carbapenems resistance-associated genes			
IMP	F GGAATAGAGTGGCTTAATTCTC R CCAAACCACTAGGTTATCT	188	Kaczmarek, Dib-Hajj et al. [35]
NDM	F GGTTTGGGGATCTGGTTTTC R CGGAATGGCTCATCACGATC	621	Eyvazi, Hakemi-Vala et al. [36]
OXA-48	F TTGGTGGCATCGATTATCGG R GAGCACTTCTTTTGTGATGATGGC	743	Lee and Choi. [37]
OXA-23	F TCTGGTTGTACGGTTCAGCA R GCAAAAGCGACAATTTTTCC	718	Smyth, O'Flaherty et al. [38]
VIM 2004	F GTTTGGTCGCATATCGCAAC R AATGCGCAGCACCAGGATAG	382	Manoharan, Chatterjee et al. [39]
KPC	F CTGTCTTGTCTCTCATGGCC R CCTCGCTGTGCTTGTATCC	795	Lee and Choi. [37]
Quinolons resistance-associated genes			
gyrA	F TACACCGGTCAACATTGAGG R TTAATGATTGCCGCCGTCGG	647	Dossouvi [40]
parE	F ATGCGTGCGGCTAAAAAAGTG R TCGTCGTCAGGATCGATAC	289	Dossouvi [41]
gyrB	F TGAAATGACCCGCCGTAAAGG R GCTGTGATAACGCAGTTTGTCCGGG	309	Dossouvi [41]
parC	F GTCTGAACTGGGCCTGAATGC R AGCAGCTCGGAATATTTCGACAA	248	Dossouvi [41]
qnrA	F TCAGCAAGAGGATTTCTA R GGCAGCACTATTACTCCC	657	Dossouvi [41]
qnrB	F TACACCGGTCAACATTGAGG R TTAATGATTGCCGCCGTCGG	469	Dossouvi [41]
qnrC	F GGGTTGTACATTTATTGAATCG R CACCTACCCATTTATTTTCA	307	Dossouvi [41]
qnrD	F TGTGATTTTTCAGGGGTTGA R CCTGCTCTCCATCCAACTTC	520	Dossouvi [41]
qnrS	F ACGACATTCGTCAACTGCAA R TAAATTGGCACCCTGTAGGC	417	Dossouvi [41]
Aminoglycosids resistance-associated genes			
aac(6')-Ib	F TTGCGATGCTCTATGAGTGGCTA R CTCGAATGCCTGGCGTGTTT	482	Dossouvi [41]
QepA	F AACTGCTTGAGCCCGTAGAT R GTCTACGCCATGGACCTCAC	596	Dossouvi [41]
AadA1	F TATCCAGCTAAGCGCGAACT R ATTTGCCGACTACCTTGGTC	447	Heidary, Momtaz et al. [42]

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Table 6: Thermo cycling conditions of PCR for 32 antimicrobials resistance-associated genes of *K. pneumoniae*

		Cycling condition					
Genes	**Initial denaturation**	**Denaturation**	**Annealing**	**Extension**	**No. Of cycles**	**Final extension**	**Reference**
β-lactams genes							
blaTEM	94°C/10min	94°C/1min	60°C/1min	72°C/2min	30	72°C/10min	Dossouvi et al. 2022[34]
blaSHV	94°C/10min	94°C/1min	60°C/1min	72°C/2min	30	72°C/10min	Dossouvi et al. 2022[34]
CTX-M	95°C/3min	94°C/1min	55°C/1min	72°C/1min	35	72°C/3min	Dossouvi et al. 2022[34]
CTX-M-2	95°C/3min	94°C/1min	56°C/1min	72°C/1min	35	72°C/7min	Dossouvi et al. 2022[34]
CTX-M-9	95°C/3min	94°C/1min	55°C/1min	72°C/1min	35	72°C/3min	Dossouvi et al. 2022[34]
CTX-M-1	95°C/3min	94°C/1min	50°C/1min	72°C/1min	35	72°C/7min	Dossouvi et al. 2022[34]
CTX-M-15	95°C/3min	94°C/1min	50°C/1min	72°C/1min	35	72°C/7min	Dossouvi et al. 2022[34]
CTX-M-8	95°C/3min	94°C/1min	52°C/1min	72°C/1min	35	72°C/7min	Dossouvi et al. 2022[34]
CTX-M-25	95°C/3min	94°C/ 1min	52°C/1min	72°C/1min	35	72°C/7min	Dossouvi et al. 2022[34]
Carbapenems resistance-associated genes							
IMP	94°C/4min	94°C/40s	52°C/40s	72°C/45s	35	72°C/4min	Kaczmarek, Dib-Hajj *et al.*
NDM	95°C/3min	94°C/30s	58°C/1min	72°C/1min	30	72°C/7min	Eyvazi, Hakemi-Vala et al. [36]
OXA-48	95°C/3min	94°C/30s	58°C/1min	72°C/1min	30	72°C/7min	Lee and Choi. [37]
OXA-23	95°C/3min	94°C/30s	58°C/1min	72°C/1min	30	72°C/7min	Smyth, O'Flaherty et al. [38]
VIM 2004	95°C/3min	94°C/30s	58°C/1min	72°C/1min	30	72°C/7min	Manoharan, Chatterjee et al. [39]
KPC	95°C/3min	94°C/30s	58°C/1min	72°C/1min	30	72°C/7min	Lee and Choi. [37]
Quinolons resistance-associated genes							
gyrA	94°C/5min	94°C/30s	60°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
parE	94°C/5min	94°C/30s	60°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
gyrB	94°C/5min	94°C/30s	60°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
parC	94°C/5min	94°C/30s	60°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
qnrA	94°C/5min	94°C/30s	55°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
qnrB	94°C/5min	94°C/30s	55°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
qnrC	94°C/5min	94°C/30s	55°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
qnrD	94°C/5min	94°C/30s	55°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
qnrS	94°C/5min	94°C/30s	55°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
Aminoglycosids resistance-associated genes							
aac(6')-Ib	94°C/5min	94°C/30s	55°C/1min	72°C/1min	30	72°C/10min	Dossouvi [41]
QepA	95°C/15 min	95°C/60s	55°C/60s	72°C/60s	30	72°C/5min	Dossouvi [41]
AadA1	96°C/6min	95°C/70s	60°C/65s	72°C/90s	33	72°C/8min	Heidary, Momtaz et al. [42]

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Table 7: Sequencing of primers used in PCR for 6 virulence-associated genes of *K. pneumoniae*

Gene	oligo sequence (5'-3')	Product size (bp)	Reference
rmpA	F ACTGGGCTACCTCTGCTTCA	535	Hossain, De Silva et al. [14]
	R CTTGCATGAGCCATCTTTCA		
mrKD	F CCACCAACTATTCCCTCGAA	240	Hossain, De Silva et al. [14]
	R ATGGAACCCACATCGACATT		
kfu	F GAAGTGACGCTGTTTCTGGC	797	Hossain, De Silva et al. [14]
	R TTTCGTGTGGCCAGTGACTC		
magA	F GGTGCTCTTTACATCATTGC	1280	Aher, Roy et al. [13]
	R GCAATGGCCATTTGCGTTAG		
uge	F TCTTCACGCCTTCCTTCACT	534	Aher, Roy et al. [13]
	R GATCATCCGGTCTCCCTGTA		
fyuA	F TGATTAACCCCGCGACGGGAA	880	Kanaan, Al-Shadeedi et al. [43]
	R CGCAGTAGGCACGATGTTGTA		

Table 8: Thermo cycling conditions of PCR for 6 virulence-associated genes of *K. pneumoniae*

		Cycling condition					
Genes	Initial denaturation	Denaturation	Annealing	Extension	No. Of cycles	Final extension	Reference
rmpA	95°C/5min	95°C/60s	53°C/60s	72°C/60s	30	72°C/5min	Hossain, De Silva et al. [14]
mrKD	94°C/4min	94°C/30s	52°C/40s	72°C/60s	30	72°C/10min	Hossain, De Silva et al. [14]
kfu	94°C/5min	94°C/60s	54°C/45s	72°C/60s	35	72°C/10min	Hossain, De Silva et al. [14]
magA	94°C/1min	94°C/30s	59°C/45s	72°C/2min	30	72°C/6min	Aher, Roy et al. [13]
uge	94°C/5min	94°C/60s	54°C/45s	72°C/60s	35	72°C/10min	Aher, Roy et al. [13]
fyuA	95°C/4min	95°C/50s	58°C/60s	72°C/45s	30	72°C/8min	Kanaan, Al-Shadeedi et al. [43]

alone in three strains (10.7%), association *mrK*D + *fyu*A in one strain (7.1%), association *mrK*D + *uge* in 7 strains (25%), and association *mrK*D + *uge* + *fyu*A in 11 strains (39.3%).

Discussion

The aim of this study was to investigate the distribution of virulence genes, phenotypic and genotypic patterns of antibiotic resistance among ESBL strains of *K. pneumoniae* isolated from HAIs from 2018 to 2021 in two hospitals (Dantec and Albert Royer) in Dakar, Senegal. In our study, all *K. pneumoniae* isolates showed MDR patterns displaying high rates of resistance to commonly used antibiotics for treating *K. pneumoniae* infections, such as β-lactams (100%), aminoglycosides (82.1%), fluoroquinolones (96.4%), cyclins (100%), Fosfomycin (28.5%) and trimetroprim-sulfamethoxazole (92.8%). A worldwide meta-analysis with 47 studies estimated the prevalence of antibiotic resistance in healthcare-associated MDR *K. pneumoniae* [20]. According to this meta-analysis, the resistance rates to various classes of antibiotics were as follows: β-lactams (91.5%), aminoglycosides (85.1%), quinolones (87.2%), cyclins (34%), sulphonamids (51%), polymyxins (14.9%) and other classes of antibiotics (38.3%).

At the molecular level, the observed high resistance to β-lactam antibiotics can be attributed to the expression of bla_{TEM} and bla_{SHV} genes that are expressed in 92.8% of the tested strains. Among these strains, 17 (60.7%) carried both bla_{SHV}, bla_{TEM} and $bla_{CTX\text{-}M}$ genes. Specifically, $bla_{CTX\text{-}M15}$, belonging to the $bla_{CTX\text{-}M1}$ group, was the only variant identified in our study and is known to be widely distributed globally [21]. The *CTX-M* groups and bla_{SHV} are the major ESBLs phenotype worldwide [22] included in HAIs [23]. The $bla_{CTX\text{-}M}$ genes are predominantly plasmid-encoded [24] and are the predominant β-lactamase conferring resistance in *K. pneumoniae* strains and other gram-negative bacterial to new broad spectrum β-lactam antimicrobials.

Ten *K. pneumoniae* strains were resistant to ertapenem and out of those, six also showed resistance to meropenem and imipenem. In Senegal, different studies have shown variable resistance rate to imipenem in healthcare-associated strains from 6.7 to 11.2% [18, 25, 26] and up to 72.2% [27]. The carbapenemase gene *OXA-48* was detected in 6 strains while it was not found in the four strains that were only resistant to ertapenem. Further investigation may be needed to identify the mechanisms behind this resistance phenotype.

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ESBLs genes can also enable bacteria to resist to other classes of antimicrobials and 82.14% and 92.85% of the studied strains are respectively resistant to at least one antibiotic in aminoglycosides and fluoroquinolones family. Twenty (20) strains were resistant to amikacin, 27 (96.4) to ciprofloxacin and 23 (82.1) to norfloxacin.

Three studies conducted in Aristide Le Dantec hospital (Senegal) have investigated the prevalence of antibiotic resistance in healthcare-associated infections (HAIs). The results of these studies have demonstrated varying levels of resistance to aminoglycosides and ciprofloxacin. Specifically, the resistance rates for aminoglycosides were reported as 27%, 80%, and 84.6%, while the resistance rates for ciprofloxacin were found to be 55%, 81.43%, and 87% [18, 26, 28]. Two others studies conducted in the neurosurgery service of Fann Hopsital (Senegal) and the urologic service of Aristide le Dantec hospital have shown prevalence for amikacin resistance respectively of 16.7% [25] and 44.5% [27]. Although it has been used for several years, resistance for fosfomycin and chloramphenicol has remained low in *Klebsiella spp* [29] compared to our study. We found respectively 8/28 (28.5%) and 7/28 (25%) resistant strains for fosfomycin and chloramphenicol antibiotics. Genes encoding quinolone plasmid-mediated resistance q*nr*B, *qnr*S and aminoside resistance gene *aac(6')-Ib* were prevalent at high rates (75%) and are mostly responsible for resistance to fluoroquinolones and aminoglycosides, in concordance with some recent studies [24, 30].

Among the virulence factors investigated, three out of the six targeted genes were detected in the K. pneumoniae strains, with varying proportions. The *mrKD* gene encoding for a type 3 fimbriae (adhesins) was found in 21/28 (75%) of the studied isolates and is involved in the adhesion to epithelial, urinary and respiratory cells and promote biofilms development [14]. This finding aligns with the well-established ubiquitous nature of these fimbriae in *K. pneumoniae* [31]. The *fyu*A gene was found in 13/28 strains (46.4%), encoding for a yersiniabactin receptor, one of the most upregulated gene in biofilm formation specially in iron-poor environments such as human urine [32]. Additionnaly, the *uge* gene was found in 19/28 strains (67.8%) and codes for uridine diphosphate galacturonate 4-epimerase, express both smooth lipopolysaccharide (LPS) with O antigen molecules and capsule polysaccharide (K antigen) on the surface of the bacteria. Therefore, this gene is essential for *K. pneumoniae* virulence and strains that carried this gene were more virulent [33].

Our research findings demonstrate the existence of multidrug-resistant strains that owe their resistance to various genes within their genome. The combination of these genes, at times, contributes to an elevated resistance level against different antibiotics. Moreover, specific virulence factors are present, and their expression has the potential to escalate the severity and/or duration of infections. A sequencing of these strains should provide a better understand of the clones all the factors of virulence and resistance present in these strains and occurrence of mobile genetic elements.

Conclusion

The emergence of multidrug resistant *K. pneumoniae* as a significant aetiologic agent in HAIs poses a growing public health concern not only for Senegal but all over the world. The successful spread of these multidrug resistant *K. pneumoniae* has been largely facilitated by the fact that *K. pneumoniae* itself is a notorious healthcare associated pathogen leading to outbreaks in hospital seetings, in addition to other factors, such as inappropriate use of antibiotics , high-density populations, poor infection control, international travel and medical tourism. This study highlights the need to establish a comprehensive antimicrobial resistance surveillance network in Senegal for *K. pneumoniae* and other major healthcare-associated bacteria. Such a network would enable monitoring of emerging resistance trends and novel resistance mechanisms within hospitals. We also recommend strengthening antimicrobial stewardship rules and implementing robust infection control measures in healthcare facilities to mitigate the selective pressures that drive the emergence and spread of multidrug-resistant strains. By adopting these strategies, we can proactively combat antimicrobial resistance and safeguard patient health in hospital settings.

Ethical Research Approval

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent for publication

All authors have given their consent for this publication.

Competing interests

The authors have not declared any conflict of interest.

Author's contributions

MMB, AC, FT and OS helped to collect samples and data. MMB, AD, AD were involved in strains isolation and to perform some antibiogram susceptibility testing and contributed to antimicrobial resistance profiles interpretation. BN, FT, CF and YD helped revised the manuscript. BSB, GCDM, AS designed the study, supervised the results analysis and interpretation, and revised the manuscript. NI is the main author hence, participated to the experiment design, performed the lab work, did the bibliographic review, analyzed and interpreted the results, designed the figures and drafted the manuscript.

All the authors have read and approved the submitted version of the manuscript.

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