Journal of Biosciences and Medicines, 2025, 13(11), 436-443
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Research on IncL/M, IncN, and IncX Plasmids in ESBL *Escherichia coli* Strains Isolated in Senegal

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How to cite this paper: Ngom, B., Diagne, R., Wade, S.F., Dasylva, M. and Sarr, M.G. (2025) Research on IncL/M, IncN, and IncX Plasmids in ESBL *Escherichia coli* Strains Isolated in Senegal. *Journal of Biosciences and Medicines*, **13**, 436-443.
https://doi.org/10.4236/jbm.2025.1311031

Received: October 3, 2025
Accepted: November 23, 2025
Published: November 26, 2025

Abstract

Introduction: *Escherichia coli* is a vertebrate intestinal commensal increasingly implicated in various intestinal and extraintestinal infections as an opportunistic pathogen. Beta-lactam antibiotics, the antibiotics of choice in the treatment of *E. coli* infections, are becoming increasingly ineffective. Indeed, the increase in acquired resistance, most often mediated by conjugative plasmids, considerably reduces the chances of successful antibiotic therapy. **Study Objective:** This work aimed to investigate the presence of IncL/M, IncN, and IncX conjugative plasmids in ESBL *E. coli* strains isolated in Senegal. **Materials and Methods:** The study involved 32 ESBL *E. coli* strains. After antibiogram and PCR characterization of the identified ESBL types, conjugation by transfer in solid and liquid media was performed to select the plasmids for the study. Subsequently, a Carattoli multiplex PCR was performed to search for IncL/M, IncN, and IncX plasmids in the transconjugant strains. **Results:** IncL/M and IncX plasmids were absent from the *E. coli* strains studied. Only the IncN plasmid, measuring 559 bp, was found in a single *E. coli* strain.

Keywords

E. coli, ESBL, Plasmids, IncL/M, IncN, IncX

1. Introduction

Escherichia coli is a Gram-negative bacterium susceptible to natural and random genetic alterations. There is a large collection of sequenced *E. coli* genome samples, whose size and genomic diversity vary depending on whether they belong to commensal or pathogenic strains [1]. Thus, within the *E. coli* species, commensal strains belonging to the normal intestinal microbiota of humans and many

animals are distinguished, and pathogenic strains are divided into diarrheal and extraintestinal pathovars [2]. Beta-lactams are antibacterial molecules widely used in the treatment of *E. coli* infections. However, the global circulation of plasmids significantly increases acquired bacterial resistance and therefore reduces the possibility of effectively treating these infections. Horizontal transfer of antibiotic resistance genes by plasmids is one of the main modes of dissemination of antibiotic resistance in Gram-negative bacteria [3].

Incompatibility group N (IncN) plasmids have a broad host range, conjugate at high frequency, and are stably maintained in the bacterial host cell through partitioning and anti-restriction systems [4]. They exhibit a relatively high prevalence in the fecal flora of healthy animals (10.9%) and in bacterial populations not preselected for antimicrobial resistance, and are one of the major vehicles for the dissemination of CTX-M-1-type extended-spectrum β-lactamase (ESBL) genes and plasmid-mediated resistance in *Escherichia coli* and *Salmonella* isolates from humans, animals, and the environment [5].

Plasmids of the L/M incompatibility group (IncL/M) are involved in the spread of OXA-48 genes [6]. Indeed, among the broad-host-range conjugative plasmids, those of the IncL/M group are among the six main groups of plasmids identified as responsible for the transmission of resistance in *Enterobacteriaceae*. They are considered to carry various β-lactam resistance genes encoding ESBLs, class A, B, and D carbapenemases, and AmpC β-lactamases [3].

Incompatibility group X (IncX) plasmids are rarely encountered and most often associated with the spread of quinolone resistance [3]. Although they have been shown to be infrequently isolated from commensal and pathogenic *E. coli* strains, IncX plasmids have recently been described in other *Enterobacteriaceae* species from various sources and geographic areas [7].

The main objective of this work is to investigate the presence of IncL/M, IncN, and IncX conjugative plasmids in ESBL *E. coli* strains isolated at the National University Hospital of Fann, Senegal, from biological samples of inpatients and outpatients.

2. Materials and Methods

Origin of the strains - thirty-two strains of *E. coli* BLSE were the subject of this study. The strains were isolated in the bacteriology-virology laboratory of the CHNU of Fann from various pathological products: blood, urine, pus, and vaginal secretions. It was also in this laboratory that the entire phenotypic study up to the antibiogram was carried out. The synergy test is the main test used to detect ESBL *E. coli* strains in the study. This test is based on the demonstration of a so-called "champagne cork" synergy between third-generation cephalosporin discs (cefotaxime, ceftazidime, cefepime, or a monobactam such as aztreonam) and an amoxicillin/clavulanic acid disc. A space of 30 mm was maintained between the centers of the discs. The results of the synergy tests showed that 87.5% of the 32 *E. coli* strains in the study exhibited champagne cork synergy, compared to 12.5%

which, in the absence of synergy, were resistant to third-generation cephalosporins and/or aztreonam, which was considered a sufficient criterion for the recruitment of these latter strains. The work of identifying the 32 strains in the study, as well as the search for resistance phenotypes, was carried out between 2009 and 2010 at the Fann bacteriology laboratory. Furthermore, the strains came from both outpatients and hospitalized patients in the various departments of the Fann National University Hospital. However, all samples came from male and female patients of Senegalese nationality. The characterization of the types of BLSE of the strains in the study, as well as the conjugations and the search for conjugative plasmids InL/M, InN, and IncX, was carried out in the Bacteriology Laboratory of the Faculty of Medicine Pierre and Marie Curie, University Paris VI, in the Research Team "ANTIBIOTICS and DIGESTIVE FLORA" of the Bacteriology Research Unit n˚ ER8 (Table 1: profile of the strains of the study). Knowing that with ESBL *enterobacteriaceae* resistance was often determined by plasmids, the study had every chance of detecting plasmids present in our ESBL *E. coli* strains within the transconjugants if our results were validated as positive after the conjugations.

Table 1. Profile of *E. coli* strains.

Strain ID	Origin of the strain	Presence or absence of the $bla_{CTX\text{-}M\text{-}15}$ gene
1890/Ur	Urine	+
2261/Ur	Urine	+
1039/Ur	Urine	+
195/Ur	Urine	+
331/Ur	Urine	+
403/Ur	Urine	+
747/Ur	Urine	+
161/Ur	Urine	+
1530/Ur	Urine	−
771/Ur	Urine	+
1420/Ur	Urine	+
1037/Ur	Urine	+
148/H	Blood	+
1287/Ur	Urine	+
1595/Ur	Urine	+
2027/Ur	Urine	+/Positive IncN
609/Ur	Urine	−
100/Ur	Urine	+

Continued

1270/Ur	Urine	+
1474/Ur	Urine	+
2226/Ur	Urine	+
1478/Ur	Urine	+
2214/Ur	Urine	+
1619/Ur	Urine	+
802/PV	Vaginal discharge	+
173/P	Pus	−
1639/Ur	Urine	+
2213/Ur	Urine	+
554/Ur	Urine	+
1399/Ur	Urine	+
273/P	Pus	+
1228/Ur2	Urine	+

*(+) = presence of the $bla_{CTX\text{-}M\text{-}15}$ gene; *(−) = absence of the $bla_{CTX\text{-}M\text{-}15}$ gene.

Regarding the selection of plasmids coding for the incompatibility groups sought in our *E. coli* strains, the liquid transfer conjugation technique was carried out. This technique gives results after five days according to the following protocol:

On day 1:

- Culture an ESBL *E. coli* strain (presumed plasmid donor strain, resistant to cefoxitin and ceftriaxone) in 2.5 ml of Trypticase-soy broth.

- Culture the reference strain *E. coli* J53 (presumed plasmid recipient strain and resistant only to rifampicin) for 6 hours at 37˚C in 10 ml of Trypticase-soy broth.

- Place 1 ml of the recipient strain suspension, 1 ml of the donor strain suspension, and 8 ml of Trypticase-soy in a tube; then incubate the tube at 37˚C (slanted) overnight.

- Prepare Trypticase-soy or Drigalski dishes (20 ml/round dish) containing rifampicin and ceftriaxone (3 dishes/conjugation) so as not to run the hot agar onto the antibiotics and homogenize them in the agar by gentle agitation.

Day 2:

- Place the Petri dishes in the incubator for 2 hours before adding 10 µL, 50 µL, and 100 µL of broth to dishes numbered 1, 2, and 3, respectively.

- Spread the inocula using a rake and incubate the dishes in the incubator overnight at 37˚C.

Day 3:

A positive reading indicates the presence of the ceftriaxone-resistant J53 receptor *E. coli* strain.

To confirm this hypothesis, the different colony types obtained on Tryptic Soy Agar (round dish, 1/4 dish/colony) should be isolated on Tryptic Soy Agar (round dish, 1/4 dish/colony).

Day 4:

Antibiograms and Api20E galleries will be performed on the various re-isolated colonies.

Day 5:

- Read the antibiogram and the conjugant galleries;

- A conjugation is positive if:

The conjugant gallery matches that of the reference *E. coli* recipient strain (J53: Api20E = 5044552);

The antibiogram reveals associated donor and recipient resistance (strain resistant to both rifampicin and ceftriaxone).

After conjugation, plasmid DNA from the transconjugants was extracted by heat shock as follows:

- One colony was placed in 250 µL of distilled water;

- The tube was placed in a dry water bath at 100˚C for 10 minutes before being transferred to a freezer at −20˚C for 5 minutes;

- Finally, the tube was centrifuged for a few seconds and used immediately or stored at −20˚C.

Carattoli's multiplex PCR 2 was used to search for IncL/M, IncN, and IncX plasmids in our transconjugant strains and not in the parental isolates. Knowledge of the base pair sizes of IncL/M, IncN, and IncX allows them to be clearly differentiated by gel electrophoresis. This approach only allows the targeted search for conjugative plasmids and excludes other non-conjugative plasmids potentially present in the parental isolates. Table 2 lists the primers used for this characterization, along with their DNA sequences and sizes.

Table 2. Primers carattoli incompatibility.

PCR Carattoli	Name	DA sequence	Target	Size
Multiplex 2	X FW	5'-aaccttagaggctatttaagttgctgat-3'	Oriγ	376
	X RV	5'-tgagagtcaatttttatctcatgttttagc-3'		
	L/M FW	5'-ggatgaaaactatcagcatctgaag-3'	repA, B, C	785
	L/M RV	5'-ctgcaggggcgattctttagg-3'		
	N RV	5'-gtctaacgagcttaccgaag-3'	repA	559
	N RV	5'-gtttcaactctgccaagttc-3'		

The reaction media for multiplex PCRs were 50 µL final solutions, including 45 µL of mixture for 5 µL of DNA. The mixture was prepared using the Phusion High-Fidelity Taq. Its composition is shown in Table 3 below.

Table 3. Composition of the mix for carattoli's multiplex 2 PCR.

5X Phusion HF	10 l
dNTP 10mM.	1 l
primer FW 1 (50 pM)	2.5 l
primer RV 1 (50 pM)	2.5 l
primer FW2 (50pM)	2.5 l
primer RV 2 (50 pM)	2.5 l
primer FW 3 (50 pM)	2.5 l
primer RV 3 (50 pM)	2.5 l
DMSO	1.5 l
Taq	0.5 l
H_2O Nuclease-free water	17 l

The Carattoli PCR program that was carried out is given in **Table 4**.

Table 4. Carattoli's PCR multiplex 2 program.

Denaturation	94˚C; 5 min	1 cycle
Denaturation	94˚C for 1 min	
Hybridization	60˚C; 30 s	40 cycles
Elongation	72˚C; 1 min	
Elongation	72˚C; 5 min	1 cycle
End of reaction	4˚C	Infinity

3. Results

Plasmids IncL/M and IncX were absent from the *E. coli* strains in our study. However, plasmid IncN was found in an *E. coli* strain isolated from urine. Furthermore, this ESBL strain belonged to the A1 phylogenetic group and carried the $bla_{CTX\text{-}M\text{-}15}$ gene.

4. Discussions

Our study revealed the presence of the IncN plasmid in one of our *E. coli* strains and, similarly, the absence of the IncL/M and IncX plasmids. Thus, this study confirmed the presence and circulation of the IncN plasmid within ESBL-infected *E. coli* strains present in Senegal.

This IncN plasmid has already been described in numerous *E. coli* strains isolated from humans and animals in many countries, including Denmark, Spain, and Italy [8]-[10]. As is also the case in our study, the IncN plasmid was often associated with the $bla_{CTXM\text{-}15}$ gene carried by *E. coli* strains isolated from acute

urinary tract infections in hospital settings, in intensive care units, and in community medicine [11].

Indeed, the transfer of bla_{CTX-M} genes within *Enterobacteriaceae* via plasmids, including IncN, among other diffusion mechanisms, is a well-documented phenomenon today [12] [13]. Additionally, IncN plasmids can carry several antimicrobial resistance genes, including qnr determinants in the *Enterobacteriaceae* family [14]; this could explain the high resistance of this *E. coli* strain carrying the IncN plasmid to norfloxacin and pefloxacin.

Finally, IncN plasmids harboring bla_{CTX-M} genes are known to be epidemic resistance plasmids dispersed throughout the world, hence the need to strengthen their surveillance and effectively combat their spread [15].

Concerning the prevalence of IncL/M and IncX plasmids in Senegal and the African sub-region, much more in-depth research, involving a significant number of isolates from various sources, should be conducted. Currently, there is very little data on the presence and circulation of IncL/M and IncX plasmids in Senegal and Africa. Similarly, a study including strains of *E. coli* and other ESBL enterobacteriaceae from various health facilities in Senegal should also be undertaken to better quantify the prevalence of IncN plasmid circulation at the national level.

5. Conclusion

Our study has made it possible to highlight for the first time in Senegal, and specifically in the bacteriology laboratory of the Fann hospital in Dakar, the presence and circulation of the IncN plasmid in a strain of uropathogenic *E. coli* and the absence of the IncL/M and IncX plasmids. Indeed, even if the IncN plasmid was only found in one strain out of the twenty-six in the study, which represents a still low percentage of 3.12%, the epidemic nature and carrier of bla_{CTX-M} and qnr genes conferring resistance to beta-lactams and fluoroquinolones respectively require more in-depth studies to better assess the extent of the circulation of this plasmid at the national level.

Conflicts of Interest

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

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