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Research Article

Research of Associated Resistance to Antimicrobial Agents of Extended Spectrum Beta-Lactamases *Escherichia Coli* and *Klebsiella pneumoniae* Strains Isolated in Senegal

Ngom B[1]*, Diagne R[2], Wade SF[1], Diop TA[1], Sow AI[3]

[1]Université Amadou Mahtar MBOW, Dakar, Sénégal/ Ecole Supérieure des Sciences Agricoles et de l'alimentation, Sénégal

[2]Université Iba Der Thiam, Thiès, Sénégal

[3]Université Cheikh Anta Diop de Dakar, Sénégal

***Corresponding author:** Babacar Ngom, Université Amadou Mahtar MBOW, Dakar, Sénégal

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Abstract

Introduction: Some strains of *Escherichia coli* and *Klebsiella pneumoniae* produce Extended Spectrum Beta-Lactamases (ESBL) may be responsible for various infections such as urinary infections. These Sick people are treated in the very serious cases by association antibiotics to class to betalactamins, aminosids and quinolons. But proliferation of multi-drug resistant strains involves decreasing therapeutic success. That's why epidemiological study must be done in all laboratories of bacteriology.

Purpose: The aim of the study was to research the resistance phenotypes of our *E. coli* and *K. pneumoniae* ESBL strains compared to others families of antibiotics.

Material and methods: Thirty two (32) Extended Spectrum beta-lactamases *E. coli* and *K. pneumoniae* strains isolated from either hospitalized patients or sick people who came for consultation were studied. Susceptibility to antimicrobial agents was determined using an antibiotic disk (Bio-Rad) diffusion method on Mueller-Hinton agar (Bio-Rad). The results were interpreted according to the Standards of the French Antibiogram Committee (CA-SFM).

Results: The study showed that most of these strains were multi-drug resistant. They were resistant to many beta-lactamines antibiotics. *E. coli* strains were also resistant at 70,34% to aminosids, at 96,72% to quinolons, at 58,3% to cotrimoxazol, at 26,1% to chloramphénicol and at 21,4% to colistin ; about *K. pneumoniae*, they were resistant at 72,6% to aminosids, at 88,95% to quinolons, at 86,7% to cotrimoxazol, at 44,4% to chloramphénicol and at 25% to colistin. But all these strains were sensitive at 100% to l'imipenem.

Keywords: *E. coli*, *K. pneumoniae*, ESBL, multi-drug resistant bacteria, Senegal

Introduction

The resistance of bacteria to antibiotics is now a "globalized" phenomenon, affecting all bacterial species of medical importance and all curent classes of antibiotics [1,2]. It is the result of the excessive dissemination of antibiotics, for many uses (human and veterinary medicine, breeding and agriculture). This is the case with beta-lactams, which are the mainstay of antibiotic therapy for enterobacterial infections [3]. In fact, some potentially pathogenic strains of this family, such as *E. coli* and *K. pneumoniae*, which produce extended spectrum betalactamases (ESBL) and are frequently isolated from urinary tract infections [4-6], can be resistant to many molecules of this class. The most worrying thing is that these strains, which spread rapidly both in hospitals and in the community, are often co-resistant to many other antibiotics such as those of the aminoglycoside and quinolone class, thus making the treatment of these very problematic infections [4,7,8].

Material and Methods

Strains

Sixty-four strains of which thirty-two *E. coli* and *K. pneumoniae* were the subject of this study. These strains were isolated from patients hospitalized in various departments of the Fann national university hospital or received in outpatient consultations. The pathological products in which these strains have been isolated are: urine, blood, pus and vaginal sample and bronchoalveolar fluid. These strains were identified according to their morphological, cultural and biochemical characteristics (Api 20E gallery -bioMérieux) and were all secretors of Extended Spectrum Beta-Lactamases (ESBL).

Antibiogram

The study of the sensitivity of strains to antibiotics (antibiogram) was carried out using the agar medium diffusion method according to the updated recommendations of the Antibiogram Committee of the

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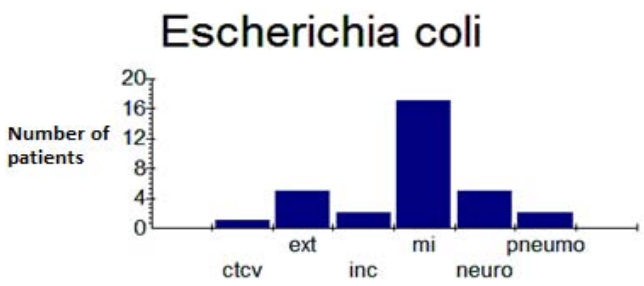

Figure 1: Distribution of *E. coli* by service.

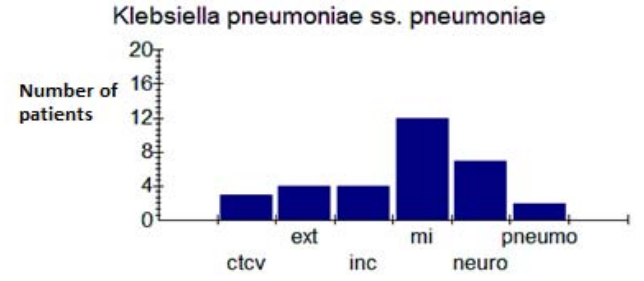

Figure 2: Distribution of *K. pneumoniae* strains according to the departments.

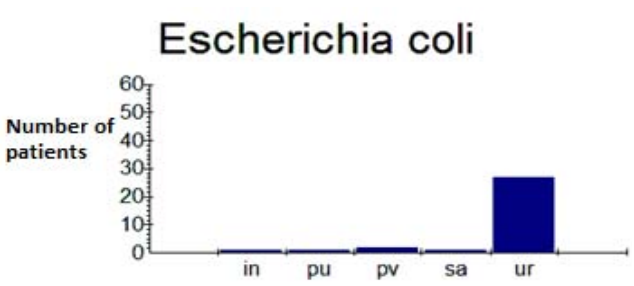

Figure 3: Distribution of *E. coli* according to pathological products.
In: Unknown; pu: Urine Sample; pv: Vaginal Sample; sa: Blood; ur: Urine

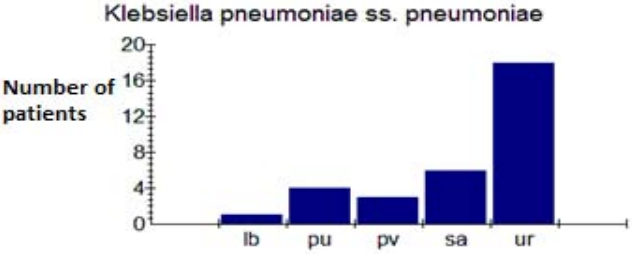

Figure 4: Distribution of *K. pneumoniae* according to pathological products.
Lb: Bronchoalveolar Fluid

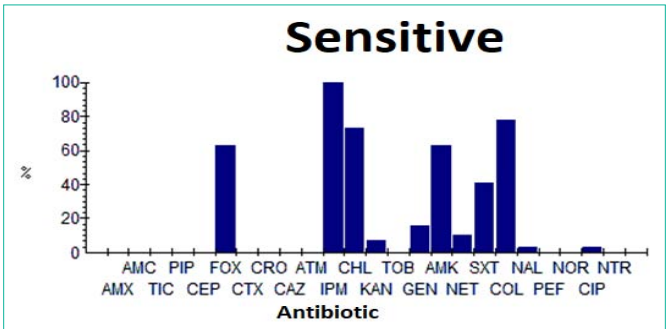

Figure 5: Antibiotic sensitivity of *E. coli.*

French Society of Microbiology (http://www.sfm.asso.fr/). The ESBL screening was performed by placing, 3 cm center to center, discs of ceftazidime, ceftriaxone, cefotaxime, cefepime and/or aztreonam around an Amoxicillin/clavulanic acid disc. The presence of a "champagne cork" synergy between Amoxicillin/clavulanic acid and one of the aforementioned discs attests to the secretion of ESBL by the strain studied. Other antibiotics, both belonging to the beta-lactam family and not, have been tested. These are amoxillin, ticarcillin, piperacillin, cephalotin, cefoxitin, imipenem, chloramphenicol, nitroxoline, colistin, cotrimoxazol (sulfametoxazol/trimetopime) aminoglycosid (kanamicin), tobramicin, gentamicin, netilmicin and amikacin) and quinolones (nalidixic acid, norfloxacin, pefloxacin and ciprofloxacin).

Results

Distribution of strains by department - Results showed a predominance of strains of *E. coli* (Figure 1) and *K. pneumoniae* (Figure 2) in the infectious disease department with respective percentages of 53.13% and 37.5% of isolates (Table 1).

Distribution of strains according to samples

The majority of strains studied were isolated from urine. In fact, 84.37% of *E. coli* (Figure 3) and 56.25% of *K. pneumoniae* (Figure 4) came from the urine. Table 2 gives the distribution of the strains according to the samples.

Antibiogram results for *E. coli*

Table 3 gives the results for antibiograms in *E. coli* in percentages. Thus, high resistance of ESBL *E. coli* has been noted to penicillins, carboxypenicillins, ureidopenicillins, first and third generation cephalosporins and aztreonam, as well as to aminoglycosides (except amikacin) and quinolones. However, these strains remain very

Table 1: Distribution of strains according to services.

Species	Number of strains	ctcv	ext	inc	mi	neuro	pneumo
Escherichia coli	32	1	5	2	17	5	2
Klebsiella pneumoniae	32	3	4	4	12	7	2

Table 2: Distribution of strains according to samples.

Species	Number of strains	in	Lb	pu	pv	sa	ur
Escherichia coli	32	1	-	1	2	1	27
Klebsiella pneumoniae	32	-	1	4	3	6	18

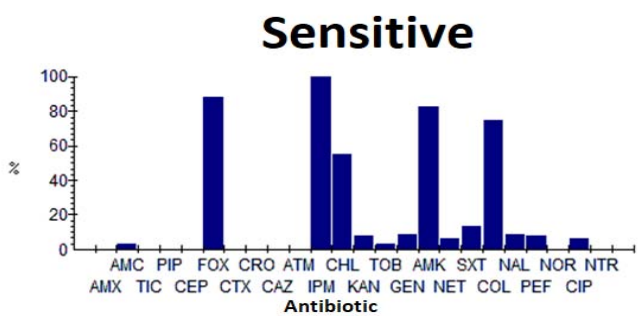

Figure 6: Antibiotic sensitivity of *K. pneumoniae.*

Table 3: Antibiogram results for *E. coli.*

Codes	Antibiotic	Nbre	%R	%I	%S
AMX_ND25	Amoxicillin	31	100	0	0
PIP_ND75	Piperacillin	31	100	0	0
TIC_ND75	Ticarcillin	31	100	0	0
AMC_ND20	Amoxicillin/clavulanic Acid	32	71,9	28,1	0
CEP_ND30	Cefalothin	30	100	0	0
CAZ_ND30	Ceftazidime	29	100	0	0
CRO_ND30	Ceftriaxone	25	100	0	0
CTX_ND30	Cefotaxime	23	100	0	0
FOX_ND30	Cefoxitin	30	10	26,7	63,3
ATM_ND30	Aztreonam	32	100	0	0
IPM_ND10	Imipenem	31	0	0	100
AMK_ND30	Amikacin	30	13,3	23,3	63,3
GEN_ND15	Gentamicin	31	80,6	3,2	16,1
KAN_ND30	Kanamycin	26	92,3	0	7,7
NET_ND30	Netilmicin	29	65,5	24,1	10,3
TOB_ND10	Tobramycin	26	100	0	0
NAL_ND30	Nalidixic Acide	32	96,9	0	3,1
NTR_ND20	Nitroxolin	16	0	100	0
CIP_ND5	Ciprofloxacin	26	96,2	0	3,8
NOR_ND5	Norfloxacin	16	93,8	6,2	0
PEF_ND5	Pefloxacin	21	100	0	0
SXT_ND1.2	Trimethoprim/Sulfamethoxazol	12	58,3	0	41,7
COL_ND50	Colistin	28	21,4	0	78,6
CHL_ND30	Chloramphenicol	23	26,1	0	73,9

sensitive to imipenem (100%), cefoxitin (63.3%) and colistin (78.6%), amikacin (63.3%) and chloramphenicol (73.9%) (Figure 5).

Antibiogram results for *K. pneumonia*

The strains of *K. pneumoniae* studied were very resistant to penicillins, carboxypenicillins, ureidopenicillins, first and third generation cephalosporins and aztreonam, as well as aminoglycosides (except amikacin) and quinolones (Table 4). However, the latter were sensitive to varying degrees to imipenem (100%), cefoxitin (88.9%) and colistin (75%), amikacin (83.9%) and chloramphenicol. (55.6%) (Figure 6).

Table 4: Antibiogram results for *K. pneumoniae.*

Codes	Antibiotiques	Nbre	%R	%I	%S
AMX_ND25	Amoxicillin	32	100	0	0
PIP_ND75	Piperacillin	32	100	0	0
TIC_ND75	Ticarcillin	29	100	0	0
AMC_ND20	Amoxicillin/Clavulanic Acid	31	61,3	35,5	3,2
CEP_ND30	Cefalothin	25	100	0	0
CAZ_ND30	Ceftazidim	29	100	0	0
CRO_ND30	Ceftriaxon	28	100	0	0
CTX_ND30	Céfotaxim	24	100	0	0
FOX_ND30	Céfoxitin	27	0	11,1	88,9
ATM_ND30	Aztreonam	24	100	0	0
IPM_ND10	Imipenem	31	0	0	100
AMK_ND30	Amikacin	31	12,9	3,2	83,9
GEN_ND15	Gentamicin	31	90,3	0	9,7
KAN_ND30	Kanamycin	24	79,2	12,5	8,3
NET_ND30	Netilmicin	32	84,4	9,4	6,2
TOB_ND10	Tobramycin	26	96,2	0	3,8
NAL_ND30	Nalidixic acid	32	84,4	6,2	9,4
NTR_ND20	Nitroxolin	10	0	100	0
CIP_ND5	Ciprofloxacin	32	93,8	0	6,2
NOR_ND5	Norfloxacin	20	95	5	0
PEF_ND5	Pefloxacin	23	82,6	8,7	8,7
SXT_ND1.2	Trimethoprim/Sulfamethoxazol	15	86,7	0	13,3
COL_ND50	Colistin	28	25	0	75
CHL_ND30	Chloramphenicol	27	44,4	0	55,6

Discussion

The study showed the circulation of ESBL-producing *E. coli* and *K. pneumoniae* strains in both hospital and community settings. Many strains have been isolated from patients who came for an outpatient consultation.

Most of the strains came from the infectious diseases department, which could be justified by nosocomial infections with *E. coli* and *K. pneumoniae* if we know that the patients hospitalized in this department are most often immunocompromised.

Most of the strains came from urine. Studies have shown that these multidrug-resistant strains express numerous virulence factors responsible for their uropathogenicity [9-11].

The strains studied were very sensitive to impipenem (100%). A few of them (10% of *E. coli*) were resistant to cefoxitin whose activity is comparable to that of cephamycin. ESBL-secreting strains only must be sensitive to this molecule. Their resistance to cefoxitin is therefore the result of a resistance mechanism other than ESBL secretion. It seems to be caused by the production of a class C beta-lactamase. In fact, work carried out in several countries [12-14] had revealed the emergence of *E. coli* and *K. pneumoniae* strains resistant to cefoxitin thanks to the production of beta-lactamase (cephalosporinase) AmpC plasmid [15-17].

The ESBL strains studied showed a high rate of co-resistance to antibiotics. Indeed, several of these strains were also resistant to several antibiotics of different classes other than beta-lactams, in particular aminoglycosid and quinolones.

E. coli were 80.6%, 100%, 65.5% and 92.3% resistant to gentamicin, tobramicin, netilmicin and kanamicin, respectively and 96.9%, 100%, 96.2% and 93.8% respectively to nalidixic acid, pefloxacin, ciprofloxacin and norfloxacin. As for *K. pneumoniae*, they were also resistant to 90.3%, 96.2%, 84.4% and 79.2% respectively to gentamicin, tobramicin, netilmicin and kanamicin and to 84.4%, 82.6%, 93.8% and 95% respectively to nalidixic acid, pefloxacin, ciprofloxacin and norfloxacin. However, these strains had high sensitivities to amikacin, 63.3% for *E. coli* and 83.9% for *K. pneumoniae*.

They were also resistant to colistin, cotimoxazol and chloramphenicol to varying degrees. *E. coli* were 21.4% resistant to colistin, 58.3% to cotrimoxazol and 26.1% to chloramphenicol and *K. pneumoniae* 25% to colistin, 86.7% to cotrimoxazol and 44.4% with chloramphenicol. These results are a perfect illustration of the multiresistant character of ESBL-producing enterobacteriaceae strains as described around the world [18], which complicated the treatment of the pathologies caused by the latter and necessitated a national surveillance of resistance. Antibiotics of these strains in order to prevent their spread and also that in the future we are faced with situations of therapeutic impasses.

Conclusion

The results of the study showed that the ESBL-secreting strains studied very often exhibited co-resistance against other families of antibiotics. They also show the spread of these strains both in hospitals and in the community. Their emergence requires very broad rigorous epidemiological surveillance of resistance to antibiotics in order to prevent their dissemination and that in the future we may be faced with situations of therapeutic impasses.

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