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Research Article

Antimicrobial Resistance and Characterization of Broad-Spectrum Batalactamases and Quinolone Resistance Genes of Urinary *E. Coli* Isolated in Senegal between 2009 and 2017

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Abstract

Introduction: *Escherichia coli* is a commensal bacterium of the digestive flora of the man responsible for urinary tract infections. The objective of our work is to determine the resistance profiles of broad-spectrum betalactamase-producing *Escherichia coli* isolated from the Fann hospital, as well as the beta-lactam resistance genes and the quinolone resistance genes.

Materials and Methods: Our study focuses on forty-six strains isolated between 2009 and 2017. The antibiograms was performed according to the diffusion method. Conventional PCR allowed determination of resistance genes to betalactamines and quinolones.

Results: These strains exhibited high resistance to amoxicillin, cefotaxime, aztreonam, nalidixic acid and ciprofloxacin.

All strains were highly sensitive to imipenem and amikacin, and cefoxitin. For the characterization of betalactamases, 85% were positive for OXA bla, 17% for TEM bla, 37% for CTXM group 1. Our study revealed a positivity for QNR A at 11% and 4% for QNR B.

Conclusion: The use of carbapenems and amikacin would be more appropriate in the treatment of broad-spectrum betalactamase-producing *E. coli* infections.

The emergence of OXA and CTXM enzymes requires national surveillance of urinary tract infections with monitoring of enzymes produced by resistant strains.

Introduction

The *E. coli* species is considered as a normal guest that is to say commensal to man's digestive microflora and to warm-blooded animals, it constitutes about 80% of our intestinal flora. However, numerous E.coli strains belonging to particular serotypes have been listed among humans as well as among animals, as being pathogenic strains responsible for various affections from a simple diarrhea to severe systemic and even deadly infections [1-3].

Thus, the *E. coli* strains can be classified into two big groups: (i) the group of the *E. coli* responsible for intra intestinal infections and (ii) the group of the *E. coli* responsible for extra intestinal infections or Ex PEC.

The ExPEC can affect the urinary tract, the circulatory system but also the central nervous system where they can cause serious pathologies, urinary tract infection, meningitis and septicemias [4]. Some ExPEC strains the Uropathogenic *E. coli* (UPEC) are by the way mainly responsible for the ITU, The ITU can manifest through more or less serious pathologies: cystitis (infection of the bladder) and pyelonephritis (infection of the kidney) [5]. Those diseases require in most cases going to the doctor's and an ant biotherapy.

In 2006 in the United States, urinary tract infections brought about eleven million medical check-ups and 500,000 hospitalizations with a cost of 35 billion dollars. [6].

That infection mainly affects women, more than 30% of women and about 10% of men suffer at least once in their lives from a urinary infection [7].

The massive use of antibiotics especially when they are taken orally, be it in human or animal medicine, contributes to the selection and the emergence of resistant bacteria, It has been accepted for a long time that there is a connection between the rate of resistant bacteria and the quantity of antibiotics used whether in hospitals or in cattle farming. Pathogenic bacteria can acquire resistance genes through horizontal transfer bacteria that are present in a milieu polluted by antibiotics. The intensive use of antibiotics thus favors the dissemination at a large scale of potentially pathogenic resistant bacteria, which constitutes a risk because antibiotic-based treatments become ineffective.

The aim of our work was on the one hand to determine the resistance profiles of the extended-spectrum and isolated betalactlamase producing *Escherichia coli* in the bacteriology

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Table 1: Primers of beta lactamases genes and the amplification conditions.

Target genes	Primers	Sequences of primers sense anti sense	Size (pb)	Reference
Bla TEM	TEM F TEM R	F : CATTTCCGTGTCGCCCTTATTC R : CGTTCATCCATAGTTGCCTGAC	800	[8]
Bla SHV	SHV F SHV R	F : AGCCGCTTGAGCAAATTAAAC R : ATCCCGCAGATAAATCACCAC	713	[8]
Bla OXA	OXA F OXA R	F : GGCACCAGATTCAACTTTCAAG R : GACCCCAAGTTTCCTGTAAGTG	564	[8]
Bla CTXM group 1	CTXM group 1:F CTXM group 1:R	F : TTAGGAARTGTGCCGCTGYA[b] R : CGATATCGTTGGTGGTRCCAT[b]	688	[8]
Bla CTXM group 8/25	CTXM group 8/25:F CTXM group 8/25:R	F : AACRCRCAGACGCTCTA R : TCGAGCCGGAASGTGTYAT	326	[8]
QNR A	QNR A F QNR A R	F : ATTTCTCACGCCAGGATTTG R : GATCGGCAAAGGTTAGGTCA	516	[9]
QNR B	QNR B F QNR BR	F : GATCGTGAAAGCCAGAAAGG R : ATGAGCAACGATGCCTGGTA	476	[9]

laboratory of Fann but on the other hand, to search for two groups of genes: betalactam resistant genes and quinolone resistant genes.

The goal of our work will be to provide the results of the achieved antibiograms and the betalactam resistant genes (OXA, CTXM, SHV, TEM) and quinolone resistant genes (QNR AB) discovered in our cohort of strains.

Materials and Methods

The strains

Our study focuses on a total of 46 isolated *E coli* strains between 2009 and 2017.

All strains come from hospitalized patients in the services of the University Hospital Center (UHC) of Fann or examined during external consultations including outpatients coming from other health structures.

Every strain has undergone a study of morphologic, cultural and biochemical characteristics, with its identification in view.

Antibiotic sensitivity test

The antibiogram of strains was realized according to the diffusion method in an agar medium. List table of tested antibiotics with disc loads. The laboratory uses the norms of the French Society of Microbiology [8,9]. The strains of our study were all ESBL producers.

The production of ESBL was searched for by the synergy test which proves to be positive with the appearance of a "champagne cork"-shaped image between the 3rd generation cephalosporins and or the aztroenam with the amoxicillin disc +clavunalic acid.

We tested the penicillins, the association amoxicillin and clavunalic acid, the carboxypenicillins, the ureidopénicillins, the imipenem, the phenicols, the aminoglycosides and the quinolones.

Gene detection with classical PCR

We then used the conventional PCR to determine the betalactamase and quinolone resistant genes contained in our collection of 46 bacterial strains.

We realized the molecular detection of the OXA, TEM and SHV, CTXM, genes for the betalactamase and the QNR A and B genes for the quinolone resistance.

For the tested isolates with PCR, the DNA was extracted by alkaline lysis with soda 320µl.

Bacterial suspension were centrifuged at 12, 000 rpm for 5 minutes. After suction of the supernatant, 25µl of Noah to 0.5 M (Prolabo) were added in the tubes. After letting the soda have effects for 20 minutes at an ambient temperature (23°C), we added 25 µl of Tris HCl pH=7.4 à 1M (Sigma, Steinheim, Germany) and 450 µl of nanopure water . The 500 µl of DNA suspension obtained were preserved at -80°C.

For the detection with PCR of TEM, SHV, CTXM and OXA genes, a final reactional solution of 50 µl was achieved by adding 5 µl of genomic DNA to a mixture of 45 µl. That mix comprised 10X buffer 5 µl, 1 µl of dNTP (10mM), 0.2 µl of polymer DNA, 2 µl of upper primers and 2 µl of lower primers of TEM, SHV, OXA genes respectively. For the CTXM group 1, 2 µl for and 1 µl rev. For the le CTXM group2 genes, 1 µl for and 1 µl rev. For the CTXM group2 genes, 1 µl for and 1 µl rev, for the CTXM8/25 genes, 2 µl for and 2 µl rev.

For the QNR genes, the mix comprised 10X buffer 5µl, dNTP 10 µl, 0, 2 µl of taq optimase, 2 µl of forward primers and 2µl of reverse primers. The thermocycler used was BIORAD de 48 wells.

The table I shows us the specific primers of betalactamases genes and the amplification conditions.

Migration

The migration of PCR products was realized on an agarose gel at 2% containing 5 µl of RED gel in 100 ml of agarose gel. In each well of the gel, 8 µl of PCR, products plus 2 µl of loading buffer (6X) was put. The 100 pb (Promega) size marker were put in the first and the last wells.

The migration was made at 150 volts for 45 minutes.

Results

Clinical strains sensitivity test results

These ESBL producing strains presented a high resistance beside amoxicillin (100%), cefotaxim (89%), aztroenam (85%), cafepime (89%), nalidixic acid, ciprofloxacin, epéfloxacin and ernofloxacin (>90%).

All strains had a strong sensitivity beside imipenem and amikacin (100%), netilmicin (98%), piperacillin + tazobactam (91%), cefoxitin (93%).

The synergy tests were positive for 33 strains (72%) and negative for 13 strains. The latter presented a resistance against ceftriaxone, ceftazidime and the aztroenam.

Table 2: Results of *E. coli* sensibility testing.

Antibiotics	Disc Load	%S	%I	%R
Amoxicillin	20 µg	0%	0%	100%
Amoxicilline + clavulanicacid	20/10 µg	76%	0%	24%
Ticarcillin	75 µg	0%	0%	26%
Piperacillin + tazobactam	30/6 µg	91%	7%	2%
Cephalothin	30 µg	0%	0%	9%
Cefoxitin	30 µg	93%	2%	4%
Cefotaxime	5 µg	4%	7%	89%
Ceftazidime	10 µg	11%	13%	76%
Aztreonam	30 µg	9%	4%	85%
Cefepime	30 µg	7%	4%	89%
Imipenem	10 µg	100%	0%	0%
Streptomycin	10 µg	0%	0%	0%
Kanamycin	30 µg	13%	20%	67%
Amikacin	30 µg	100%	0%	0%
Gentamicin	10 µg	24%	11%	65%
Tobramycin	10 µg	9%	17%	74%
Netilmicin	10 µg	98%	2%	0%
Nalidixicacid	30 µg	7%	0%	93%
Ciprofloxacin	5 µg	7%	0%	93%
Sulfonamides	300 µg	20%	2%	76%
Tetracycline	30 µg	9%	17%	74%
Chloramphenicol	30 µg	91%	0%	9%
Nitroxolin	100 µg	98%	0%	2%
Péfloxacine	5 µg	9%	0%	91%
Colistin	50 µg	100%	0%	0%
Ernofloxacine	5 µg	7%	0%	93%

Characterization of betalactamases

All the studied strains were negative for the SHV bla and the CTXM bla group 8/25. 85% were positive for the OXA bla, 17% for the TEM bla, 8.5% of the strains were positive for the TEM and OXA association and 37% for the CTXM group 1.

Regarding quinolone -resistant genes, our study revealed a positivity for the QNR A of 11% and 4% for the QNR B.

Discussion

The results of our study show a strong prevalence of the OXA gene (85%) among our extended-spectrum betalactamase producing *Escherichia coli* strains. (ESBL) In addition to the OXA gene, TEM and SHV genes were searched for and we found out a frequency of 17% for the TEM gene. On the other hand, no SHV gene was detected. We must also note that we found out among certain strains more than one gene at a time. The genes of these carbapenemases are more often plasmidic, in majority in the hospital strains but their community diffusion has already been reported. These carbapenemases are present in antibiotic-multiresistant strains. So for the detection of these genes, we used extended spectrum betalactamase producing *E. coli* strains and we noted a high frequency of OXA and TEM genes among the latter.

In the North of Lebanon, Tabbouche Sana and al. 2011, reported results a bit similar to our study. Indeed their study was about 73 *E. coli* ESBL strains and the OXA gene was detected with a frequency of 45% followed by the TEM gene (22%). The SHV gene was also present with a frequency of 4% [10]. We did not find any SHV gene in our study unlike Tabbouchesana and al. and this could be explained by the fact that they worked on a higher number of strains.

During the study of a monitoring of extended spectrum betalactamase producing strains, in two hospitals and an ambulatory laboratory in Portugal, 17 *E. coli* strains possessed the TEM and OXA genes and more than 95% of those strains came from urine samples. [11].

In Turkey, Elif Burcu Bali and al. 2010, found among 42 *E. coli* ESBL strains a high frequency of the TEM gene namely 72.72% and 4 strains carrying SHV genes. However, they did not detect any OXA gene contrary to our study. [12].

European studies on enterobacteria have confirmed the persistence of ESBL producing strains carrying TEM and SHV genes [13]. In Turkey, a study led by Ozgumus and al. 2007, revealed that 15% of enterobacteria strains are ESBL producing, 5 of which carried the TEM gene and 12 both TEM and SHV genes [14].

In Thailand, Kiratisin and al. 2008, studied 235 ESBL producing *E. coli* strains and found out that 77% carried the TEM gene and 3.8 % the SHV gene. Besides, few strains carried the OXA gene [15].

In Italy, the prevalence of ESBL producing *Escherichia coli* strains increases with a predominance of strains carrying TEM gene (45.4%) [16]. In India, Trupti BP and al. in 2015 found among ESBL TEM and SHV genes with respectively frequencies of 48.7% and 7.6% [17]. In Iran Eftekhar and al. 2012, found in their study SHV genes 43% and TEM genes 35.2% [18].

Our study carried out in Senegal (Africa) revealed a predominance of OXA genes among ESBL producing *E. coli* strains coming from urine samples. Compared with the results of other studies carried out in different countries, we can say that the prevalence and the predominance of carbapenemases genes vary from one country to another.

We did research on the betalactamases of the CTXM type, mainly the CTXM group1 and 8/25.

The CTXM are divided into 5 phylogenetic groups based on their frequency of amino acids. 37% of our collection of strains carried the CTXM group1 but no strain presented the CTXM group 8/25 gene.

In Syria, Ibrahim Al Subol and Nihad Youssef Ibrahim Al Subol and Nihad Youssef worked on a collection of 159 *E.coli* strains 98 of which were ESBL producers. They reported a portage of the CTXM group 1 gene higher than our results. 76% of the strains carried the CTXM group 1 gene [19]. The CTX M group 2 was not found in that study [19].

The research about that enzyme in Cameroon between 2011 and 2012 revealed on 39 ESBL producing E.coli strains a rate of 100% of CTXM group 1. That study did not detect any TEM or SHV enzyme [20].

It is common to use quinolones or fluoroquinolone such as nalidixic acid, ciprofloxacin and norfloxacin for the treatment of infections of the gastro-intestinal and urinary tract. In the 1960s, quinolones were privileged as extremely important treatments in healthcare. However, in the 1990s, quinolone-resistant isolates spread worldwide [21]. The increasing frequency of pharmacoresistance among bacteria in the past years constitutes a major public health issue.

Several mechanisms of resistance to quinolones have been described one of which is the protection of the targets of the quinolones by the proteins qnr [22]. This plasmid resistance to quinolones related to the qnr genes has been reported in several countries in the world. It is an independently acquired resistance but it can be connected to the production of ESBL. We searched for these qnr genes in our study through PCR among 46 ESBL producing *E. coli* strains coming from urine samples and a global frequency of 9% was detected with 10%of QNRA and 4.3% of QNRB.

In Iran, Abbas Mokhtari and al. 2016, found out of 94 *E. coli* strains 19% of qnrA, 88.30% of qnrB and 78.72% of qnrS [23]. Moreover, Farzaneh Firoozeh and al. 2013 found in 140 *E. coli* strains prevalences of 12.1% of qnr A and 7.8% of qnrB [24].

A study was conducted in Côte d'Ivoire by C Bonni-Cissé and al. 2012. They found a prevalence of qnr genes of 23.4% 14.9% of which were qnrB and 8.5% qnrA [25]. In Morocco, Bouchakour M and al. 2010 found a general prevalence of qnr gene of 36% (n=14; qnrA, 10.25%; qnrB, 23.07%; qnrS, 2.56%) The qnr genes were identified in *E. coli*, *Klebsiella pneumoniae* and *Enterobacteria cloacae* with respectively a frequency of 18.7%, 50%, and 62%. [26]. All these studies show the diversity of the prevalence of QNR genes in the countries of the world. The presence of these genes among bacteria largely contributes to the spread of the resistance to quinolones given that this resistance to quinolones is a complex process involving various mechanisms such as chromosomal mutations and resistance genes transferred by plasmids.

Conclusion

Our collection of ESBL producing *E coli* strains responsible for urinary infections revealed that the enzymes of OXA type are the most frequent followed by the enzymes of CTXM type. The QNRA and QNRB enzymes are present but at relatively weak rates with most of the time a QNR A rate higher than that of QNRB. However, the antibiotic sensitivity test results have shown a high resistance against fluoroquinolones which are largely used in urinary infections in Fann University Hospital in Dakar. The use of carbapenemase and amikacin would be more judicious in the treatment of infections with extended spectrum betalactamase producing *E coli*.

The emergence of OXA and CTXM enzymes requires a national observation of urinary infections with a proper monitoring of the enzymes produced by resistant strains. We must also note that other studies are also necessary like a genetic characterization of enzyme types, which are produced. A global reinforcement of research laboratories in our countries would be a priority action in order to reach that objective.

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