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Full Length Research Paper

Prevalence of ESBL genes and antibiotic susceptibility patterns of *Escherichia coli* and Klebsiella species isolated from specimen of patients suffering of hospital acquired infections in National Hospital of Niamey, Niger

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The nosocomial infections associated with bacterial resistance are a public health problem in hospitals. The aim of the present study was to determine the antibiotic susceptibility patterns and distribution of the blaTEM, blaCTX M, blaSHV and blaOXA ESBL resistance genes in *Escherichia coli* and *Klebsiella* species isolated from specimen of patients suffering from nosocomial infections. This was a prospective study that included the results of specimen exahation, their corresponding antibiotic susceptibility testing, and the results of conventional polymerase chain reaction concerning the beta-lactamase genes of identified isolates. The study was conducted over a period of nine months, from January 2023 to September 2023. Eighty-four isolates with a phenotypic beta-lactamase profile were isolated from urine and pus specimens collected from patients suffering from nosocomial infections. Of the 84 isolates, *Escherichia coli* represented 83.33% and Klebsiella 16.67%. These bacteria were isolated from urine in 84.30 and 64.30% of cases for *Escherichia coli* and Klebsiella, respectively. The pathogens also showed a high level of resistance and co-resistance to the tested antibiotics. *E. coli* showed 100% resistance to amoxicillin, 100% to amoxicillin + clavulanic acid, 98.57% to ceftriaxone, and 94.29% to gentamicin but was sensitive to meropenem at 97.14%. Klebsiella isolates showed 100% resistance to amoxicillin + clavulanic acid, 75% to ceftriaxone, 91.67% to gentamicin, but were sensitive to meropenem at 91.66%. Concerning the beta-lactamase genes, the CTX-M 1 was the most prevalent in the isolates, ranging from 97.14 to 78.57% for *E. coli* and Klebsiella, respectively. Improving hospital hygiene and prescribing antibiotics appropriately after conducting antibiotic sensitivity tests are fundamental in the fight against nosocomial infections.

Key words: Extended-spectrum beta-lactamases genes, *Escherichia coli*, Klebsiella *species*, Niamey, nosocomial infections.

INTRODUCTION

Infections acquired during the provision of health, called hospital-acquired infections, are a significant public health problem worldwide (Pittet et al., 2008; Allegranzi and Pittet, 2007). In developing countries, the risk of infection is 2 to 20 times higher, and the proportion of infected patients can exceed 25% (Pittet et al., 2008). More than 1.4 million patients develop a serious infection at some point while being treated for an entirely different condition (Allegranzi and Pittet, 2007). Antimicrobial resistance is a global concern for effective healthcare delivery. Studies have revealed that the form of antimicrobial resistance is particularly acute, with rates of methicillin resistance in *Staphylococcus aureus* exceeding 50% in several countries, and resistance in *Escherichia coli* to third-generation cephalosporins exceeding 70% in some countries (Borg et al., 2009). The germs responsible for nosocomial infections are multi-resistant bacteria, including *Enterobacter cloacae* (extended-spectrum beta-lactamase secretor), methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Déguénonvo et al., 2015). Six pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii*, and Pseudomonas, were the pathogens most implicated in the occurrence of deaths due to microbial resistance, with 929,000 cases in 2019 (Antimicrobial Resistance Collaborators, 2022). In hospitals, the treatment of infections caused by these multi-resistant bacteria is becoming increasingly problematic (Carle, 2009).

Some of these bacteria have become resistant to many antibiotics, including carbapenems and third-generation cephalosporins, which are the best products available for treating multidrug-resistant bacteria (WHO, 2017). Moreover, resistant ESBL-producing enterobacteria are among the priority pathogens for which it is necessary to find new antibiotics (WHO, 2017). ESBL enzymes confer resistance to penicillins, cephalosporins, monobactams, and other antibiotic classes (Manyahi et al., 2017). These pathogens express a high rate of acquired resistance to the majority of antibiotics from the beta-lactam group by producing enzymes called extended-spectrum beta-lactamases (ESBLs), which inactivate first, second, and third-generation cephalosporins (Rawat and Nair, 2010). The genes that code for these enzymes are carried by plasmids and coexist with genes for resistance to other antibiotics, hence the origin of the multi-resistance of enterobacteria producing extended-spectrum beta-lactamases (Rawat and Nair, 2010). In Niger, data on ESBL resistance genes are fragmentary and do not allow us to assess the relationship between multi-resistant enterobacteria and the occurrence of hospital-acquired infections.

METHODS

Isolation and identification of bacterial species

Enterobacteriaceae strains were isolated from pathological products (urine and pus) from patients suffering from healthcare-associated infections at the Niamey National Hospital in Niger. The samples were processed in accordance with the pre-established protocols available in the laboratory. The urine samples were systematically inoculated on CLED agar (cystine lactose deficient electrolyte), and those of us were inoculated on MacConkey agar with crystal violet, Chapman (Mannitol salt agar), and fresh sheep blood agar. Incubation conditions were 37°C under aerobiosis for 18-24 hours for CLED, MacConkey, and Chapman media, and 37°C under 5 to 10% CO2 for fresh blood agar. Strain identification was carried out after Gram staining, presumptive tests (oxidase, catalase), and by using the Api 20E system in the biology laboratory of the National Hospital of Niamey. The Api 20E method is a standardized technique that allows for the biochemical identification of enterobacteria from isolated colonies.

Antibiotic susceptibility testing and phenotypic detection of ESBL

The antibiotic sensitivity test was carried out using the disk diffusion method on Mueller-Hinton agar medium, following the recommendations of EUCAST/CASFM (2021). The antibiotics were stored at -20°C before use. The antibiotics used were amoxicillin, amoxicillin-clavulanic acid, cefoxitin (30 µg), gentamicin (10 µg), ceftazidime (30 µg), meropenem (10 µg), ciprofloxacin (5 µg), and amikacin (10 µg). The antibiotic susceptibility tests were conducted on strains of *Escherichia coli* and *Klebsiella* species isolated from pathological products originating from patients with hospital-acquired infections. The antibiotics nitrofurantoin and fosfomycin were tested only on enterobacteria isolated from urine. All strains were tested for ESBL detection using the double-disk technique. The production of extended-spectrum beta-lactamases was demonstrated by the double-disk synergy technique described by Olonitola et al. (2007). The amoxicillin+clavulanic acid (AMC) disk was placed between the ceftazidime, ceftriaxone, or cefotaxime (third-generation cephalosporin C3G) disks at a distance of 2 to 3 cm from center to center on a Mueller-Hinton agar plate. After an incubation period of 18 to 24 hours, a dome-shaped culture-free zone appears between the AMC discs and those of the C3G, known as a "champagne cork". The strains presenting an ESBL were stored at -80°C. These strains were then sent to the molecular biology laboratory of the Gaston Muraz center in Bobo-Dioulasso for the search for ESBL genes using conventional PCR, for the period from July 2023 to October 2023.

Extraction of bacterial DNA

The method used was the Chelex® 100 method. It consisted of emulsifying the bacteria in a tube containing a suspension of 200 µl of Chelex® 100. The mixture was then brought to 56°C for 30 to 55 h and vortexed before being incubated at 95°C for 5 h. After this final incubation, the samples were centrifuged at 13,000 rpm to separate

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Table 1. PCR types and primers used.

PCR name	ß-Lactamase (s) targeted	Primer name	Sequence (5' –3')	Length (bases)	Annealing positiona	Amplicon size (bp)	Primer concentration (pmol/mL)
Multiplex TEM, SHV and OXA-1-like	TEM variants including TEM-1 and TEM-2	MultiTSO-T_for	CATTTCCGTGTCGCCCTTATTC	22	13–34	800	0.4
		MultiTSO-T_rev	CGTTCATCCATAGTTGCCTGAC	22	812–791		0.4
	SHV variants including SHV-1	MultiTSO-S_for	AGCCGCTTGAGCAAATTAAAC	21	71–91	713	0.4
		MultiTSO-S_rev	ATCCCGCAGATAAATCACCAC	21	783–763		0.4
	OXA-1	MultiTSO-O_for	GGCACCAGATTCAACTTTCAAG	22	201–222	564	0.4
		MultiTSO-O_rev	GACCCCAAGTTTCCTGTAAGTG	22	764–743		0.4
Simplex CTX-M group 1	CTX-M group 1	SimplexCTXMGp1	TTAGGAARTGTGCCGCTGYAb	20	61–80	688	0.4

Table 2. Prevalence of ESBL-positive enterobacteria according to pathological product.

Strains	Urine (%)	Pus (%)	Total (%)
Escherichia coli	59(84.30)	11(15.70)	70(100)
Klebsiella spp	9(64.30)	5(35.70)	14(100)

Most of the strains were isolated from urine specimen.

the Chelex resin from the DNA. The supernatants were used for PCR.

Amplification

DNA samples (2 µl) were tested using a double PCR method. A first multiplex PCR for the search for *blaTEM*, *blashv* ,*oxa* -1 like genes, in a reaction mixture of 18µl per sample (4µl of Master mix, 1µl of primer F10 µl, 1 µl of primer R10 µl, 12 µl of water for PCR and 2 µl of DNA) and a second simplex PCR for the detection of the *CTX-M* group 1 gene . For the two PCRs the amplification program was carried out according to the diagram below: an initial denaturation at 95°C for 5 h, 30 cycles of denaturation at 95°C for 30 s, hybridization at 60°C for 60 s, elongation at 72°C for 60 s and a final elongation at 72°C for 7 h.

Revelation of PCR amplification products

The amplification products (amplicons) were detected after electrophoresis in a 1.5% agarose gel. For the preparation of the 1.5% agarose gel, 3 g of powdered agarose were dissolved in 200 ml of Tris Borate EDTA 1X solution. The mixture was heated until clear and then cooled to laboratory temperature. After cooling, 15 µl of ethidium bromide was added. The agarose was well mixed and poured into the gel electrophoresis tank for 30 h. The molecular weight size marker used was 100 bp (SOLIS BIODYNE Estonia). The molecular weight size marker used was 100 pb (SOLIS BIODYNE Estonia). The three genes blaTEM, blashv ,oxa -1 like were detected at 800,713, 564 bp respectively (Figure 2) whereas CTX-M group 1gene was detected at 688 bp (Figure 3). Interpretation was made by comparing the band sizes of different samples to those of the molecular weight marker bands and the positive and negative controls. The positive control was said to be positive if the band migrates at the correct size. The negative control was said to be negative if no band migrates. A sample was considered positive if the band appears at the correct size. A sample was considered negative if no bands appear or if only spurious bands appeared. PCR types and primers used are shown in Table 1.

RESULTS

During this study, 84 strains of enterobacteria were isolated and tested with antibiotics. These strains mainly came from patients suffering from nosocomial urinary infections, that is, 83.30% for *Escherichia coli* and 64.30% for Klebsiella (Table 2). Regarding the level of antibiotic resistance, the bacteria showed a high level of resistance. *Escherichia coli* showed 100% resistance to amoxicillin and 98.57% resistance to ceftriaxone but maintained a sensitivity of 97.14% to meropenem (Table 3). Klebsiella strains also showed a high level of resistance, that is, 100% resistance to amoxicillin + clavulanic acid and 75% for ceftriaxone, but with a sensitivity of 91.66% for meropenem (Table 4). The majority of *Escherichia coli* strains (36.90%) came from patients aged over 60, while Klebsiella strains (47.36%) were more

Table 3. Antibiotic sensitivity of *Escherichia coli* to antibiotics.

Antibiotics	Resistant N (%)	Sensitive N (%)	Total N
Amoxicillin	70 (100)	0 (0)	70
Amoxicillin+clavulanic acid	70 (100)	0 (0)	70
Cefoxitin	42 (60)	18 (40)	70
Ceftriaxone	69 (98.57)	1 (1.43)	70
ceftzadime	69 (98.57)	1 (1.43)	70
Gentamycin	66 (94.29)	4 (5.71)	70
Amikacin	45 (64.29)	15 (35.71)	70
Ciprofloxacin	61 (87.14)	19 (13.10)	70
Meropenem	2 (2.86)	68 (97.74)	70
Nitrofurantoin	4 (36.36)	7(63.64)	11
Fosfomycin	7 (63.34)	4 (36.66)	11

Escherichia coli isolates were 100% resistant to amoxicillin but sensitive to meropenem to 97.14%.

Table 4. Antibiotic sensitivity of Klebsiella to antibiotics.

Antibiotics	Resistant N (%)	Sensitive N (%)	Total N
Amoxicillin+clavulanic acid	14 (100)	0 (0)	19
Cefoxitin	11(91.67)	1 (8.33)	19
Ceftriaxone	9 (75)	5(25)	19
ceftazidime	9 (75)	5(25)	19
Gentamycin	11 (91.67)	1(8.33)	19
Amikacin	3(25)	9 (75)	19
Ciprofloxacin	11(91.67)	1(8.33)	19
Meropenem	1(8.33)	11(91.66)	19
Nitrofurantoin	5(55.55)	4(44.45)	11
Fosfomycin	**3(33.33)**	**6(66.67)**	**11**

Klebsiella spp isolates were 100% resistant to amoxicillin +clavulanic acid but sensitive to meropenem to 91.66%.

prevalent in the 0- to- 20-year age group (Figure 1). *Escherichia coli* ESBL isolates were more prevalent in the over 60 age group, whereas *Klebsiella* species ESBL isolates were more prevalent in the 0- to- 20-year age group.

DISCUSSION

The level of resistance of bacteria responsible for hospital acquired infections has been described in several studies around the world. In Niger, the data concerning this public health problem remain nuanced. This study remains the first concerning the resistance and diversity of genes coding for ESBLs in multi-resistant enterobacteria causing hospital acquired infections.

These strains mainly came from patients suffering from nosocomial urinary infections, to the extent of 84.42 and 64.28% for *Escherichia coli* and *Klebsiella* respectively. These results were similar to those reported in Poland (Trześniewska-Ofiara et al., 2022) where *Escherichia coli* and *Klebsiella* remained the most common types of pathogens in hospital urinary tract infections in healthcare units on selected hospital wards. This confirms reports by other authors that *E. coli* is the most common etiological agent of urinary tract infections in many countries (Serretiello et al., 2021).

The presence of these micoorganisms as part of the intestinal microflora, poor hygiene and hospitalizations duration may be the main hypothesis that explains the role played by these pathogens in the occurrence of nosocomial urinary tract infections. Also with regards to antibiotic resistance, the level of resistance of the strains studied was somehow alarhg with the isolates showing high level of antibiotic resistance. Concerning resistance to cephalosporins expressed in ESBL, similar results have been found in Vietnam where ESBL rates above 70% have been reported (Trang et al., 2023); although concerning *Escherichia coli* EBSL, the pathogen EBSL prevalence rate has raised from 19 to 54% from 2002 to 2017 in all

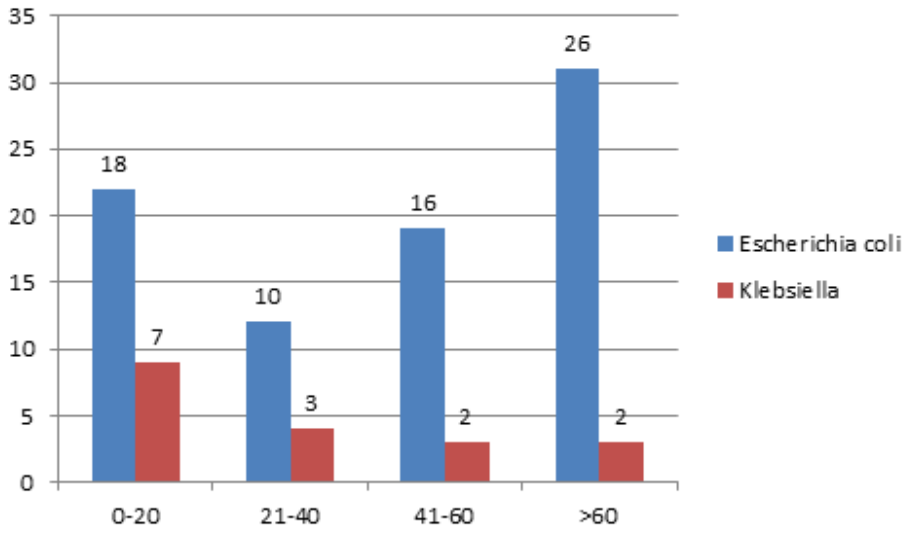

Figure 1. Prevalence of ESBL in bacterial isolates according to age group.

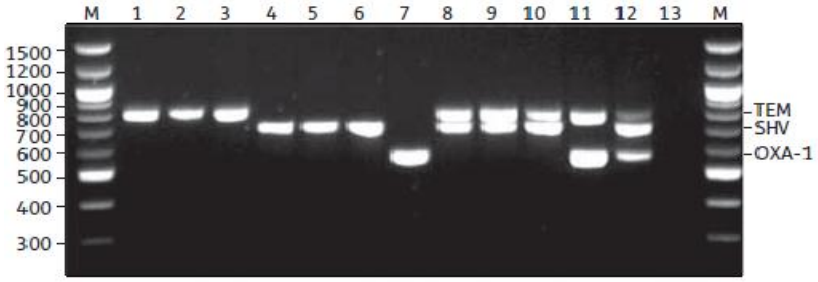

Figure 2. Positive PCR results for *blaTEM*, *SHV*, *OXA-1* like.

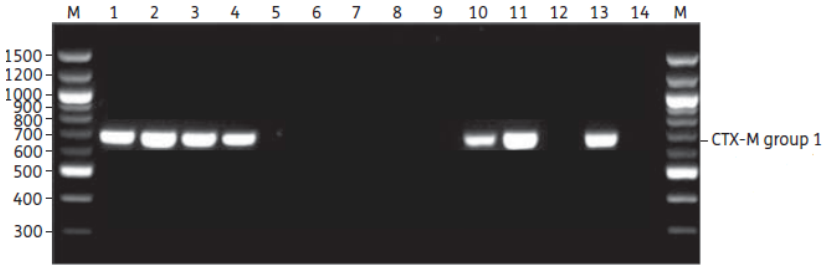

Figure 3. Positive PCR results for CTX-M1

(Santé Publique France, 2019) and was the most prevalent between all isolates encountered during nosocomial infections surveys in france (Daniau et al., 2018).

The misuse of antibiotics coupled with the transmission of resistance deterhants could explain this trend. *Escherichia coli* showed 100% resistance to amoxicillin, 98.57% resistance to ceftriaxon but maintained a sensitivity of 97.14% to meropenem. *Klebsiella* strains also showed a high level of resistance, 100% resistance to amoxicillin + clavulanic acid, 75% for ceftriaxon, even though the microorganism has shown a sensitivity of 91.66% for meropenem. Such high levels of resistance to third-generation cephalosporins have also been reported in Vietnam, reaching 87.3% among enterobacteriaceae isolated from clinical samples (Trang et al., 2023).

Table 5. Prevalence of ESBL resistance genes in bacterial strains.

Strains	Numbers	TEM (%)	SHV (%)	OXA-1 LIKE (%)	CTX-M 1 (%)
Escherichia coli	70	41(58.57)	3(4.28)	34(48.57)	68(97.14)
Klebsiella species	14	10(71.42)	0(0)	6(42.85)	11(78.57)

CTX-M 1 genes were more prevalent in *Escherichia coli* ESBL isolates and in Klebsiella species ESBL isolates.

However, despite the high level of resistance to belactamines constituting the most recommended therapeutic arsenal in healthcare establishments for treatment, the strains maintained a high sensitivity to carbapenems. In our study the rate of sensitivity to carbapenems was 97.14 and 91.66% for *Escherichia coli* and *Klebsiella* respectively. This rate, although relatively high was lower than Vietnam (Trang et al., 2023) where all the ESBL enterobacteria sampled were sensitive to carbapenems. Also in France (Daniau et al., 2017) in 2017 in another study on infections associated in health care establishments; the resistance of enterobacteria to carbapenems has been estimated at 0.6%. This resistance rate nevertheless forced clinicians to use these molecules considered to be the last line in the treatment of patients. The over prescription of antibiotics; probabilistic antibiotic therapy and the use of urinary catheters in patients with nosocomial urinary infections may likely contribute to the emergence of this resistance to carbapenems.

Concerning the different ESBL genes (Table 5) harboured by the isolates, our findings were similar to those reported in other studies in bacterial strains mostly causing nosocomial infections (Alam et al., 2019; Ghaima et al.,2018). Concerning the prevalence rate particularly with regards to *blaTEM*, and *CTX-M1*, the results found during our work showed a high rate of *blaTEM and CTX-M1* for *Escherichia coli* and Klebsiella ESBL studied. These results were similar to those found in Togo (Diagbouga et al., 2016; Salah et al., 2017), where prevalence rates of *TEM* (82.31%) and *CTX-M1* (95.73%) has been reported. This high level of these antibiotic resistance genes could be explained by the acquisition of plasmids encoding these different mechanisms.

Considering the prevalence rate of *SHV*, our prevalence rate was respectively 4.28 and 0% for *Escherichia coli* and *Klebsiella*. This rate was similar to that found in Spain (Karmele et al., 2003) on strains of *Escherichia coli* resistant to amoxicillin + clavulanic acid where only two strains out of 51 studied carried the *blaSHV* gene indicating therefore a prevalence of 3.92%. Our rate was significantly lower than that found in Togo (Diagbouga et al., 2016, Salah et al., 2017) with a *blaSHV* gene prevalence of 45.12% was reported. Until the end of the 1990s, these enzymes were considered to be the most prevalent in the acquisition of ESBL and were associated to the occurrence of hospital acquired infections (Silva et al., 2000). This low prevalence could be justified by the size of our sample knowing that Klebsiella strains were more likely to be sources of ESBL than *Escherichia coli.*

The prevalence rate of *bla* OXA-1 was 48.57 and 42.85% for the E coli and Klebsiella strains under study. Regarding *Escherichia coli* strains, these results reflect other data reported in England with a rate found of 50.9% (David et al., 2019). However, with regard to *Klebsiella*, our results were doubly higher than those found in another study in India (David et al., 2003) with a rate of 20.3%. The acquisition of these enzymes has been variously discussed in different studies around the world. Thus the presence of OXA-1 enzymes had been associated with resistance to the combination of penicillin + β-lactamase inhibitor in enterobacteria and non-enterobacteria (David et al., 2008, Sugumar et al., 2014). This assertion was highlighted in a single study (Sugumar et al., 2014) and many of the isolates were resistant to carbapenems, proof that the presence of oxa-1 enzymes was possibly associated with other resistance mechanisms. Our results will be better understood by furthering the investigations to look for other resistance genes associated with the presence of oxa - 1 like genes in our isolates.

Conclusion

Nosocomial infections caused by multidrug-resistant bacteria constitute a serious public health problem. This resistance is likely encoded by transferable plasmids, allowing bacteria to acquire new resistance mechanisms. Collaboration between hygienists, biologists, and prescribers is fundamental in order to reduce the heavy burden of nosocomial infections in hospitals, especially in low-income countries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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