Full Length Research Paper

Phenotypic detection of extended spectrum beta-lactamase in multidrug-resistant *Escherichia coli* from clinical isolates in Niamey, Niger

Alio Mahamadou Fody¹, Laouali Boubou², Ali Moussa³, Hadiza Ibrahim Bawa¹, Ali Konaté¹, Chaibou Yaou¹, Cheikna Zongo¹, Chaibou Salaou², Alhousseini Daouda⁵, Ramatou Sidikou⁴, Alfred S. Traoré¹ and Nicolas Barro¹


²Laboratoire de Biologie, Hôpital National de Niamey (HNN), BP 238 Niamey, Niger.

³Laboratoire de Biologie, Hôpital National Lamordé (HNL), BP 10146 Niamey, Niger.

⁴Laboratoire de Biotechnologies, Faculté des Sciences et Technologies Université Abdou Moumouni de Niamey B.P.: 12022, Niamey, Niger.

⁵Laboratoire de Biochimie Hôpital National de Niamey (HNN) BP 238, Niamey, Niger.

Received 22 March, 2017; Accepted 21 April, 2017

Extended spectrum beta lactamase (ESBL) producing *Enterobacteriaceae* is one of the main causes of antibiotic treatment failure in hospitals. The aim of this study was to evaluate the prevalence of ESBL produced by multidrug-resistant (MDR) *Escherichia coli* isolated from various clinical samples (urine, stool, pus, blood culture) in the “Hôpital National de Niamey” and the “Hôpital National Lamordé” of Niamey, Niger. Samples were processed using standard bacteriological methods. Isolates were identified by biochemical tests and confirmed on API 20 E system (Bio-Mérieux, France). Antibiotic susceptibility was determined using the disk diffusion method on Mueller-Hinton (MH) agar plates (Liofilchem, Italy). Producing of extended spectrum beta-lactamase was performed using simple double-disk synergy test (DDST) and double-disk synergy test using cloxacillin. A total of two hundred and seventeen (217) multidrug-resistant *E. coli* were isolated from various clinical samples. Among these isolates, 57 (26.3%) were extended spectrum beta-lactamase producers. From clinical sources, prevalence of ESBL producing *E. coli* was observed in urine samples (26.7%), stool samples (26.3%), pus samples (25%) and blood samples (25%). ESBL producing *E. coli* were observed in the age groups under 5 years (24.9%), 26 to 45 (38.1%) and over 65 years (50%). This study showed a notable prevalence of extended spectrum beta-lactamase *E. coli* isolated from various clinical samples in two hospitals of Niamey, suggesting the rational and judicious use of antibiotics by clinicians.

Key words: Extended spectrum beta lactamase (ESBL), multidrug-resistance, *Escherichia coli*, prevalence, Niamey, Niger.

INTRODUCTION

*Escherichia coli* is a commensal of the human gut and one of the most frequently isolated bacteria from clinical
specimens (Quinet et al., 2010). It plays an important role as a member of the gut microbiota; however, pathogenic strains also exist, including various diarrheagenic *E. coli* pathotypes and extraintestinal pathogenic *E. coli* that cause illness like bacteremia, bladder infections, meningitis or pus (Dias et al., 2009; Smith et al., 2010; Fratamico et al., 2016). The discovery of antibiotics has been a humanity relief because these remedies have significantly reduced the incidence of infectious diseases, especially in developing countries (Guessennd et al., 2008). *E. coli* is one of the most common clinical pathogens causing nosocomial infection. For a long time, the widespread use of antibiotics to treat *E. coli* infectious disease has rapidly increased the multidrug resistance (MDR) of *E. coli* (Trecarichi et al., 2012; Kanwar et al., 2013) especially with those strains producing ESBL (Bush, 2001; Woerther et al., 2013). The appearance of ESBL stated in the 1980s and widely distributed in the world (Knothe et al., 1983; Bradford, 2001) and conferred increased resistance to beta-lactams except carbapenems and cephapemycins (Patterson, 2001; Masterton et al., 2003). ESBLs are plasmid mediated and the genes encoding these enzymes are easily transferable among different bacteria (Todar, 2012). Most of these plasmids not only contain DNA encoding ESBLs but also carry genes conferring resistance to several non-β-lactam antibiotics (Rankin and Svara, 2011). The presence of ESBL in clinical isolate has been documented as a very serious problem and a significant trait to: quick survival of patients in the hospital, high economic burden, loss of hours in life’s activities and high treatment failure (CDC, 2010). The phenotypic methods are currently the gold standard in determination of susceptibility or resistance of clinical isolates. The most widely used methods to screen ESBL are E-test, or double-disk synergy test (DDST) (EUCAST, 2014). Several reports have described the prevalence of ESBLs in the Middle East North Africa region and most of the Gulf Cooperation Countries (Zowawi et al., 2013). However, there is insufficient scientific data on the prevalence of ESBLs available from the State of Niger.

This study aimed to determine the prevalence of ESBL-producing among MDR *E. coli* isolates from various clinical samples at “Hôpital national de Niamey” and “Hôpital national lamordé”, Niger.

**MATERIALS AND METHODS**

**Study design and site**

The present prospective study was conducted on routine specimens received at the bacteriology laboratory of “Hôpital National de Niamey” and the “Hôpital National Lamordé”, with a capacity of 800 and 500 beds, respectively. Bacterial isolates that were resistant to third generation of cephalosporin were collected from March 2014 to June 2014 and then from October 2014 to June 2015 (13 months) simultaneously in two hospitals. They were isolated during diagnosis analysis of biological specimens. Different clinical specimens such as blood, pus, urine, stool, and vaginal swab were collected.

**Isolation and identification of *E. coli***

Bacterial isolates were obtained from various clinical specimens. Stool samples were inoculated on eosin methylene blue agar (EMB, Merseyside UK), urine samples on cystine lactose electrolyte deficient agar (CLED, Liofichem), pus and vaginal swabs were cultured on blood and chocolate agar and were incubated at 37°C for 18 to 24 h. Blood samples were inoculated and incubated in culture bottles with Bact/Alert 3D 60 Biomérieux at 37°C. The isolates were identified according to the procedures described by Cheesbrough et al. (2005) and were confirmed using gallery API 20 E system (Biomérieux, France). The clinical isolates were preserved at −70°C for further analysis.

**Antibiotic susceptibility testing**

Antibiotic susceptibility was determined using the disk diffusion method on Mueller-Hinton (MH) agar plates (Liofichem, Italy) according to the recommendations of “Comité de l’Antibiogramme de la Société Francaise de Microbiologie” (CA-SFM, 2012). The following antimicrobials were tested: amoxicillin (25 µg), amoxicillin + clavulanic acid (20 +10 µg), cephalothin (30 µg), cefotaxime (30 µg), cefazidime (30 µg), ceftriaxone (30 µg), ofloxacine (5 µg), nalidixic acid (30 µg), aztreonam (30 µg), amikacin (30 µg), gentamicin (15 µg), ciprofloxacin (5 µg), nitrofurant (300 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and imipenem (10 µg). Quality control was done using *E. coli* ATCC 25922. Multidrug resistant *E. coli* was defined as resistance to at least three classes of antibiotics.

**Detection of extended-spectrum beta-lactamase (ESBL) by DDST**

All MDR *E. coli* isolated were screened for ESBL. The double-disk synergy test (DDST) was performed for the phenotypic detection of ESBL producers according to the CA-SFM recommendations, using cefazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg) disks and were placed 25 mm (center to center) from the amoxicillin/clavulanic acid (20/10 µg) disk on Mueller–Hinton agar (CA-SFM, 2012). After inoculation, the plates were incubated at 37°C; the presence of a keyhole effect was recorded 24 h after incubation. The DDST was performed in parallel to the antibiogram.

**Detection of extended-spectrum beta-lactamase by DDST using cloxacillin**

Cloxacillin test was performed for MDR isolates naturally producing inducible céphalosporinase ( AmpC). The production of ESBL was inferred by a synergy image as previously described (Drieux et al., 2008). From July 2014 to September 2014 and then from July 2015
Table 1. Global antimicrobial susceptibility of MDR E. coli.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance percent (%)</th>
<th>Susceptible percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic</td>
<td>93.1</td>
<td>6.9</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>98.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>35.9</td>
<td>64.1</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>92.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>97.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>83.9</td>
<td>16.1</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>77.4</td>
<td>22.6</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>91.2</td>
<td>8.8</td>
</tr>
<tr>
<td>Amikacin</td>
<td>10.6</td>
<td>89.4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>36.9</td>
<td>63.1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>82.9</td>
<td>17.1</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>77.4</td>
<td>22.6</td>
</tr>
<tr>
<td>Nitrofurant</td>
<td>21.7</td>
<td>78.3</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>95.4</td>
<td>4.6</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1.4</td>
<td>98.6</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of ESBL among MDR E. coli.

<table>
<thead>
<tr>
<th></th>
<th>Positive ESBL</th>
<th>Negative ESBL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DDST</td>
<td>DDST + cloxacillin</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>26.3</td>
<td>73.7</td>
</tr>
</tbody>
</table>

Ethical consideration

All isolates obtained were from biological specimens on clinical routine examinations of patients, with the authorization of the laboratory director and hospital director.

Data analysis

Data were analyzed using Excel, Microsoft® Office 2013. Chi square was used to determine the statistical significance of the data. Statistical significant difference was considered with a p-value < 0.05.

RESULTS

Antimicrobial susceptibility testing

During the study period, two hundred and seventeen (217) MDR E. coli were isolated from the two hospitals. High resistance to beta-lactams was observed, mainly with ampicillin (100%), amoxicillin + clavulanic acid (93.1%), cephalothin (98.2%), cefotaxime (92.6%), ceftazidime (97.2%) and ceftriaxone (83.9%) as compared to quinolone with ofloxacin (77.4%), ciprofloxacin (84.9%) and nalidixic acid (91.2%). Resistance to the nobactams was 77.4% to aztreonam, and the sulfonamides was 95.4% to trimethoprim-sulfamethoxazole (Table 1).

Prevalence of ESBL among MDR E. coli

Among the 217 MDR E. coli isolated, screening for ESBL production showed a global prevalence of 26.3% (57) as shown in Table 2. The difference between percentages of ESBL-producing (26.3%) and non-ESBL-producing (73.7%) E. coli was highly significant (P = 0.0001). The DDST showed 22.6% (49) of ESBL E. coli prevalence. Those MDR E. coli (168) that were negative to ESBL were tested with DDST using cloxacillin. Of these isolates, ESBL prevalence of 4.8% (9) was observed (P = 0.0013). This indicated a significant difference between the two methods.

Prevalence of ESBL E. coli according to demographic characteristic of the studied patients

The demographic characteristic of the studied patients is summarized in Table 3. Of the 217 MDR E. coli, 150 (69.1%) were isolated from outpatients, and 67 (30.9%) from inpatients (P= 0.0002). However, ESBL prevalence of 25.3 and 28.4% from outpatients and inpatients were

Table 2. Prevalence of ESBL among MDR E. coli.

<table>
<thead>
<tr>
<th></th>
<th>Positive ESBL</th>
<th>Negative ESBL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DDST</td>
<td>DDST + cloxacillin</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>26.3</td>
<td>73.7</td>
</tr>
</tbody>
</table>

Prevalence of ESBL among MDR E. coli

Among the 217 MDR E. coli isolated, screening for ESBL production showed a global prevalence of 26.3% (57) as shown in Table 2. The difference between percentages of ESBL-producing (26.3%) and non-ESBL-producing (73.7%) E. coli was highly significant (P = 0.0001). The DDST showed 22.6% (49) of ESBL E. coli prevalence. Those MDR E. coli (168) that were negative to ESBL were tested with DDST using cloxacillin. Of these isolates, ESBL prevalence of 4.8% (9) was observed (P = 0.0013). This indicated a significant difference between the two methods.

Prevalence of ESBL E. coli according to demographic characteristic of the studied patients

The demographic characteristic of the studied patients is summarized in Table 3. Of the 217 MDR E. coli, 150 (69.1%) were isolated from outpatients, and 67 (30.9%) from inpatients (P= 0.0002). However, ESBL prevalence of 25.3 and 28.4% from outpatients and inpatients were
observed respectively (P= 0.78). The distribution of ESBL producers based on gender indicates that women had a higher prevalence rate of 28.3% than men, 24.6% (P= 0.78). There was no significant difference between the gender distributions and the source patients. Otherwise, samples were collected from patients ranging in age from 1 month to over 65 years. The highest prevalence (50%) of ESBL was observed among the age group over 65 years followed by the age group of 26 to 45 years (38.1%), then the age group under five years (24.5%), age group 6 to 25 (23.1%) and the least in age group of 46 to 65 years (11.5%). Low prevalence of ESBL E. coli was observed in “Hôpital National de Niamey” with 10.1%; as compared “Hôpital National Lamordé” (37.5%) (p= 0.0001). This difference was significant among the two hospitals.

**Prevalence of ESBL E. coli according to biological specimens**

Most of the MDR E. coli were isolated from the urine samples (67.3%) followed by stool samples (26.3%) as shown in Table 4. However, only 26.7 and 26.3% of urine and stool isolates, respectively were ESBL-producers. On the other hand, in spite of their small number, 25% of isolates from pus and blood samples were ESBL producers.

<table>
<thead>
<tr>
<th>Biological samples</th>
<th>MDR E. coli n (%)</th>
<th>ESBL E. coli n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>48 (37.5)</td>
<td>29 (24.6)</td>
</tr>
<tr>
<td>Stools</td>
<td>26 (12.8)</td>
<td>6 (23.1)</td>
</tr>
<tr>
<td>Pus</td>
<td>26 (12.8)</td>
<td>6 (23.1)</td>
</tr>
<tr>
<td>Blood</td>
<td>26 (12.8)</td>
<td>6 (23.1)</td>
</tr>
<tr>
<td>Vaginal swabs</td>
<td>26 (12.8)</td>
<td>6 (23.1)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The number of infections due to ESBL E. coli is increasing, especially in African countries (Manyahi et al., 2014). In this study, the authors investigated the prevalence of ESBL production by MDR E. coli isolated from clinical samples sent to the two main hospitals of Niamey. The antimicrobial susceptibility tests showed an important level of resistance of antibiotics classes. Thus, for beta-lactams classes, resistance frequency of 93.1% was observed for amoxicillin-clavulanate, 92.6% for cefotaxim and 97.2% for ceftazidim. Similar results have been reported in Nigeria with a prevalence of 89.71% for amoxicillin-clavulanate 79.47% for cefotaxim and ceftazidim 41.03% (Odumosu and Akintimehin, 2015). On the other hand, co-resistance was shown for different antibiotics such as ofloxacin (77.4%), ciprofloxacin (84.9%) and nalidixic acid (91.2%). Similar results were observed in Ivory Coast with a prevalence of 70.2% for ciprofloxacin and 76.8% for nalidixic acid (Guessennd et al., 2008). Such level of resistance could be due to abusive prescription of antibiotics by professionals of health care without prior laboratory investigations or parallel care at home, self-medication and also the use of street drugs which is very spread in Africa (Yandai et al., 2014). Nevertheless, this study showed a susceptibility of 95.4% for imipenem, this frequency was similar to published data in Burkina Faso, which showed a susceptibility of 100% for imipenem (Sanou et al., 2015).

In all the 217 MDR E. coli isolated in this study, ESBL prevalence of 26.3% was observed. Similar prevalence was found in some African countries such as in Benin (22%) (Ahoyo et al., 2007), Nigeria (20%) (Onwuezobe and Orok, 2015), Niger (30.9%) (Woerther et al., 2011) and Chad (20.09%) (Yandai et al., 2014). As compared to previous studies, the current study results are lower than that observed in Burkina Faso (38.3%) (Dembélé et al., 2015) and Senegal (52%) (Lo et al., 2014). However, these results are higher than that observed in Tanzania (15.1%) (Mshana et al., 2016) and the Libyan community (13.4%) (Ahmed et al., 2014). This wide variation in prevalence was probably due to differences in type of samples collected.

The study showed the prevalence of ESBL producers
based on gender and indicated that females had a higher prevalence rate (28.3%) than males (24.6%). No differences were apparent between ESBL-producing *E. coli* with gender distribution (Akanbi et al., 2013). However, contradictory observations were found in Nigeria (Yusuf et al., 2013).

In this study, the majority of ESBL-producing *E. coli* was isolated from inpatients (28.4%) as compared to outpatients (25.3%). The rates of ESBL-producing *E. coli* were higher among inpatients (22.82%) than the outpatients (18.11%) as reported in Chad (Yandai et al., 2014).

Among the patients studied, the highest prevalence was observed in age group over 65 years (50%) followed by age group 45 to 65 years (38.1%) and the age group under 5 years (24.8%). Higher median age was observed among individuals colonized with ESBLs as reported from Tanzania (Mshana et al., 2016). Although, previous study in Guinea-Bissau showed that ESBL *E. coli* prevalence was high in all age groups, among the youngest 27% were carriers in the ages 0 to 3 months. This indicates that colonization with ESBL-producing bacteria often occurs early in life in this population (Isendahl et al., 2012).

This study showed that MDR *E. coli* were isolated from a variety of clinical samples. Thus, it was found that urine samples had the highest proportion of ESBL (26.7%) followed by stool samples (26.3%). However, the same prevalence (25%) was observed from blood and pus samples. The major ESBL *E. coli* producer (18.2%) was isolated from urine samples (Raut et al., 2015). Ouedraogo et al. (2016) found that blood cultures had the highest proportion of ESBL isolates. In previous study, the distribution of ESBL producers was most prevalent in blood (22.2%) and urine samples (17.6%). This was followed in that order by stool (15.8%), urogenital swabs (14.3%) and wound swab (13.5%) while the least prevalence was observed in ear swab specimens (3.2%) (Yusuf et al., 2013). This difference of ESBL producers among clinical source could be due to lower number of some samples. Therefore, infection control strategies, with the rational use of antibiotics could be an important factors to reduce the spread of ESBL. Further investigations, including molecular characterization of different ESBL was necessary to understand the spread of resistant bacteria.

**Conclusion**

This study demonstrated the high occurrence of ESBL produced by *E. coli* isolated in two hospitals of Niamey. All the age groups were concerned with high resistances of the ESBL-producing isolates to antibiotics classes. Urine and stool samples had a higher prevalence of ESBL producers in Niamey. Hence, it was necessary to develop a nosocomial infection control and antimicrobial surveillance system in all health centers in order to avoid emergence and clonal spread of ESBL *E. coli*.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

This study was supported by “CAMPUS FRANCE”, “CRSBN/UFR-SVT” (Université Ouaga I Pr Joseph Ki ZERBO.), the “Hôpital National de Niamey” and the “Hôpital National Lamordé” of Niamey. The authors thank the laboratory technician for their assistance in collection and processing samples. They also thank the “Université ABDOU MOUMONI de Niamey” for his collaboration.

**REFERENCES**


Guessendi N, Kacou-N’douba A, Gbonon V, Yapi D, Ekaza E, Dosso
M. Courvalin P. (2008). Prevalence and resistance profile of beta-

lactamase enterobacteria A Broad spectrum (ESBL) in Abidjan Côte

Isendahl J, Turje-Rogaucka I, Maguba C, Rodrigues A, Giske CG,

Pontus Naucloer (2012). Fecal Carriage of ESBL-Producing E. coli

and K. pneumoniae in Children in Guinea-Bissau: A Hospital-Based


Kanwar N, Scott HM, Norby B, Lonneragan GH, Vinasco J, McGowan M,


Effects of ceftriaxone and chlorotetracycline treatment strategies on

antimicrobial susceptibility and on tet (A), tet (B), and bla CMY-2

resistance genes among E. coli isolated from the feces of feedlot


Transferable resistance to cefotaxime, cefoxitin, cefamandole and

cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia


Lo S, Ka R, Ba Diallo A, Diallo OF, Diagne R, Dia ML, Sarr AM, Sow AI

(2014). Sensitivity to antibiotics of enterobacteriaceae isolated from

urine at the Regional Hospital Center of Saint Louis (Senegal) from


Manyahi J, Matee Mi, Majigo M, Moyo S, Mshana SE, Lyamuuya EF

(2014). Predominance of multi-drug resistant bacterial pathogens

causing surgical site infections in Muhimbili National Hospital.

Tanzania. BMC Res. Notes 7(1) 500.


antimicrobial treatment in nosocomial infections-the clinical challenges.


Mshana SE, Falgenhauer L, Mirambo MM, Mushir MF, Moremi N, Julius


blaCTX-M-15 in varieties of Escherichia coli genotypes from humans in


Odumosu BT, Akintimehin AR (2015). Occurrence of Extended

Spectrum Beta Lactamase Producing Enterobacteriaceae Isolates in


Onwuzoebi AI, Orok FE (2015). Extended spectrum beta-lactamase

producing uropathogens in asymptomatic pregnant women attending


Ouedraogo AS, Sanou M, Kiasou A, Sanou S, Solaré H, Kabore F,

Poda A, Abergak S, Bouzini N, Sano I, Nacro B, Sangaré L, Carrière C,


High prevalence of extended-spectrum ß-lactamase producing

enterobacteriaceae among clinical isolates in Burkina Faso. BMC Infect.

Dis. 16(1):326.

Patterson JE (2001). Antibiotic utilization: is there an effect on


Quinet B, Mitranche D, Salauze B, Carbonne A, Bingen E, Fournier S,

Moissenet D, Vu-Thien H (2010). Description and investigation of a

nosocomial epidemic of colonization and Escherichia coli infections

producing extended-spectrum beta-lactamase in a neonatal unit.