ANTIMICROBIAL SUSCEPTIBILITY OF EXTENDED-SPECTRUM BETALACTAMASE PRODUCING ENTEROBACTERIAEAE CAUSING URINARY TRACT INFECTIONS IN OUAGADOUGOU, BURKINA FASO

D.S. KPODA1,2, N. GUINSSEND, N. S. SOMDA1, A. AJAYI1, J.I. BONKOUNGOU1,2, F. KONAN1, M. B. OUATTARA1, M. SOMDA1, J. SIMPORE1, R. OUEDRAOGO1, M. K. DRABO1, L. SANGARE1, M. DOSSO1, A. S. TRAORE1

1 Université Ouaga 1 Pr Joseph Ki-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso, 2 Laboratoire National de Santé Publique, 09 BP 24, Ouagadougou 09, Burkina Faso, 3 Centre Hospitalier Universitaire Yalgado Ouedraogo, 03 BP 7021, Ouagadougou 03, Burkina Faso, 4 Centre Hospitalier Universitaire Pédagogique Charles De Gaulle, 01 BP 1198 Ouagadougou 01, Ouagadougou, Burkina Faso, 5 Hôpital Saint Camille, 09 BP 444, Ouagadougou 09, Burkina Faso, 6 Institut Pasteur de Côte d’Ivoire, 01 BP 490, Abidjan 01, Côte d’Ivoire, 6 Department of Microbiology, University of Lagos Akoka, Nigeria

Correspondence : KPODADissivielStéphane, Tel : 00226 70077357 ; podadiissin@yahoo.fr

ABSTRACT

Objective: To determine the frequency of the extended-spectrum beta lactamase producing Enterobacteriaceae (ESBL) and other antibiotic resistant bacteria in urinary tract isolates.

Study Design: prospective and experimental study.

Methodology: Place and duration of study : Yalgado Ouedraogo University Hospital Center, Charles De Gaulle Pediatric Hospital Center, Saint Camille Hospital and National Public Health Laboratory, Ouagadougou, from November 2014 to October 2015. All Enterobacteriaceae strains isolated from urinary samples of patients were identified using API 20E chemical gallery (BioMerieux, France). All strains were subjected to an array of 14 antibiotics to study their drug susceptibility by using Kirby-Bauer disk diffusion method. Detection of ESBL was carried out by double disk diffusion technique. Statistical analysis was performed by Microsoft Excel and Anova one-way GraphPad Prism version 5.01. Chi-square (χ2) test was used to determine significance. A p< 0.05 was considered to be statistically significant.

Results: A total of 324 isolates of Enterobacteriaceae were identified during the study period, including 211 (65%) E. coli, 75 (23%) Klebsiella spp., 18 (6%) Enterobacter spp., 11 (3%) Proteus spp., 5 (2%) Citrobacter spp., Serratia spp. 3 (1%). All the clinical isolates were susceptible to imipenem. Resistance to amikacin was 14% (45/324); gentamicin 54% (175/324); tobramycin 58% (187/324); nalidixic acid 72% (234/324), ciprofloxacin 63% (204/324) and to cotrimoxazole 83% (269/324). The overall rate of the EBSL producing strains was 35% (114/324). Their susceptibility to antibiotics was (imipenem, amikacin, cefoxitin and fosfomycin) 100% (114/114), 93% (106/114), 74% (84/114) and 84% (96/114) respectively. ESBL positivity within individual organisms was highest in Escherichia coli 64% (73/324) followed by Klebsiella spp. 28% (32/114), Enterobacter spp. 3% (4/124), Proteus spp. and Citrobacter spp. 2% (2/124).

Conclusion: The results showed a high frequency of Enterobacteriaceae, especially Escherichia coli and Klebsiella spp. The data points to the need of routine detection and surveillance of ESBL producing bacteria in Burkina Faso.

Keywords: Antimicrobial susceptibility, Enterobacteriaceae, Urine, Burkina Faso

SENSIBILITE DES ENTEROBACTERIES PRODUCTRICES DE BETA-LACTAMASES A SPECTRE ELARGI ISOLEES DES INFECTIONS URINAIRES, OUAGADOUGOU, BURKINA FASO

Résumé

Objectif : Déterminer la fréquence des entérobactéries productrices de bêta-lactamasas à spectre élargi (BLSE) et la résistance aux autres antibiotiques utilisés dans le traitement des infections urinaires.

Type de l’étude : Etude prospective et expérimentale

Méthodologie : Lieu et période : Centre Hospitalier Universitaire Yalgado Ouedraogo, Centre Hospitalier Universitaire Pédagogique Charles De Gaulle, Hôpital Saint Camille et Laboratoire National de Santé Publique, Ouagadougou, de Novembre 2014 à Octobre 2015. Toutes les entérobactéries isolées des urines de patients et identifiées sur galerie API 20E (BioMérieux, France), Quatorze (14) antibiotiques ont été utilisés pour tester la sensibilité des souches cliniques par la méthode de diffusion des disques selon Kirby-Bauer. La détection des souches productrices de BLSE a été faite en utilisant la technique de test à double synergie. Le logiciel Excel et Anova one-way GraphPad Prism version 5.01 ont été utilisés pour l’analyse statistique et le test de χ2 au seuil de p < 0.05 était considéré statistiquement significatif.

Résultats : Un total de 324 souches d’Enterobacteriaceae ont été collectées durant la période d’étude composées de 211 (65%) souches de E. coli, 75 (23%) Klebsiella spp., 18 (6%) Enterobacter spp., 11 (3%) Proteus spp., 5 (2%) Citrobacter spp., Serratia spp. 3 (1%). Toutes les entérobactéries productrices de BLSE étaient sensibles à l’imipénème. La résistance à l’amikacine était de 14% (45/324); la gentamicine était de 54% (175/324); la tobramycine était de 58% (187/324); l’acide nalidixique était de 72% (234/324), la ciprofloxacine était de 63% (204/324) et la co-trimoxazole était de 83% (269/324). La fréquence des souches productrices de BLSE était de 35% (114/324). Leurs taux de résistance aux autres antibiotiques étaient de 100% (114/114), 93% (106/114), 74% (84/114) et 84% (96/114) respectivement à l’amikacine, à la cefoxitine et à la fosfomycine. La fréquence des souches productrices de BLSE par espèce établie chez Enterobacteriaceae était de 64% (73/324) suivie de Klebsiella spp. 28% (32/114), Enterobacter spp. 3% (4/124), Proteus spp. et Citrobacter spp. 2% (2/124).
INTRODUCTION

Urinary tract infections (UTI) is one of the most common infectious diseases ranking next to upper respiratory tract infection, it is an important cause of morbidity and mortality in human. Infected urine, renal calculi, obstructive uropathy, vesico ureteral reflux and avoiding disorders can lead to urinary stasis and may predispose to the development of UTIs and complications [1]. It has been estimated that nearly 10% of the human population will experience an UTI during the life time [2, 3, 4]. Resistance to commonly-prescribed antibiotics is an expanding global problem and has been observed in both developed and developing countries [5, 6, 7, 8]. Enterobacteriaceae are the major causative organisms of UTIs and are responsible for more than 81% of UTIs cases. Escherichia coli is the most prevalent causative organisms of UTIs and is solely responsible for more than 69% of the infections [1, 9, 10]. Bacterial resistance to antibiotics has emerged even to newer, more-potent antibacterial agents [11]. A number of epidemics have recently occurred caused by multiple resistant organisms [12, 13].

In Burkina Faso, UTIs due to Enterobacteriaceae are common and represent a frequent cause of morbidity in outpatients as well as a frequent cause of nosocomial infections in many hospitals. Most infections are treated on an empirical basis. Clinical experience has indicated the presence of numerous cases of infection resistant to conventional antibiotics therapy. Microbial resistance rates to commonly prescribed antibiotics have increased recently. Updated knowledge of urinary tract infections Enterobacteriaceae, the frequency of ESBL strains and the susceptibility patterns to other antibiotics is important for the proper selection and use of antibiotic and for the development of an appropriate prescribing policy. The aim of this study was to determine the frequency of ESBL strains and the susceptibility patterns of other antibiotic resistant bacteria of clinical importance responsible for urinary tract infections in Ouagadougou, Burkina Faso.

Materiel and methods

Study population and settings

This study was an experimental study of beta-lactam and other antibiotics resistance expression of Enterobacteriaceae. The socio demographic data and Enterobacteriaceae strains were obtained from patients who came for an etiological diagnosis for bacterial infection from November 2014 to October 2015.

The Enterobacteriaceae strains were obtained from the following 3 health centers in Ouagadougou: Yalgado Ouedraogo University Hospital (CHU-YO), the largest public medical institution, Charles De Gaulle Pediatric University Hospital (CHUP-CDG), the referral public pediatric hospital with 120 beds and Saint Camille Hospital (HOSCO), the confessional hospital.

All Enterobacteriaceae strains collected at these 3 different sites were transported to the LNSP Bacteriology-Virology Laboratory for Enterobacteriaceae investigation. Strains identification was performed using API 20 E gallery (Biomerieux, Marcy-L’Étoile, France) after 24 hours incubation at 37°C. The isolated and identified strains were seeded on Mueller-Hinton (MH) agar for 18 to 24 hours in order to have young and pure colonies. All clinical isolates were stored at -30°C for future investigations at Institut Pasteur de Côte d’Ivoire (IPCI).

Antibiotic Susceptibility Testing

All isolates were tested for susceptibility to 14 different antimicrobial agents using the disk diffusion method on Mueller-Hinton agar (BioRad, France) following the European Committee on Antimicrobial Susceptibility Instructions (EUCAST) guidelines (CASFM/EUCAST, 2016). E. coli ATCC 25922 and ATCC 35218 were used as a control. The antimicrobial disks (Bio-Rad, France) used were: gentamicin (15 µg), amikacin (30 µg), tobramycin (15 µg), Amoxicillin (25 µg), amoxicillin + clavulanic acid (20 µg), cefepime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), cotrimoxazole (1.25 / 23.75 µg), nalidixic acid (30 µg) and fosfomycin (50 µg).

ESBL strains screening

The double disk synergy tests (DDST) to detect ESBL-producing isolates was performed on Mueller-Hinton agar, placing cefepime, cefotaxim, and ceftriaxone discs around the amoxicillin + clavulanic acid disk at a distance of 3 cm from the center to center, according to the European Committee on Antimicrobial Susceptibility Instructions (EUCAST) guidelines (CASFM/EUCAST, 2016) [14].

Determination of multiple antibiotic resistance index

Multiple antibiotic resistance index (MAR) was determined using the formula $\text{MAR} = \frac{y}{x}$, where $x$ was the number of antibiotics to which test isolate displayed resistance and $y$ is the total number of antibiotics to which the test organism has been evaluated [15].

Statistical analysis

Statistical analysis was performed with Excel and ANOVA one-way GraphPad Prism version 5.01. Chi-square ($\chi^2$) test was used to calculate probabilities and determine significance. A p-value of less than 0.05 was considered to be statistically significant ($p<0.05$).
RESULTS
Characteristic of the study population and distribution of strains
(162 men and 162 women) in 3 hospital centers in Ouagadougou, Burkina Faso. The mean age of these patients was 33.7 ± 2 years and the sex ratio 1.7.

Out of 324 Enterobacteriaceae isolates, 211 (65%) were E. coli, 75 (23%) Klebsiella spp., 18 (6%) Enterobacter spp., 11 (3%) Proteus spp., 5 (2%) Citrobacter spp., Serratia spp., 3 (1%) as shown in Figure 1.

The most frequent urinary Enterobacteriaceae isolated were E. coli, Klebsiella species, and Enterobacter species. Other urinary tract bacteria were isolated in relatively few number. These included Citrobacter spp., Proteus spp., Serratia spp. and Providencia spp.

A total of 324 clinical isolates belonging to Enterobacteriaceae family were obtained from patients.

TABLE 1: AGE WISE DISTRIBUTION OF ESBL-PRODUCING ENTEROBACTERIACEAE IN DIFFERENT AGE GROUPS

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Total number of isolates (n=324)</th>
<th>ESBL positive (n=114)</th>
<th>Percent ESBL positive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15 years</td>
<td>67</td>
<td>28</td>
<td>42%</td>
<td>0.5321*</td>
</tr>
<tr>
<td>15-45 years</td>
<td>135</td>
<td>44</td>
<td>33%</td>
<td></td>
</tr>
<tr>
<td>46-60 years</td>
<td>38</td>
<td>12</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>84</td>
<td>30</td>
<td>36%</td>
<td></td>
</tr>
</tbody>
</table>

*p = 0.5321, no significant statistically

TABLE 2: RESISTANCE RATE OF 44 ESBL-PRODUCING ENTEROBACTERIACEAE TO ANTIBIOTICS IN OUAGADOUGOU, BURKINA FASO

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance rate</th>
<th>1+R(%)</th>
<th>S(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td></td>
<td>37(84)</td>
<td>7(16)</td>
</tr>
<tr>
<td>Amikacin</td>
<td></td>
<td>1(2)</td>
<td>43(98)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td></td>
<td>36(82)</td>
<td>8(18)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td></td>
<td>44(100)</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin/clavulanicid</td>
<td></td>
<td>9(20)</td>
<td>35(80)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td></td>
<td>8(18)</td>
<td>36(82)</td>
</tr>
<tr>
<td>Ceftriaxin</td>
<td></td>
<td>42(95)</td>
<td>2(5)</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td></td>
<td>43(98)</td>
<td>1(2)</td>
</tr>
<tr>
<td>Cefepime</td>
<td></td>
<td>42(95)</td>
<td>2(5)</td>
</tr>
<tr>
<td>Imipenem</td>
<td></td>
<td>0</td>
<td>44(100)</td>
</tr>
<tr>
<td>Nalidixicacid</td>
<td></td>
<td>38(86)</td>
<td>6(14)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>40(91)</td>
<td>4(9)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td></td>
<td>44(100)</td>
<td>0</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td></td>
<td>12(27)</td>
<td>32(73)</td>
</tr>
</tbody>
</table>

Antibiotics susceptibility test
The antibiotic resistance profile of the urinary tract isolates is shown in figure 3. All the strains isolated showed high resistance to amikacin 88% (286/324), amoxicillin/clavulanicid 38% (124/324), gentamicin 54% (175/324), tobramycin 58% (187/324), ciprofloxacin 63% (204/324), nalidixic acid 72% (234/324) and to cotrimoxazole 83% (269/324).
However, there was low resistance toamikacin 14% (45/324)and fosfomycin 18% (57/324) (Figure 3). All strains were susceptible to toimipenem. Escherichia coli and Klebsiella pneumonia showed high rate of resistance to gentamicin, tobramycin, amoxicillin and all the 3rd generation cephalosporins (Figure 3).
Occurrence of ESBL-producing Enterobacteriaceae

The overall rate of ESBL-producing Enterobacteriaceae in UTIs in both male and female patients was found to be 35% (114/324). The frequency of ESBL strains within individual organism group was E. coli 64% (73/324), Klebsiella spp. 28% (32/324), Enterobacterspp. 3% (4/324), Proteus spp. and Citrobacter spp. 2% (2/324) as shown in Figure 2. E. coli strains producing ESBL were higher than those of the other species.

Considering the clinical isolates origin, ESBL strains rate was 24% (78/324) at CHU-YO, 4% (13/324) at Saint Camille Hospital (HOSCO) and 7% (23/324) at CHUP-CGD. The mean age of patients with ESBL producing organisms was 32.38 years compared to 27.66 years for patients with non-ESBL strains. ESBL production among various age groups ranged from 32% to 42%. However, there was no statistically significant difference between the age groups (Table 1) with respect to ESBL production ($p = 5321$). ESBL producing strains showed high susceptibility rates with imipenem 100% (114/114), amikacin 93% (106/114), cefoxitin 74% (84/114) and fosfomycin 84% (96/114) (Table 2).

**DISCUSSION**

In this study, we investigated the frequency of ESBL production by Enterobacteriaceae isolates in three hospitals in Ouagadougou, Burkina Faso. A total of 324 clinical isolates belonging to Enterobacteriaceae family were obtained from urine samples from these 3 centers in Ouagadougou, Burkina Faso from November 2014 to October 2015. These bacteriawere isolated from 162 females and 162 males. The mean age was 33.7 ± 2 years and the sex ratio 1.7.

UTIs are the most common nosocomial infections, comprising about 35% of such occurrences in both hospitals and nursing homes [16]. More than 95% of UTIs are caused by a single bacterial specie and Escherichia coli is by far the most frequent infecting organism in acute infections[17]. The spectrum of strains isolated from urinary samples in this study is not different from those reported in literature.

In this study, all of the 324 Enterobacteriaceae strains, Escherichia coli predominated followed by Klebsiella spp. and Enterobacterspp. Citrobacterspp. and Proteus spp. were less significant. In several others studies in Burkina Faso, Sudan, India, Pakistan and Ivory Coast [18,19,20,21,27] the authors reported that Escherichia coli was also the most common isolate followed by Klebsiella spp., Enterobacterspp. and Proteus spp.

E. coli remains an essential bacterium in urinary tract infections. Wilson and Gaido [28] also reported that E. coli is the major bacterial etiology of urinary tract infections. This corroborates the high frequency of E. coli isolates reported in our study. The antibiotic susceptibility tests revealed in our study high levels of resistance to certain molecules used in common practice. Our study also revealed that 88% (286/324) of isolates were resistant to amoxicillin, which may be due to the frequent possible misuse of this antibiotic. The resistance rate to amoxicillin + clavulanic acid was 38% (124/324). In contrast with the report of Adonis-Koffi et al. [30] in Ivory Coast who obtained a resistance rate of 68% to amoxicillin + clavulanic acid.

The high level resistance of the Enterobacteriaceae 3rd and 4th generation cephalosporin antibiotics were also observed (cefepim showed 58%, cefotaxim 59% and ceftriax 63%). The most active molecule among the aminoglycosideantibiotic family was amikacin and the resistance rate of strain was 14%; BonniCisse et al. [27] did observe the same trend in Ivory Coast. Other aminoglycoside antibiotics tested were gentamicin and tobramycin with resistance rates of 54% and 58% respectively. Leski et al. [29] in Sierra Leone however reported a high percentage (73%) resistance to gentamicin. Furthermore, the clinical strains showed very high resistance to ciprofloxacin and nalidixic acid and the
resistance rates were 63% (204/324) and 72% (234/324) respectively. Results of our study is similar to that of Guessennd et al. [25] conducted from 2005 to 2006 in which the resistancerate of quinolones was 71% to ciprofloxacin and 77% to nalidixic acid. Fosfomycin had the best activity among antibiotics used, where strains showed 82% susceptibility. We observed high resistance rate to cotrimoxazole with 83% (269/324), this is in line with reports of BonniCisse et al.[27] and Guessennd et al. [25] in which they reported 76% and 91% resistance to cotrimoxazole respectively in Ivory Coast. These high rates could be attributed to the use of cotrimoxazole in chemoprophylaxis in the treatment of opportunistic infections in immunocompromised patients.

The resistance to antibiotics and ESBL production spares no country in the world. The frequencies of ESBL producing strains vary from country to country and from species to species in the world [22, 23]. Studies carried out by Ouedraogo et al. [24] in Burkina Faso, Mohanty et al. [26] in India reported the overall prevalence of 58% and 69% ESBL producing bacteria respectively. Our study showed a prevalence of 35% ESBL producing strain which is relatively high and is almost similar to other data which showed high prevalence.

This resistance is often associated with antibiotics such as aminoglycosides and quinolones (Table 2). The emergence of these Bacteria Multi-Resistance (BMRs) in hospitals of Ouagadougou could lead to therapeutic failures despite the administration of aminoglycosides and 3rd generation cephalosporins. While the issue of antibiotic resistance has long been considered a concern in hospitals for nosocomial infections, in recent years the problem has been extended to include community medicine [32]. One of the reasons is the high consumption of antibiotics in human medicine, the illicit sale of antibiotics on the streets, and the increased movement of colonized or infected patients between hospitals and community settings [32].

However, the good susceptibility of the ESBL-producing Enterobacteriaceae to imipenem (100%) (114/114) and also to amikacin (93%) (106/114), cefoxitin (74%) (84/114) and fosfomycin (94%) (96/114) makes them the molecules of choice in alone treatment or in combination of other antibiotics (Table 2).

These results should act as an impetus for the establishment of antibiotic control policies. Indeed, currently there is no restriction in the use of antibiotics in Burkina Faso.

Conclusion
UTI antibiotics therapy should be guided by antimicrobial susceptibility as increasing numbers of urinary isolates are developing resistance to commonly use antibiotics. Increasing antimicrobial resistance of Enterobacteriaceae has led to reconsideration of traditional treatment of recommendations in many areas. This experimental study should be followed by several studies on antimicrobial resistance among patients in Burkina Faso hospitals and other regions of West Africa as there is relatively few data concerning the antibiotic susceptibility spectrum of bacteria isolated from patients with UTIs.

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