Molecular Characterization of Carbapenemase-Producing Enterobacteria in Children with Diarrhea in Rural Burkina Faso

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Abstract

Background and objective: In recent years, carbapenemase-producing Enterobacteriales (CPE) resistance to antibiotics has dramatically increased leading to limitations of their treatment options. In the present study, we investigated the occurrence of carbapenemase-producing Escherichia coli and Salmonella in rural Burkina Faso.

Materials and methods: Salmonella isolates were serotyped according to the Kauffman White scheme. Diarrheagenic Escherichia coli (DEC) strains was identified using 16-plex Polymerase Chain Reaction (PCR), whereas antibiotic susceptibility was realized using the disk diffusion method. Furthermore, multiplex PCR assays were carried out using oligonucleotides to detect the presence of genes of the blaTEM, blaSHV, blaKPC, blaOXA, blaVIM, blaIMI, blaNDM, and blaIMP types in all E. coli and Salmonella strains.

Results: The study highlighted high resistance rates of the identified bacteria to common antibiotics. Likewise, two strains of E. coli were impenem resistant with carbapenemase-encoding genes. The genes detected were Klebsiella pneumoniae carbapenemase (KPC), Verona integrin-encoded metallo-β-lactamase (VIM) and Imipenemase (IMP-2) reaching a rate of 40% each in E. coli strains. However, no Salmonella carbapenemases blaKPC, blaVIM or blaIMP were detected.

Conclusion: This study showed that for a real-time infection control and prompt application of antimicrobial chemotherapy, characterization of carbapenemase-producing Enterobacteriales in patients is crucial.

Keywords: Antibiotics, Carbapenemase-Producing Enterobacteriales, children, Burkina Faso.

Conflict of Interest: The authors stipulate that they have no conflict of interest in regard to this study.
INTRODUCTION

New antimicrobial resistance mechanisms are emerging and spreading globally, hampering our ability to effectively treat common infectious diseases. This has extended illness, disability and increased death rates. As a result, antimicrobial resistance represents a major challenge for public health worldwide. One of the most worrying threats is the emergence and rapid dissemination of carbapenem resistant Gram-negative bacteria, following the spread of carbapenemase-producing Enterobacteriales (CPE) [2, 3]. In bacteria of animal and human origins, beta-lactam resistance, which includes resistance to extended-spectrum beta-lactams, has now been increasingly observed [4]. In Enterobacteriaceae, the carbapenemases have been previously classified into the three following classes: class A [ie K. pneumoniae carbapenemase (KPC) enzymes], class B [ie metallo-beta-lactamases (MBL)] including New Delhi metallo-beta-lactamase (NDM), Verona integrin-encoded metallo-beta-lactamase (VIM), imipenemase (IMP), and Class D [ie oxacillinase (OXA)-48 and related variants] [5-7]. Enterobacteriaceae can be resistant to carbapenems through intrinsic or acquired mechanisms. The chromosomally-encoded mechanisms can occur by (a) production of chromosomal carbapenemases from the group of class A serine carbapenemases [8] or (b) efflux pumps or (c) reduction in outer membrane permeability through porin loss [9]. The acquired resistance is a plasmid-mediated mechanism through which the mobile carbapenemases are easily transmitted between bacteria [10].

The recent spread of CPE is one of the major public health threats worldwide [11] because carbapenems are among the mainstay of therapy for treating severe infections directly related to multidrug-resistant bacteria producing extended-spectrum beta-lactamases (ESBLs) [12]. Carbapenemases are defined as beta-lactamases that hydrolyze almost all beta-lactam antibiotics. According to some recent studies, the most prevalent carbapenemases in Enterobacteriaceae are blaKPC (Ambler class A), blaOXA, blaNDM, blaVIM (class B) and blaOXA-48 like (class D) [11, 12]. Although several studies reported the occurrence of carbapenemases producing bacteria in Africa [13], to our best knowledge, no study focusing on the molecular characterization of Carbapenemase-producing E. coli and Salmonella has been undertaken in Burkina Faso. Therefore, the objective of the present study was to carry out a molecular characterization of carbapenemase-Producing Escherichia coli and Salmonella isolates recovered from children in two rural hospitals in Burkina Faso.

MATERIAL AND METHODS

Study design and population

A prospective cross sectional study was conducted to determine the serotypes and antimicrobial susceptibility of diarrheagenic E. coli and Salmonella among children visiting hospitals in rural settings of Burkina Faso.

Settings

This study was conducted in north (Gourcy, distance 140 km) and western (Boromo, distance 185 km) of the capital Ouagadougou, Burkina Faso (Fig. 1). The main sources of income in these rural settings are subsistence farming, animal husbandry and small scale trade.

Specimen collection

Two hundred and seventy five (275) faecal samples were taken in 2009-2010 by trained health staff personnel using a swab transport system (M40 transystemAmies agar gel without charcoal; Copan Italia Spa, Brescia, Italy) and transported to laboratory within 24h of their collection for analysis. A questionnaire was used to collect demographic information (e.g., age and sex) of each patient.

Salmonella isolation and serotyping

Selenite broth (Emapol, Pologne) was used for the enrichment of specimens followed by an incubation at 37°C for 18h. Subsequently, samples were cultured on Heekto Enteric agar (Liofilchim, Italy) and incubated at 37°C for 24h. The identity of typical-looking Salmonella colonies on Heekto was examined by using orthonitrphenyl-β-D-galactopyranoside (ONPG), citrate, mannitol, lysine decarboxylase tests and the Klger Haja medium (Liofilchim, Italy). Finally the isolates were confirmed by API 20E (BioMérieux, Marcy l’Etoile, France). All Salmonella isolates were serotyped by the Salmonella Reference Laboratory. Isolates were serotyped with the somatic O and flagellar H anti-sera according to the Kaufman White scheme [14].

E. coli isolation and identification

Stool samples were plated on eosin methylene blue agar (Liofilchim, Italy), and the plates were incubated at +37°C for 18–24 h. After incubation, the suspected colonies were selected and streaked onto Mueller Hinton agar plate (Liofilchim, Italy). Confirmation was carried out by a biochemical microbiology method based on negative urinease (Bio-Rad, France), negative citrate (Liofilchim, Italy), positive indole (Bio-Rad, France), positive lactose (Liofilchim, Italy), and positive orthonitrphenyl-β-D-galactopyranoside (ONPG) (BioMérieux, France). E. coli strains isolated were confirmed by API 20E (bioMérieux, France).

The 16-plex PCR was used to detect simultaneously 16 genes from the five main pathogroups of E. coli (enterohemorrhagic E. coli: EHEC, enteropathogenic E. coli: EPEC, enteraggregative E. coli: EAEC, enteroinvasive E. coli: EIPEC and enterotoxigenic E. coli: ETEC) as described by Antikainen et al. [15]. The genes investigated and primers used are listed in Table 1 [15-17].

Antimicrobial susceptibility testing

Antibiotic susceptibility was determined on Mueller–Hinton agar (Liofilchim, Italy) using the standard disc diffusion procedure as described by the European Committee of Antimicrobial Susceptibility Testing (EUCAST) [18]. Nineteen antibiotics belonging to 7 different families (Table 2) were tested: amoxicillin (25 µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefixime (10 µg), piperacillin (75 µg), piperacillin-tazobactam (100 +10 µg), imipenem (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (1.25 ± 23.75 µg), aztreonam (30 µg), colistin sulfate (50 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), gentamicin (15 µg), netilmicin (10 µg) and tobramycin (10 µg) (Bio-Rad, France). The diameters of the antibiotic susceptibility halos were recorded according to the recommendations of EUCAST. Intermediate (I) susceptibility of pathogens was classified as resistant (R).

Detection of antibiotic resistance genes

The strains that were resistant to imipenem and amoxicillin-clavulanate were PCR screened. DNA for PCR analysis was extracted from the isolates using the heat lysis method [19]. A loopful of bacterial growth from Mueller Hinton agar (Liofilchim, Italy) plate was suspended in 1 ml of sterile water, and the mixture was boiled for 10 min at +100 °C and
centrifuged for 10 min at 12000 rpm at +4°C. The obtained supernatant was collected and used for PCR reactions. Multiplex PCR assays were carried out using oligonucleotides (Table 3) to detect the presence of genes of the blaKPC, blaVIM, blaIMP, blaTEM, blaOXA, and blaCTX-M types in all E. coli and Salmonella strains of the study.

About 2.5 μl of supernatant were added to 22.5 μl reaction mixture containing 5U of Taq DNA polymerase (Accu Power, Korea), deoxyribonucleic triphosphate (10 mM), buffer GC (10X), MgCl2 (25 mM), and PCR primers (10 μM). We performed PCR conditions as followed: 5min at +94°C, followed by 35 amplification cycles of +94°C for 30s, 59±4 °C for 60s and +72°C for 60s with a final extension of +72°C for 10min on a thermal cycler (Gene Amp 9700, Applied Biosystems). Our reaction products were separated by electrophoresis in (1.5% weight/volume) agarose gel, stained with a Redsafe solution (Prolabo, France) and visualized under ultraviolet (UV) light (Gel Logic 200).

Ethics approval and consent to participate

Permission to conduct the study was obtained from the hospital authorities of Burkina Faso, and informed verbal consent was obtained from the parents/guardians of every child before sample collection. The National Ethical Committee (s) of Burkina Faso (N° 39) approved the study protocol.

RESULTS

Bacterial isolates

Of the 275 stool samples, five isolates were confirmed as E. coli strains: 3 EAE, and 2 atypical EPEC. Nine Salmonella isolates were detected belonging to the following serotypes: Salmonella Poona, S. Typhimurium, S. Oualam, S. Virchow, S. Duisburg and S. Hvittingfoss.

Antimicrobial resistance

The five E. coli strains were resistant to amoxicillin-clavulanic acid, amoxicillin and tetracycline (5/5); four strains were resistant to trimetoprim-sulfamethoxaxol, colistin-sulfate and piperacillin (4/5); three strains showed resistance to cefotaxim, ceftriaxone, aztreonam, ceftaxime and cepfine (3/5). Whereas, resistance to chloramphenicol and also to imipenem was shown in two of isolates (2/5).

All the nine Salmonella strains were resistant to amoxicillin (9/9), eight were resistant to amoxicillin-clavulanic acid (8/9), six were resistant to tetracycline, ceftaxime and cepfine (6/9); five were resistant to ceftriaxone, cefotaxim and colistin-sulfate (5/9). Less than five strains were resistant to aztreonam, trimetoprim-sulfamethoxaxol and piperacillin while no resistance was shown to imipenem (Fig. 2).

Carbapenemase genes

The results of PCR amplification of blaKPC, blaVIM, blaIMP, blaTEM, blaoxa, blCTX-M and blaCTX-M genes in E. coli and Salmonella isolates are given in table 4. blaKPC gene (Fig. 4), blaoxa gene and blaimp gene were carried by two E. coli isolates which were resistant to imipenem. blaoxa gene (Fig. 3) was found in all the five E. coli strains (5/5) and in three of the Salmonella strains (3/9). blCTX-M gene (Fig. 4) was shown in one E. coli strain and in none of the Salmonella. blTEM and blaoxa were reported in none of the E. coli and Salmonella strains.

All E. coli strains were isolated in children of one year old while the sex distribution was 4/5 for male and 1/5 for female. The Salmonella were reported in children of one year old (4/9), two years old (2/9) and three years old (3/9) with a sex distribution of 5/9 for male and 4/9 for female (table 3).

Figure 1: Map of Burkina Faso. In square black dots = Gourcy and Boromo where the study was conducted.

https://commons.wikimedia.org/wiki/File:Burkina_Faso_administrative_divisions_-_de_-_monochrome.svg
**Figure 2:** Resistance to individual antimicrobial among E. coli and Salmonella strains.

Figure legend: AMC = Amoxicillin-clavulanic acid, AMX = Amoxicillin, CTX = Cefotaxime, ATM = Aztreonam, IPM = Imipenem, CRO = Ceftriaxone, FEP = Cefepime, CFM = Cefixime, TET = Tetracycline, CHL = Chloramphenicol, SXT = Trimethoprim-sulfamethoxazole, CIP = Ciprofloxacin, NAL = nalidixic acid, CST = Colistin sulfate, GMI = Gentamicin, TZP = Piperacillin-tazobactam, PIP = Piperacillin, NTM = Netilmicin, TMN = Tobramycin, I = Intermediate, R = Resistant.

**Figure 3:** blaOXA gene detected in E. coli.

Figure legend: Lane M: hyperlader (100 bp), Lane 1: blaOXA positive control (813 pb), Lane 2-8: positive samples for blaOXA gene (813 pb), Lane T: negative sample.
**Figure 4.** blaCTX-M and blaKPC genes detected in E. coli.

Figure legend: Lane M = hyperlader 100 bp; Lane A = blaCTX-M positive control (544 pb), Lane B, D et E = positive samples for blaCTX-M gene (544 pb), Lane B, C, D et E = positive samples for blaKPC gene (300 pb), T: negative sample.

**Table 1**: Oligonucleotides primers used for multiplex PCR reaction

| Pathotype   | Target gene | Primer sequence (5’ to 3’) | Size (bp) | [C] (μM) | Reference |
|-------------|-------------|----------------------------|-----------|----------|-----------|
| Typical EPEC | bfpB        | MP3-bfpB-F: GACACCTCATTGCCTGAAGTCC | 910       | 0.1      | [16]      |
|             |             | MP3-bfpB-R:CCAGAACACCTCGTTATGC |           | 0.1      |           |
| EHEC and EPEC | eaeA       | eae-F: TCAATGCAGTTCCCTATCATTT | 482       | 0.1      | [16]      |
|             |             | eae-R: GTAAAGCTTACCTACCCAACTTG |           | 0.1      |           |
|             | escV        | MP3-escV-F: ATTCTGGCTTCTTCTTATTTGCTG | 544      | 0.4      | [16]      |
|             |             | MP3-escV-R: CGTCCCTTTTACAAACTTCCTCAG |           | 0.4      |           |
|             | Ent         | ent-F: TGGGCTAAAAGAAGACACACTG | 629       | 0.4      | [16]      |
|             |             | ent-R: CAAGCATTCTGATTATCTCACC |           | 0.4      |           |
| EHEC        | EHEC-hly    | hlyEHEC-F: TTCGGAAGAAGTGAAGACATA | 688       | 0.1      | [15]      |
|             |             | hlyEHEC-R: TCACCAGATTTTCTCATCCTAATG |           | 0.1      |           |
|             | Stx1        | MP4-stx1A-F: GCAGTATTACGTTAGATAGACGC | 244      | 0.2      | [16]      |
|             |             | MP4-stx1A-R: AATGGCAGCTTCTACCCAAATTG |           | 0.2      |           |
|             | Stx2        | MP3-stx2AF: GTTCTTGACATCTTCTGTCTGATTG | 324      | 0.4      | [16]      |
|             |             | MP3-stx2AR: AGGGTGAAAGCTTCTGTGTGAC |           | 0.4      |           |
| EAEC        | astA        | MP-astA-F: TGGGCTAAAAGAAGACACACTG | 102       | 0.4      | [16]      |
|             |             | MP-astA-R: ACGGTAAAGGCTTCTGTGTGAC |           | 0.4      |           |
|             | aggR        | MP2-aggR-F: ACCGAGATTTGCTGTTAAAG | 400       | 0.2      | [16]      |
|             |             | MP2-aggR-R: AATAGAATCTGCTGACGAC |           | 0.2      |           |
|             | Pic         | MP2-pic-F: AGGCCCTTTCCGCAAAGGC | 1111      | 0.2      | [16]      |
|             |             | MP2-pic-R: AAATGCTAGTGAACAGGATTG |           | 0.2      |           |
|             | invE        | MP2-invE-F: CGATAGATTGCAGAAATATATCCCG | 766      | 0.2      | [16]      |
|             |             | MP2-invE-R: CGATCAAGATCTGAGAAATAC |           | 0.2      |           |
| EIEC        | ipaH        | ipaH-F: GAAACCCCTCCTGTCCATCAG | 437       | 0.1      | [17]      |
|             |             | ipaH-R: GACGGCTGACCACTTCTGTGAGTAC |           | 0.1      |           |
| ETEC        | elt         | MP2-elt-F: GACAGAGGTTTCTGCGTTAGTG | 655       | 0.1      | [16]      |
|             |             | MP2-elt-R: CTCTTCAAGATGGTTTCTTGGGAGTC |           | 0.1      |           |
|             | estA        | MP4-estA-F: CCTCTTCTTCTGCACTGAACATCTG | 157      | 0.4      | [16]      |
|             |             | MP4-estA-R: CAGCGAGGATTACAACAAATGGTCAGCCTAC |           | 0.4      |           |
|             | estB        | MP2-estB-F: CTGCTTTTCTACCTTGCTCCTCAG | 171      | 0.2      | [16]      |
|             |             | MP2-estB-R: CGTACAGAGGATTACAACACAC |           | 0.2      |           |
| E. coli     | uidA        | MP2-uidA-F: FATGCGACTCCAGCTGTTTTG | 1487      | 0.2      | [17]      |
|             |             | MP2-uidA-R: AAAGGTGTGGGCTCAATATCAACAGGT |           | 0.2      |           |
### Table 2: Zones of inhibition of the tested antibiotics

| Families                  | Antibiotics                                      | [C]a (µg) | Ø b (mm) | R (Ø<) | S (Ø≥) |
|---------------------------|--------------------------------------------------|-----------|----------|---------|--------|
| Aminopenicillins          | Amoxicillin- clavulanic acid (AMC)               | 30        | 19       | 19      | 19     |
|                           | Amoxicillin (AMX)                                | 25        | 19       | 19      | 19     |
|                           | Piperacillin (PIP)                               | 75        | 17       | 17      | 20     |
|                           | Piperacillin-tazobactam (TZP)                    | 100/10    | 17       | 17      | 20     |
| Cephalosporins C3G        | Ceftriaxone (CRO)                                | 30        | 20       | 23      |        |
|                           | Cefixime (CFM)                                   | 10        | 17       | 17      |        |
| β-lactams                 | Cefotaxime (CTX)                                 | 30        | 17       | 20      |        |
| Cephalosporines C4G       | Cefepime (FEP)                                   | 30        | 21       | 24      |        |
| Monobactam                | Aztreonam (ATM)                                  | 30        | 21       | 24      |        |
| Carbapenemes              | Imipenem (IPM)                                  | 10        | 16       | 22      |        |
| Quinolones                | Nalidixic acid (NAL)                             | 30        | 14       | 19      |        |
| Fluoroquinolones          | Ciprofloxacin (CIP)                              | 5         | 19       | 22      |        |
| Cyclines                  | Tetracycline (TET)                               | 30        | 15       | 18      |        |
| Phenicols                 | Chloramphenicol (CHL)                            | 30        | 17       | 17      |        |
| Sulfamides                | Trimethoprim-sulfamethoxazole (SXT)              | 1.25/23.75| 13       | 16      |        |
| Polymyxines               | Colistin sulfate (CST)                           | 50        | 15       | 15      |        |
|                           | Gentamycin (GM)                                  | 15 (10 IU)| 14       | 17      |        |
| Aminoglycosides           | Netilmicin (NTM)                                 | 10        | 12       | 15      |        |
|                           | Tobramycin (TMN)                                 | 10        | 14       | 17      |        |

### Table 3: Oligonucleotides used to amplify carbapenemases genes

| Primer name | Target genes | Primers sequence (5’to3’) | Size (bp) |
|-------------|--------------|----------------------------|-----------|
| KPC- F      | blaKPC2      | GCT CAG GCG CAA CTG TAA G  | 300       |
| KPC- R      | blaKPC2      | AGC ACA GCG GCA GCA AGA AAG|           |
| VIM- F      | blavim       | CAG ATT GCC GAT GGT GTT TGG| 390       |
| VIM- R      | blavim       | AGG TGG GCC ATT CAG CCA GA |           |
| IMP- F      | blaimp       | GGA ATA GAG TGG CTT AAT TCTC| 232       |
| IMP- R      | blaimp       | GTG ATG CTT CYC CAA YTT CACT|          |
| TEM- F      | blatem       | ATG AGT ATT CAA CAT TTC CG | 1080      |
| TEM- R      | blatem       | CCA ATG CTT ATT CAG TGA GG |          |
| SHV- F      | blashv       | TTA TCT CCC TGT TAG CCA CC | 768       |
| SHV- R      | blashv       | GAT TTG GTT ATT TCG CTC GG |          |
| OXA- F      | blaOXA       | ATG AAA AAC ACA ATA CAT ATC| 813       |
| OXA- R      | blaOXA       | AAT TTA GTG TGT TTA GAA TGG|          |
| CTX-M- F    | blactxM      | ATG TGC AGY ACC AGT AAR GT |          |
| CTX-M- R    | blactxM      | TGG GTR AAR TAR GTS ACC AGA| 544       |
**DISCUSSION**

Increasing numbers of antibiotic-resistant *Enterobacteriaceae* are responsible of serious problems in infection control. This phenomenon also contributes to the global spread of Carbapenemase-producing bacteria becoming therefore especially worrisome. It has been shown that *Enterobacteriaceae* spp., such as *Escherichia coli* and *Klebsiella pneumoniae*, are common human pathogens and asymptomatic colonizers of the human gastrointestinal tract and environmental niches. Our study reported for the first time the occurrence of Carbapenemase-producing *E. coli* and *Salmonella* in children with diarrhea in rural settings of Burkina Faso.

The isolated strains were mainly resistant to amoxicillin-clavulanic acid, amoxicillin, tetracycline, trimetoprim-sulfamethoxazole, colistin-sulfate, piperacillin, ceftazidime, ceftriaxone, aztreonam, cefixime and cefepime (between 60% and 100%). Particularly, two *E. coli* harbored resistance patterns to imipenem. In contrast, no resistance to imipenem was observed in *Salmonella* strains. Similar results concerning *E. coli* resistance to imipenem were reported in India. All isolated *E. coli* and *Salmonella* were 100% sensitive to netilmicin in agreement with data reported in Bangladesh.

In the present study, the most detected carbapenemase was * blaOXA* which was encoded by eight of the isolates (57.14%). In agreement to our result, a recent review showed that OXA-48-like enzymes associated with *Enterobacteriaceae* are one of the most concerning developments in carbapenem resistance in the last decade and are still globally ascending. * blaCTX-M* gene was shown in one *E. coli* strain while * blaTEM* and * blaSIV* were reported in none of the strains. Unlike our results, studies conducted in India reported a high prevalence of CTX-M-type ESBL among ESBL-*E. coli* isolated from clinical specimens. Interestingly, the strain encoding * blaCTX-M* gene in our study also harbored * blaOXA* gene. Indeed, enteric Gram-negative bacteria with the blaOXA-48-like genes could co-harbored genes encoding ESBL (*blaCTX-M, blaSIV, blaTEM*) * blaOXA* and * blaTEM* were carried by two *E. coli* isolates while no *Salmonella* strains harboured these genes. Similarly, a recent study reported no *blaKPC, blaVIM* and * blaIMP* genes on carbapenemase producing *Salmonella enterica* isolates in the United Kingdom whereas reported 2 *blaOXA48* strains and one *blaNDM* on the same isolates. This is expected because carbapenemase-producing *Salmonella* strains are rarely isolated. In contrast, resistance to carbapenems was observed in CIP-R *Salmonella* KentuckyX1-ST198-SGI1 isolates in which carbapenemases *blaVIM–2* and *blaOXA–48* have been detected.

Our results show that carbapenemase-producing *Enterobacteriaceae* (CPE) remain one of the most urgent healthcare threats. Indeed, carbapenemase-encoding genes are already widespread in many parts of the world. A recent study on wastewater used for urban agriculture in Ouagadougou (Burkina Faso) concluded that raw sewage used as fertilizer could be contributing to the spread of resistance bacteria among humans and animals. Furthermore, a study has shown that bacteria producing carbapenemase are currently spreading among pets and because of the proximity between humans and animals, these bacteria can contaminate humans. For example, it has been shown that poultry flocks contribute to the global dissemination of *Salmonella* Kentucky ST198-X1-SGI1CIP-R strain in developing countries. Since subsistence, farming and animal husbandry are the primary economic activities for the local populations in Boromo and Gourcy, the spread of these bacteria poses serious health concerns.

**Table 4**: Carbapenemase-producing *Enterobacteriaceae*

| Strains                      | Sex | Age (year) | Carbapenemase genes | Total N |
|------------------------------|-----|------------|---------------------|---------|
| *Escherichia coli* pathotyps |     |            |                     |         |
| 025 B (EAEC)                 | M   | 1          | -                   | -       | +       | -       | 1       |
| 039 B (EAEC)                 | F   | 1          | -                   | -       | +       | -       | 1       |
| 043B (Atypical EPEC)         | M   | 1          | +                   | +       | +       | -       | 4       |
| 044B (EAEC)                  | M   | 1          | +                   | +       | +       | +       | 5       |
| 046B (Atypical EPEC)         | M   | 1          | -                   | -       | -       | +       | 1       |
| *Salmonella* serovars        |     |            |                     |         |
| 084B (S. Duisburg)           | M   | 3          | -                   | -       | -       | +       | 1       |
| 057B (S. Poona)              | M   | 2          | -                   | -       | -       | -       | 0       |
| 066B (S. Typhimurium)        | M   | 1          | -                   | -       | -       | -       | 0       |
| 068B (S. Typhimurium)        | M   | 2          | -                   | -       | +       | -       | 1       |
| 078B (S. Ouakam)             | M   | 1          | -                   | -       | -       | +       | 1       |
| 063G (S. Hvittingfoss)       | F   | 1          | -                   | -       | -       | -       | 0       |
| 087G (S. Poona)              | F   | 1          | -                   | -       | -       | -       | 0       |
| 112G1 (S. Virchow)           | F   | 3          | -                   | -       | -       | -       | 0       |
| 112G2 (S. Virchow)           | F   | 3          | -                   | -       | -       | -       | 0       |
Three specific genes were detected in two E. coli strains: Klebsiella pneumoniae carbapenemase (KPC), Verona integron-encoded metallo-β-lactamase (VIM) and imipenemase (IMP-2). To our best of knowledge, this is the first report of blaVIM gene in E. coli in Burkina Faso. However, KPC producers have been described, mostly from nosocomial K. pneumoniae isolates, and E. coli strains in Israel but also from other enterobacterial species. Indeed, since their identification the first time in the USA in 1996, Klebsiella pneumoniae carbapenemases (KPCs) have spread internationally among Gram-negative bacteria, especially K. pneumoniae, although their precise epidemiology is diverse across countries and regions worldwide. Furthermore, because of its extensively identification worldwide, K. pneumoniae may have contributed to the spread of the blaKPC genes.

As far as the class B metallo-β-lactamases (MBLs) is concerned, our results corroborate the existing reports. Endemicity of VIM- and IMP-type enzymes has been reported in Greece, Taiwan and Japan, although outbreaks and single reports of VIM and IMP producers have been shown in many other countries.

It has been shown that carbapenemases can hydrolyze almost all β-lactams, and are easily transferable among enterobacterial species. These genes are found in multidrug-resistant isolates consistent with the result found in the present study. Therefore, its spread in Enterobacteriaceae is a public health issue. For example, invasive infections by carbapenem-resistant strains have been found to be associated with high morbidity and mortality rates.

Otherwise, several risk factors of colonization and infection with carbapenemase-producing Enterobacteriales (CPE) including severe underlying illness, prolonged hospital stay, the presence of invasive medical devices, and antibiotic use have been shown. According to previous studies, CPE have been associated with adverse clinical and economic outcomes such as increased mortality, increased length of stay, setting up an effective therapy scheme, decreased functional status on discharge, and increased cost of health care. Young children (those under one year old) were severely infected with carbapenem-resistant E. coli. This is a matter of public health issues because the emergence of MBL-producing bacteria greatly limits treatment options.

The most frequent MBLs reported to date belong to the VIM and IMP types and have been described extensively worldwide.

The main limitation of the present study consists of the low number of isolates which makes generalizability difficult. Moreover, the use of phenotypic approach limited to imipenem MIC is not adapted for the detection of all carbapenemase type. For instance, enterobacteria strains carrying blaOXA48 carbapenemase could present low imipenem MIC (0.5 mg/L) suggesting the use of temocillin for phenotypic detection of blaOXA48.

CONCLUSIONS

Infections by carbapenem-resistant bacteria are difficult to treat successfully. This study highlights the need for rapid identification of MBL-producing Gram-negative species both for appropriate treatment and for timely implementation of infection control measures. In developing countries like Burkina Faso, phenotypic methods may be useful for routine detection of Carbapenemase production, particularly when PCR is not available.

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CONFLICT OF INTEREST

The authors stipulate that they have no conflict of interest in regard to this study.

REFERENCES

1. World Health Organization (WHO). Antimicrobial resistance; 2018. https://www.who.int/medicines/areas/rational_use/who-amr-aml-report-20181109.pdf
2. Gauthier L, Dortet L, Cotellon G, Creton E, Cuzon G, et al. Diversity of carbapenemase-producing Escherichia coli isolates, France 2012-2013. Antimicrob Agents Chemother. 2018; 62: pii: e00266-18.
3. Vousi M, Touati A, Mairi A, Creton E, Cuzon G, Ponties V, et al. Emergence of Carbapenemase-Producing Escherichia coli Isolated from Companion Animals in Algeria. 2016; Microbial Drug Resist. 22:342-346.
4. Li XZ, Mehrotra M, Ghimire S, Adewoje Y. Beta-Lactam resistance and beta-lactamas in bacteria of animal origin. Vet Microbiol. 2007; 121:197-214.
5. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Dailos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: An evolving crisis of global dimensions. Clin Microbiol Rev. 2012; 25:682-707.
6. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! Trends Mol Med. 2012; 18:263-273.
7. Nordmann P, Cornaglia G. Carbapenemase-producing Enterobacteriaceae: A call for action! Clin Microbiol Infect. 2012; 18:411-412.
8. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev. 2007; 20:440-458.
9. Rupp E, Wöther P-L, Barbier F. Mechanisms of antimicrobial resistance in gram-negative bacilli. Ann Intensive Care 2015; 5 (21): Article 61.
10. Lowman W, Bamford C, Govind C, Swe Swe Han K, Kularatne R, Senekal M, et al. The SASCAM CRE-WG: consensus statement and working guidelines for the screening and laboratory detection of carbapenemase-producing Enterobacteriaceae. South Afr J Infect Dis. 2014; 29:5-11.
11. Shcherchan B, Hayakawa K, Miyoshi-Akiyama T, Ohmagari N, Kikunae T, Nagamatsu M, et al. Clinical Epidemiology and Molecular Analysis of Extended-Spectrum B-Lactamase-Producing Escherichia coli in Nepal: Characteristics of Sequence Types 131 and 648. Antimicrob Agents Chemother. 2015; 59:3424-32.
12. Mariappan S, Sekar U, Kamalanathan A. Carbapenemase-producing Enterobacteriaceae: Risk factors for infection and impact of resistance on outcomes. Int J App Basic Med Res. 2018; 7:32-39.
13. Ortega A, Saez D, Bautista V, Fernandez-Romero S, Lara N, Aracil B, et al. Carbapenemase-producing Escherichia coli is becoming more prevalent in Spain mainly because of the polyclonal dissemination of OXA-48. J Antimicrob Chemother. 2016; 71: 2131-38.
14. Popoff MY, Bockemuhl J, Gheselign LL. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res Microbiol. 2004; 155:568-70.
15. Antikainen J, Tarkka E, Haukka K, Siitonen A, Vaara M, Kirveskari J. New 16plex PCR method for rapid detection of Diarrheagenic Escherichia coli directly from stool samples. Euro J Clin Microbiol Infect Dis. 2009; 28: 999-908.
16. Müller D, Greune L, Neussipp G, Karch H, Fruth A, Tschäpe H, et al. Identification of unconventional intestinal pathogenic Escherichia coli isolates expressing intermediate virulence factor profiles by using a novel single-step multiplex PCR. Appl Environ Microbiol. 2007; 73: 3380-90.
