Molecular Characterization of Carbapenemase-Producing Escherichia coli and Salmonella in children with diarrhea in rural Burkina Faso.

Rene DEMBELE (simavedemb@gmail.com)  
University of Dedougou

Ali Konaté  
Universite de Ouagadougou

Issiaka Soulama  
National Centre for Research and Training on Malaria

Wendpoulomdé A. D. Kaboré  
University Joseph KI-ZERBO of Ouagadougou

Assèta Kagambèga  
University Joseph KI-ZERBO of Ouagadougou

David Coulibaly N'Golo  
Universite Felix Houphouet-Boigny

Oumar Traoré  
University of Dedougou

Abdoulaye Seck  
Universite Cheikh Anta Diop Faculte de Medecine de Pharmacie et d’Odontologie

Alfred S. Traoré  
University Joseph KI-ZERBO of Ouagadougou

Nathalie K. Guessennd  
Universite Felix Houphouet-Boigny

Amy Gassama-Sow  
Universite Cheikh Anta Diop

Nicolas Barro  
University Joseph KI-ZERBO of Ouagadougou

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Abstract

Background In recent years, Carbapenemase-producing Enterobacteriaceae (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, using bacterial strains obtained from previous studies.

Results 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 used to characterize bla KPC, bla VIM and bla IMP genes in carbapenemase-producing E. coli and Salmonella. The study highlighted high resistance rates of the identified bacteria to common antibiotics. Likewise, two strains of E. coli were imipenem 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. However, no Carbapenemase-encoding genes were detected in Salmonella isolates.

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

Background

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 [1]. As a result, antimicrobial resistance represents a major challenge for public health worldwide. One of the most worrying threat is the emergence and rapid dissemination of carbapenem resistant Gram-negative bacteria, following the spread of carbapenemase-producing Enterobacteriaceae (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-β-lactamase (NDM), Verona integrin-encoded metallo-β-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 intrinsic 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 (Table 1; [10]).
Table 1
The plasmid-mediated mechanisms of resistance [10]

| Type of mechanism of resistance | Examples                                                                                                                                 |
|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| (a) Carbapenemases              | (1) Class A serine carbapenemases                                                                                                                                                               |
|                                 | Klebsiella pneumoniae carbapenamse (KPC)                                                                                                                                                        |
|                                 | Guiana extended spectrum (GES)                                                                                                                                                                  |
|                                 | (2) Class B metallo-β-lactamases (MBLs)                                                                                                                                                         |
|                                 | Verona integron-encoded metallo-β-lactamase (VIM)                                                                                                                                              |
|                                 | Active on imipenem (IMP)                                                                                                                                                                       |
|                                 | Sao Paulo metallo-β-lactamase (SPM)                                                                                                                                                             |
|                                 | Seoul imipenemase (SIM)                                                                                                                                                                        |
|                                 | German imipenemase (GIM)                                                                                                                                                                       |
|                                 | New Delhi metallo-β-lactamase (NDM)                                                                                                                                                             |
|                                 | (3) Class D serine carbapenemases                                                                                                                                                              |
|                                 | OXA β-lactamases (e.g. OXA-48 and variants)                                                                                                                                                     |
| (b) Amp-C beta-lactamase OR extended spectrum beta-lactamase (ESBL) production along with efflux pumps or porin loss |                                                                                                                                                                                                   |

The recent spread of CPE is one of the major public health threats worldwide [11] because carbapenems are among the main stay of therapy for treating severe infections directly related to multidrug-resistant bacteria producing extended-spectrum β-lactamases (ESBLs) [12]. Carbapenemases are defined as β-lactamases that hydrolyze almost all beta-lactam antibiotics. According to some recent studies, the most prevalent carbapenemases in Enterobacteriaceae are bla\textsubscript{KPC} (Ambler class A), bla\textsubscript{VIM}, bla\textsubscript{IMP}, bla\textsubscript{NDM} (class B) and bla\textsubscript{OXA-48} like (class D) [11, 13]. Although several studies reported the occurrence of carbapenemases producing bacteria in Africa [12], 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.

Methods
Bacterial strains
Bacterial strains were obtained from our previous studies [14, 15] conducted in Gourcy and Boromo municipalities (Fig. 1). The 16-plex PCR was used to detect simultaneously 16 genes from the five main pathogroups of E. coli (enterohemoragic E. coli: EHEC, enteropathogenic E. coli: EPEC, enteroaggregative E. coli: EAEC, enteroinvasive E. coli: EIEC and enterotoxigenic E. coli: ETEC) as described by Antikainen et al. [16]. The genes investigated and primers used are listed in Table 2 [16–18]. A questionnaire was used to collect demographic information (e.g., age and sex) of each patient).
Table 2
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: GACACCTCA TTGCTGAAG TCG              | 910       | 0.1      | [18]      |
|                   |             | MP3-bfpB-R: CCAGAACA CCTCCGTTAT GC               |           | 0.1      |           |
| EHEC and EPEC     | eaeA        | eae-F: TCAATGCA GTTCCGTTA TCAGTT                 | 482       | 0.1      | [18]      |
|                   |             | eae-R: GTAAGGTC GTTTACCCCC AACCTG               |           | 0.1      |           |
| escV              |             | MP3-escV-F: ATTCTGGC TCTCTTCTT TTTATGCG TG      | 544       | 0.4      | [18]      |
|                   |             | MP3-escV-R: CGTCCCCCCT TTTACAAAECT TCATCGC      |           | 0.4      |           |
| Ent               | ent-        | ent-F: TGGGCTAA AAGAAGACA CACTG                 | 629       | 0.4      | [18]      |
|                   |             | ent-R: CAAGCATC CTGATTATCT CACC                 |           | 0.4      |           |
| EHEC              | EHEC-hly    | hlyEHEC-F: TTCTGGGAA ACAGTGACG CACATA           | 688       | 0.1      | [17]      |
|                   |             | hlyEHEC-R: TCACCGATC TTCTCATCCC AATG           |           | 0.1      |           |
| Pathotype | Target gene | Primer sequence (5' to 3') | Size (bp) | [C] (µM) | Reference |
|-----------|-------------|-----------------------------|-----------|---------|-----------|
| Stx1      | MP4-stx1A-F:CGATGTGTA CGGTGGTCT ACTGTGACAGC | 244 | 0.2 | [18] |
|           | MP4-stx1A-R:AATGCCAC GCTTCCCAAG AATTG | | | 0.2 |
| Stx2      | MP3-stx2AF:GTTC TGGCATCT TCGTCTGAT TATTGAAG | 324 | 0.4 | [18] |
|           | MP3-stx2A-R:AGCGTAAG GCTTCTGCT GTGAC | | | 0.4 |
| EAEC      | astA        | MP-astA-F TGCCATCAA CACAGTAT ATCCG | 102 | 0.4 | [18] |
|           | MP2-astA-R ACGGCTTTTG TGTCCCTTC CAT | | | 0.4 |
| aggR      | MP2-aggR-F:ACGCAAGAG TTCCCGATAAAG | 400 | 0.2 | [18] |
|           | MP2-aggR-R:AATACAGA ATCGTCAGCA ATCAGC | | | 0.2 |
| Pic       | MP2-pic-F:AGCCGGTTT CCGCAGAAG CC | 1111 | 0.2 | [18] |
|           | MP2-pic-R:AAATGTCA GTGAACCGA CGATTGG | | | 0.2 |
| Pathotype | Target gene | Primer sequence (5’ to 3’) | Size (bp) | [C] (µM) | Reference |
|-----------|-------------|----------------------------|----------|----------|-----------|
| EIEC      | invE        | MP2-invE-F: CGATAGAT GCGAGAAA TATATCCCG | 766      | 0.2      | [18]      |
|           |             | MP2-invE-R: CGATCAAG AATCCCTAATC AGAAGAATAC AC |          | 0.2      |           |
|           | ipaH        | ipaH-F: GAAAAACCT CCTGGTCCA TCAGG | 437      | 0.1      | [19]      |
|           |             | ipaH-R: GCCGGTCA GCCACCCCT TGAGAGTAC |          | 0.1      |           |
| ETEC      | elt         | MP2-LT-F: GAAACAGAG GTTTCTGCG TTAGGTG | 655      | 0.1      | [18]      |
|           |             | MP2-LT-R: CTTTCAATG GCTTTTTTT TTGGAGTC |          | 0.1      |           |
|           | estA        | MP4-STIa-F: CCTCTTTTA GYCACACAR CTGAATCAS TTG | 157      | 0.4      | [18]      |
|           |             | MP4-STIa-R: CAGGCAGGA TTACAACAAAA GTTACAG |          | 0.4      |           |
|           | estB        | MP2-STI-F: TGCTTTTTT CACCTTTTG CTC | 171      | 0.2      | [18]      |
|           |             | MP2-STI-R: CGGTACAAAG CAGGATTAC AACAC |          | 0.2      |           |
Antimicrobial susceptibility testing and ESBL production

Antibiotic susceptibility was determined on Mueller–Hinton agar using the standard disc diffusion procedure as described by the European Committee of Antimicrobial Susceptibility Testing (EUCAST) [19]. Nineteen antibiotics belonging to 7 different families 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), gentamycin (15 µg), netilmicin (10 µg) and tobramycin (10 µg) (Bio-Rad, France). The diameters of the antibiotic sensitivity halos were recorded according to the recommendations of EUCAST. Intermediary (I) susceptibility of pathovars was classified as resistant (R). A double synergy test was used for ESBL-producing strains testing. This consisted of placing discs (2–3 cm diameter) of ceftriaxone and cefotaxime around an amoxicillin-clavulanic acid disc on the bacterial plate.

Detection of antibiotic resistance genes

DNA for PCR analysis was extracted from the isolates using the heat lysis method [20]. A loopful of bacterial growth from Mueller Hinton agar (Liofilchem, 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 bla<sub>KPC</sub>, bla<sub>VIM</sub> and bla<sub>IMP</sub> types in carbapenemase-producing E. coli and Salmonella strains [21, 22].
Table 3

Oligonucleotides used to amplify carbapenemases genes

| Primer name | Target | Primers sequence (5' to 3') | Size (bp) | Reference |
|-------------|--------|-----------------------------|-----------|-----------|
| KPC-F       | bla<sub>KPC</sub> | GCT CAG GCG CAA CTG TAA G | 300       | [21]      |
| KPC-R       |        | AGC ACA GCG GCA GCA AGA AAG |           |           |
| VIM-F       | bla<sub>VIM</sub> | CAG ATT GCC GAT GGT GTT TGG | 390       | [22]      |
| VIM-R       |        | AGG TGG GCC ATT CAG CCA GA |           |           |
| IMP-F       | bla<sub>IMP</sub> | GGA ATA GAG TGG CTT AAT TCTC | 232       | [22]      |
| IMP-R       |        | GTG ATG CGT CYC CAA YTT CAC T |           |           |

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: 5 min at +94 °C, followed by 35 amplification cycles of +94 °C for 30 s, 59 ± 4 °C for 60 s and +72 °C for 60 s with a final extension of +72 °C for 10 min 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).

Results

Bacterial isolates

Of the 275 stool samples, about five isolates were confirmed as E. coli strains: 3 EAEC (60%), and 2 atypical EPEC (40%). Nine Salmonella isolates were detected belonging to the following serotypes: Salmonella Poona, S. Typhimurium, S. Ouakam, S. Virchow, S. Duisburg and S. Hvittingfoss.

Antimicrobial resistance

The identified E. coli strains were 100% resistant to amoxicillin-clavulanic acid, amoxicillin and tetracycline; 80% resistant to trimetoprim-sulfametoxazol, colistin-sulfate and piperacillin; 60% resistant to cefotaxim, ceftriaxone, aztreonam, cefixime and cefepime. Whereas, resistance to chloramphenicol
was shown in 40% of isolates. Antimicrobial susceptibility testing revealed that two E. coli strains were imipenem–resistant.

The Salmonella trains were 100% resistant to amoxicillin, 89% to amoxicillin-clavulanic acid, 67% to tetracycline, cefixime and cefepime; 56% resistant to ceftriaxone, cefotaxim and colistin-sulfate, 30–50% resistant to aztreonam, trimetoprim-sulfametoxazol and piperacillin while no resistance was shown to imipenem (Fig. 2). Thus, global prevalences of carbapenemase-producing E. coli and Salmonella enterica in the study children were 1.81% and 0%, respectively.

**Carbapenemase genes**

The carbapenemase genes have been reported in two strains (one atypical EPEC and one EAEC) which were resistant to imipenem. Each of these two isolates carried the following genes: bla\textsubscript{KPC}, bla\textsubscript{VIM} and bla\textsubscript{IMP−2}. No carbapenemase genes were detected in Salmonella isolates (Table 4). All five strains were isolated in children of one year old while the sex distribution was 4/5 (80%) for male and 1/5 (20%) for female. The five E. coli isolates were carbapenemase and ESBL-producing strains (Table 4).
| Code (E. coli pathovars) | Sex | Age (year) | Carbapenemase-producing Enterobacteriaceae | Carbapenemase genes + ESBL phenotypes | Total N (%) |
|-------------------------|-----|------------|------------------------------------------|-------------------------------------|-------------|
|                         |     |            |                                          | bla\textsubscript{KPC}     | bla\textsubscript{VIM} | bla\textsubscript{IMP-2} |            |
| 025 B (EAEC)            | M   | 1          |                                          | -/+                               | -            | -                        | 0 (0)     |
| 039 B (EAEC)            | F   | 1          |                                          | -/+                               | -            | -                        | 0 (0)     |
| 043 B (Atypical EPEC)   | M   | 1          |                                          | +/+                               | +            | +                        | 3 (50)    |
| 044 B (EAEC)            | M   | 1          |                                          | +/+                               | +            | +                        | 3 (50)    |
| 046 B (Atypical EPEC)   | M   | 1          |                                          | -/+                               | -            | -                        | 0 (0)     |
| Code (S. serovars)      |     |            |                                          |                                    |              |                          |           |
| 084 B (S. Duisburg)     | M   | 3          |                                          | -/+                               | -            | -                        | 0 (0)     |
| 057 B (S. Poona)        | M   | 2          |                                          | -/-                               | -            | -                        | 0 (0)     |
| 066 B (S. Typhimurium)  | M   | 1          |                                          | -/-                               | -            | -                        | 0 (0)     |
| 068 B (S. Typhimurium)  | M   | 2          |                                          | -/+                               | -            | -                        | 0 (0)     |
| 078 B (S. Ouakam)       | M   | 1          |                                          | -/-                               | -            | -                        | 0 (0)     |
| 063 G (S. Hvittingfoss) | F   | 1          |                                          | -/+                               | -            | -                        | 0 (0)     |
| 087 G (S. Poona)        | F   | 1          |                                          | -/+                               | -            | -                        | 0 (0)     |
| 112 G1 (S. Virchow)     | F   | 3          |                                          | -/-                               | -            | -                        | 0 (0)     |
| Code (E. coli pathovars) | Sex | Age (year) | Carbapenemase genes + ESBL phenotypes | Total N (%) |
|-------------------------|-----|------------|---------------------------------------|-------------|
| 112G2 (S. Virchow)      | F   | 3          | -/-                                   | 0 (0)       |

**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 [23]. Indeed, it is a major public health concern, mainly within communities. Our study reported for the first time the occurrence of carbapenemase-producing E. coli in children with diarrhea in rural settings of Burkina Faso.

The isolated strains were mainly resistant to amoxicillin-clavulanic acid, amoxicillin, tetracycline, trimetoprim-sulfametoxazol, colistin-sulfate, pipercillin, cefotaxime, 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 [12]. Susceptibility to netilmicin and ciprofloxacin appears to follow the general trend observed elsewhere in the world.

Our finding on overall prevalence of E. coli harboring carbapenemase genes in the 275 children was 1.81%. This result is similar to 2.5% reported in pets in Africa [3] suggesting that bacteria producing carbapenemase are currently spreading among these pets [3] and because of the proximity between humans and animals, these bacteria can contaminate humans. Indeed, animals could be reservoirs of gene transmission to 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 [24]. 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.

Oatherwises, we detected no carbapenemase genes in Salmonella strains. 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 [24].

Three main genes were detected in E. coli strains: Klebsiella pneumoniae carbapenemase (KPC), Verona integrin-encoded metallo-β-lactamase (VIM) and Imipenemase (IMP-2) with a rate of 40% each. To our best of knowledge, this is the first report of blaKPC 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 [25]. As K. pneumoniae was identified extensively worldwide, a study suggested that it may have contributed to the spread of the blaKPC genes [26].

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 [27, 8], although outbreaks and single reports of VIM and IMP producers have been shown in many other countries [27].

It has been shown that carbapenemases can hydrolyze almost all β-lactams, and are easily transferable among enterobacterial species [28]. These genes are found in multidrug-resistant isolates consistent with the result found in the present study [28]. 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 [29].

Otherwise, several risk factors of colonization and infection with carbapenemase-producing Enterobacteriaceae (CPE) including severe underlying illness, prolonged hospital stay, the presence of invasive medical devices, and antibiotic use have been shown. [30–33]. 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 [34–37]. 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 [38]. The most frequent MBLs reported to date belong to the VIM and IMP types and have been described extensively worldwide [39].

To conclude, 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.

**Abbreviations**

CPE: Carbapenemase-producing *Enterobacteriaceae*, DEC: Diarrheagenic *Escherichia coli*; KPC: *Klebsiella pneumoniae* Carbapenemase; VIM: Verona integron-encoded metallo-β-lactamase; IMP: Imipenemase; NDM: New Delhi metallo-β-lactamase; ESBLs: extended-spectrum β-lactamases; EHEC: enterohemoragic *E. coli*; EPEC: enteropathogenic *E. coli*; EAEC: enteroaggregative *E. coli*; EIEC: enteroinvasive *E. coli*; ETEC: enterotoxigenic *E. coli*.

**Declarations**

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Authors' contributions

Conceptualization, R.D, A.G-S and N.B; Methodology, R.D, A.K₁ and I.S; Original draft preparation, R.D; Writing-Review and Editing, R.D, A.K₁, I.S, W.A.D.K. All authors have read and approved the final manuscript.

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Availability of data and materials

Not applicable.

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° 2009-39) approved the study protocol.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1. Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food/Center for Research in Biological, Food and Nutritional Sciences/Graduate School of Science and Technology/University Joseph Ki-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.
2. Training and Research Unit in Applied Sciences and Technologies/University of Dedougou, BP 176 Dedougou, Burkina Faso.
3. National Centre for Research and Training on Malaria, 01 BP 2208 Ouagadougou 01, Burkina Faso.
4. Institute of Sciences, 01 BP 1757 Ouagadougou 01, Burkina Faso.
5. Molecular Biology Platform, Pasteur Institute of Abidjan, Ivory Coast, 01 BP 490 Abidjan 01.
6. Laboratory of Biological and Medical Analyzes, Pasteur Institute of Dakar, Dakar, Senegal.
7. Faculty of Medicine, Pharmacy and Odontology, University of Cheikh Anta DIOP of Dakar, Senegal.
8. Laboratory of Bacteriology-Virology, Unit of Antibiotics, Natural Substances and Surveillance of Resistance of Microorganisms to Antimicrobials/ Pasteur Institute of Abidjan, Ivory Coast, 01 BP 490 Abidjan 01.
9. Laboratory of Bacteriology-Virology/Unit of Training and Research of Medical Sciences/University Felix Houphouet BOIGNY, 01 BP V34 Abidjan 01, Ivory Coast.
10. Unit of Experimental Bacteriology, Pasteur Institute of Dakar, 36 avenue Pasteur, BP 220, Dakar, Senegal.

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**Figures**

**Figure 1**

Map of Burkina Faso. In dark = Gourcy and Boromo where the study was conducted.
Figure 2

Resistance to individual antimicrobial among E. coli and Salmonella strains Legend : AMC = Amoxicillin-clavulanic acid, AMX = Amoxicillin, CTX = Cefotaxime, ATM = Aztreoname, IPM = Imipenem, CRO = Ceftriaxone, FEP = Cefepime, CFM = Cefixime, TET = Tetracycline, CHL = Chloramphenicol, SXT = Trimethoprim-sulfamethoxazole CIP = Ciprofloxacine, NAL = nalidixic acid, CST = Colistin sulfate, GMI = Gentamicin, TZP = Piperacillin-tazobactam, PIP = Piperacillin, NTM = Netilmicin, TMN = Tobramycin, I = Intermediate, R = Resistant.