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

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Research

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Abstract

Background: In recent years, carbapenemase-producing *Enterobacterales* (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.

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 bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{TEM}, bla_{SHV}, bla_{OXA} and bla_{CTX-M} 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 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 in *E. coli* strains. However, no *Salmonella* carbapenemases *bla*KPC, *bla*VIM or *bla*IMP were detected.

Conclusions: This study showed that for a real-time infection control and prompt application of antimicrobial chemotherapy, characterization of carbapenemase-producing *Enterobacterales* 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 *Enterobacterales* (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 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 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_{KPC} (Ambler class A), bla_{VIM}, bla_{IMP}, bla_{NDM} (class B) and bla_{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

Specimen collection

This study was conducted in north (Gourcy, distance 140 km) and western (Boromo, distance 185 km) of the capital Ouagadougou, Burkina Faso (Fig. 1). 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 24 h 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 18 h. Subsequently, samples were cultured on Hecktoen Enteric agar (Liofilchem, Italy) and incubated at 37 °C for 24 h. The identity of typical-looking *Salmonella* colonies on Hektoen was examined by using orthonitrophenyl-ß-D-galactopyranoside (ONPG), citrate, mannitol, lysine decarboxylase tests and the Kliger Hajna medium (Liofilchem, 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 Kauffman White scheme [14].

E. coli isolation and identification

Stool samples were plated on eosin methylene blue agar (Liofilchem, 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 (Liofilchem, Italy). Confirmation was carried out by a biochemical microbiology method based on negative urease (Bio-Rad, France), negative citrate (Liofilchem, Italy), positive indole (Bio-Rad, France), positive lactose (Liofi lchem, Italy), and positive orthonitrophenyl-β-D-galactopyranoside (ONPG) (bioMerieux, 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* (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. [15]. The genes investigated and primers used are listed in Table 1 [15–17].

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: GACACCTCATTGCTGAAGTCG	910	0.1	[16]
		MP3-bfpB-R:CCAGAACACCTCCGTTATGC		0.1	
EHEC and EPEC	*eaeA*	eae-F:TCAATGCAGTTCCGTTATCAGTT	482	0.1	[16]
		eae-R:GTAAAGTCCGTTACCCCAACCTG		0.1	
	escV	MP3-escV-F:ATTCTGGCTCTCTTCTTCTTTATGGCTG	544	0.4	[16]
		MP3-escV-R:CGTCCCCTTTTACAAACTTCATCGC		0.4	
	Ent	ent-F:TGGGCTAAAAGAAGACACACTG	629	0.4	[16]
		ent-R:CAAGCATCCTGATTATCTCACC		0.4	
EHEC	EHEC-*hly*	hlyEHEC-F: TTCTGGGAAACAGTGACGCACATA	688	0.1	[15]
		hlyEHEC-R: TCACCGATCTTCTCATCCCAATG		0.1	
	Stx1	MP4-stx1A-F:CGATGTTACGGTTTGTTACTGTGACAGC	244	0.2	[16]
		MP4-stx1A-R:AATGCCACGCTTCCCAGAATTG		0.2	
	Stx2	MP3-stx2AF:GTTTTGACCATCTTCGTCTGATTATTGAG	324	0.4	[16]
		MP3-stx2A-R:AGCGTAAGGCTTCTGCTGTGAC		0.4	
EAEC	*astA*	MP-astA-F TGCCATCAACACAGTATATCCG	102	0.4	[16]
		MP2-astA-R ACGGCTTTGTAGTCCTTCCAT		0.4	
	aggR	MP2-aggR-F:ACGCAGAGTTGCCTGATAAAG	400	0.2	[16]
		MP2-aggR-R:AATACAGAATCGTCAGCATCAGC		0.2	
	Pic	MP2-pic-F:AGCCGTTTCCGCAGAAGCC	1111	0.2	[16]
		MP2-pic-R:AAATGTCAGTGAACCGACGATTGG		0.2	
EIEC	*invE*	MP2-invE-F:CGATAGATGGCGAGAAATTATATCCCG	766	0.2	[16]
		MP2-invE-R:CGATCAAGAATCCCTAACAGAAGAATCAC		0.2	
	ipaH	ipaH-F: GAAAACCCTCCTGGTCCATCAGG	437	0.1	[17]
		ipaH-R:GCCGGTCAGCCACCCTCTGAGAGTAC		0.1	
ETEC	*elt*	MP2-LT-F: GAACAGGAGGTTTCTGCGTTAGGTG	655	0.1	[16]
		MP2-LT-R: CTTTCAATGGCTTTTTTTTGGGAGTC		0.1	

Pathotype	Target gene	Primer sequence (5' to 3')	Size (bp)	[C] (µM)	Reference
	estA	MP4-STIa-F : CCTCTTTTAGYCAGACARCTGAATCASTTG	157	0.4	[16]
		MP4-STIa-R : CAGGCAGGATTACAACAAAGTTCACAG		0.4	
	estB	MP2-STI-F : TGTCTTTTTCACCTTTCGCTC	171	0.2	[16]
		MP2-STI-R CGGTACAAGCAGGATTACAACAC		0.2	
E. coli	*uidA*	MP2-uidA-F:ATGCCAGTCCAGCGTTTTTGC	1487	0.2	[17]
		MP2-uidA-R:AAAGTGTGGGTCAATAATCAGGAAGTG		0.2	

Legend: EAEC = enteroaggregative *E. coli,* EPEC = enteropathogenic *E. coli,* EIEC = enteroinvasive *E. coli,* EHEC = enterohemorrhagic *E. coli,* ETEC = enteroenterotoxigenic *E. coli,* µM = micromolaire, [C] = concentration, pb = “paire de base”

Antimicrobial susceptibility testing

Antibiotic susceptibility was determined on Mueller–Hinton agar (Liofilchem, 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-sulfametoxazole (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 pathovars was classified as resistant (R).

Table 2
Zones of inhibition of the tested antibiotics

Families		Antibiotics	[C][a] (µg)	Ø [b](mm)	
				R (Ø≤)	S (Ø≥)
β-lactams	Aminopenicillins	Amoxicillin- clavulanic acid (AMC)	30	19	19
		Amoxicillin (AMX)	25	19	19
		Piperacillin (PIP)	75	17	20
		Piperacillin-tazobactam (TZP)	100/10	17	20
	Cephalosporins C3G	Ceftriaxone (CRO)	30	20	23
		Cefixime (CFM)	10	17	17
		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
Aminoglycosides		Gentamycin (GMI)	15 (10 IU)	14	17
		Netilmicin (NTM)	10	12	15
		Tobramycin (TMN)	10	14	17

[a] = concentration, [b] = diameter

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 (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_{KPC}, bla_{VIM}, bla_{IMP}, bla_{TEM}, bla_{SHV}, bla_{OXA} and bla_{CTX-M} types in all *E. coli* and *Salmonella* strains of the study.

Table 3
Oligonucleotides used to amplify carbapenemases genes

Primer name	Target genes	Primers sequence (5'to3')	Size (bp)
KPC- F	bla_{KPC2}	GCT CAG GCG CAA CTG TAA G	300
KPC-R		AGC ACA GCG GCA GCA AGA AAG	
VIM- F	bla_{VIM}	CAG ATT GCC GAT GGT GTT TGG	390
VIM-R		AGG TGG GCC ATT CAG CCA GA	
IMP- F	bla_{IMP}	GGA ATA GAG TGG CTT AAT TCTC	232
IMP-R		GTG ATG CGT CYC CAA YTT CAC T	
TEM- F	bla_{TEM}	ATG AGT ATT CAA CAT TTC CG	1080
TEM-R		CCA ATG CTT ATT CAG TGA GG	
SHV- F	bla_{SHV}	TTA TCT CCC TGT TAG CCA CC	768
SHV-R		GAT TTG CTG ATT TCG CTC GG	
OXA- F	bla_{OXA}	ATG AAA AAC ACA ATA CAT ATC	813
OXA-R		AAT TTA GTG TGT TTA GAA TGG	
CTX-M- F	bla_{CTX-M}	ATG TGC AGY ACC AGT AAR GT	544
CTX-M-R		TGG GTR AAR TAR GTS ACC AGA	

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, five isolates were confirmed as *E. coli* strains: 3 EAEC, and 2 atypical EPEC. 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 five *E. coli* strains were resistant to amoxicillin-clavulanic acid, amoxicillin and tetracycline (5/5); four strains were resistant to trimetoprim-sulfametoxazol, colistin-sulfate and piperacillin (4/5); three strains showed resistance to cefotaxim, ceftriaxone, aztreonam, cefixime and cefepime (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, cefixime and cefepime (6/9); five were resistant to ceftriaxone, cefotaxim and colistin-sulfate (5/9). Less than five strains were resistant to aztreonam, trimetoprim-sulfametoxazol and piperacillin while no resistance was shown to imipenem (Fig. 2).

Carbapenemase genes

The results of PCR amplification of bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{TEM}, bla_{SHV}, bla_{OXA} and bla_{CTX-M} genes in *E. coli* and *Salmonella* isolates are given in Table 4. bla_{KPC}, bla_{VIM} and bla_{IMP-2} were carried by two *E. coli* isolates which were resistant to imipenem. bla_{OXA} gene was found in all the five *E. coli* strains (5/5) and in three of the *Salmonella* strains (3/9). bla_{CTX-M} gene was shown in one *E. coli* strain and in none of the *Salmonella*. bla_{TEM} and bla_{SHV} were reported in none of the *E. coli* and *Salmonella* strains.

Table 4
Carbapenemase-producing *Enterobacteriaceae*

Strains	Sex	Age (year)	Carbapenemase genes							Total N
			bla_{KPC2}	bla_{VIM}	bla_{IMP}	bla_{TEM}	bla_{SHV}	bla_{OXA}	bla_{CTX-M}	
Escherichia coli pathotyps										
025 B (EAEC)	M	1	-	-	-	-	-	+	-	1
039B (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

Legend : N = Number ; + = presence ; - = absence

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).

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 [20]. It has been shown that *Enterobacterales* spp., such as *Escherichia coli* and *Klebsiella pneumoniae*, are common human pathogens and asymptomatic colonizers of the human gastrointestinal tract and environmental niches [21]. 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-sulfametoxazol, colistin-sulfate, piperacillin, 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]. All isolated *E. coli* and *Salmonella* were 100% sensitive to netilmicin in agreement with data reported in Bangladesh [22].

In the present study, the most detected carbapenemase was bla_{OXA} 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 [23]. bla_{CTX-M} gene was shown in one *E. coli* strain while bla_{TEM} and bla_{SHV} 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 [24, 25]. Interestingly, the strain encoding bla_{CTX-M} gene in our study also harbored bla_{OXA} gene. Indeed, enteric Gram-negative bacteria with the blaOXA-48-like genes could co-harbor genes encoding ESBL (bla_{CTX-M}, bla_{SHV}, bla_{TEM}) [26]. bla_{KPC2}, bla_{VIM} and bla_{IMP-2} were carried by two *E. coli* isolates while no *Salmonella* strains harboured these genes. Similarly, a recent study reported no *bla*KPC, *bla*VIM and *bla*IMP genes on carbapenemase producing *Salmonella enterica* isolates in the United Kingdom but reported 2 *bla*OXA48 strains and one *bla*NDM on the same isolates [27]. 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 *bla*VIM − 2 and *bla*OXA − 48 have been detected [28].

Our results show that carbapenemase-producing *Enterobacterales* (CPE) remain one of the most urgent healthcare threats. Indeed, carbapenemase-encoding genes are already widespread in many parts of the world [23]. 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 [29]. Furthermore, a study has shown that bacteria producing carbapenemase are currently spreading among pets [3] 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 [28]. 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.

Three specific genes were detected in two *E. coli* strains: *Klebsiella pneumoniae* carbapenemase (KPC), Verona integrin-encoded metallo-β-lactamase (VIM) and Imipenemase (IMP-2). To our best of knowledge, this is the first report of bla_{KPC} 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 [30].

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 [31]. Furthermore, because of its extensively identification worldwide, *K. pneumoniae* may have contributed to the spread of the *bla*KPC genes [32].

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 [33, 8], although outbreaks and single reports of VIM and IMP producers have been shown in many other countries [33].

It has been shown that carbapenemases can hydrolyze almost all β-lactams, and are easily transferable among enterobacterial species [34]. These genes are found in multidrug-resistant isolates consistent with the result found in the present study [34]. 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 [35].

Otherwise, several risk factors of colonization and infection with carbapenemase-producing *Enterobacterales* (CPE) including severe underlying illness, prolonged hospital stay, the presence of invasive medical devices, and antibiotic use have been shown [36–39]. 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 [40–43]. 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 [44]. The most frequent MBLs reported to date belong to the VIM and IMP types and have been described extensively worldwide [45].

The main limitation of the present study consists of the low number of isolates which makes generalizability difficult. Morever, the use of phenotypic approach limited to imipenem MIC is not adapted for the detection of all carbapenemase type. For instance, enterobacteria strains carrying *bla*OXA48 carbapenemase could present low imipenem MIC (0.5 mg/L) suggesting the use of temocillin for phenotypic detection of *bla*OXA48 [46].

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.

Abbreviations

CPE: carbapenemase-producing *Enterobacterales*; 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

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.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization, R.D, A.G-S and N.B; Methodology, R.D, A.K_1 and I.S; Original draft preparation, R.D; Writing-Review and Editing, R.D, A.K_1, I.S, W.A.D.K., O.T. All authors have read and approved the final manuscript.

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Figures

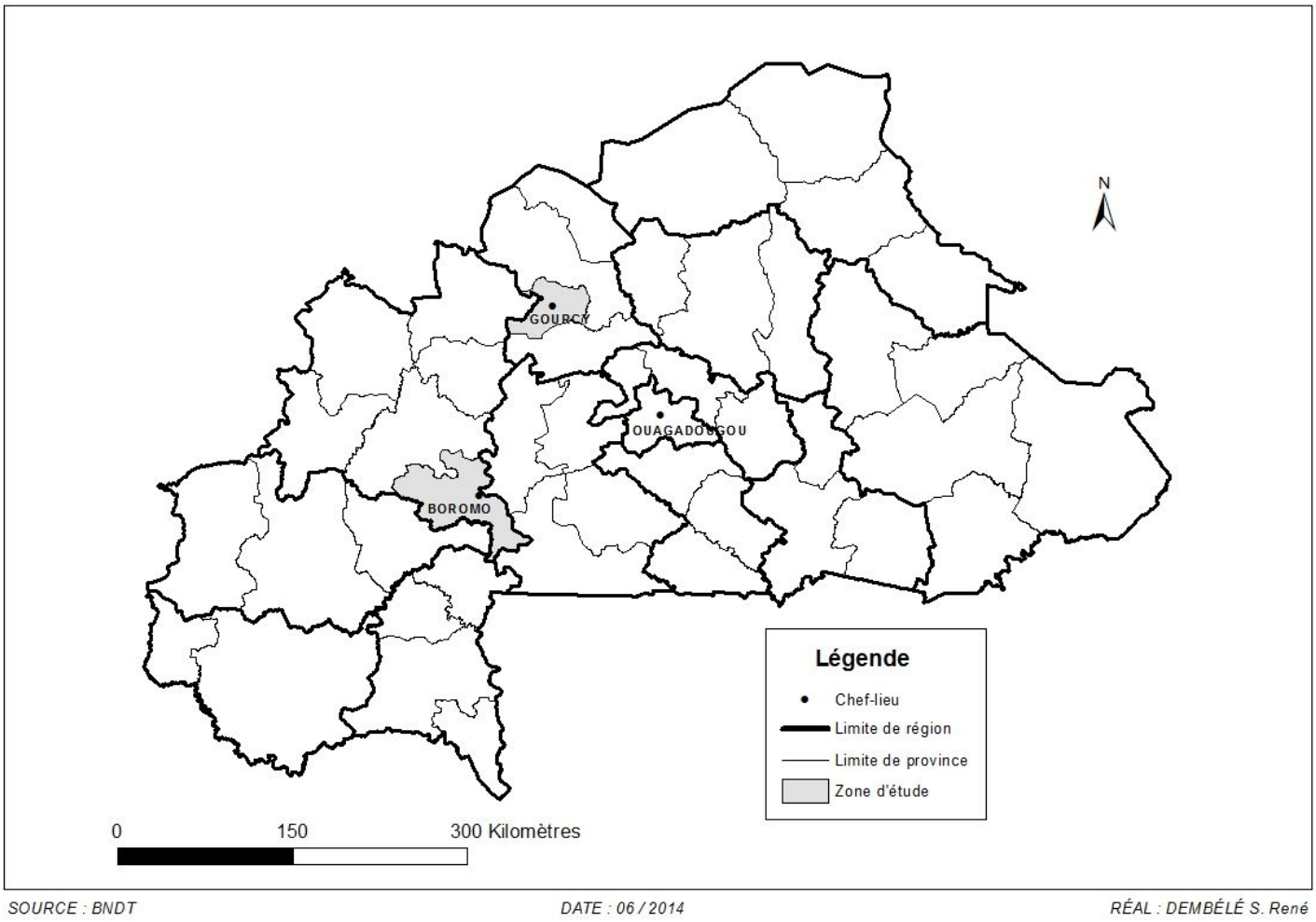

Figure 1

Map of Burkina Faso. In dark = Gourcy and Boromo where the study was conducted. Legend: Software : Quantum GIS (QGIS 2.2), Valmiera, https://qgis.org/downloads/

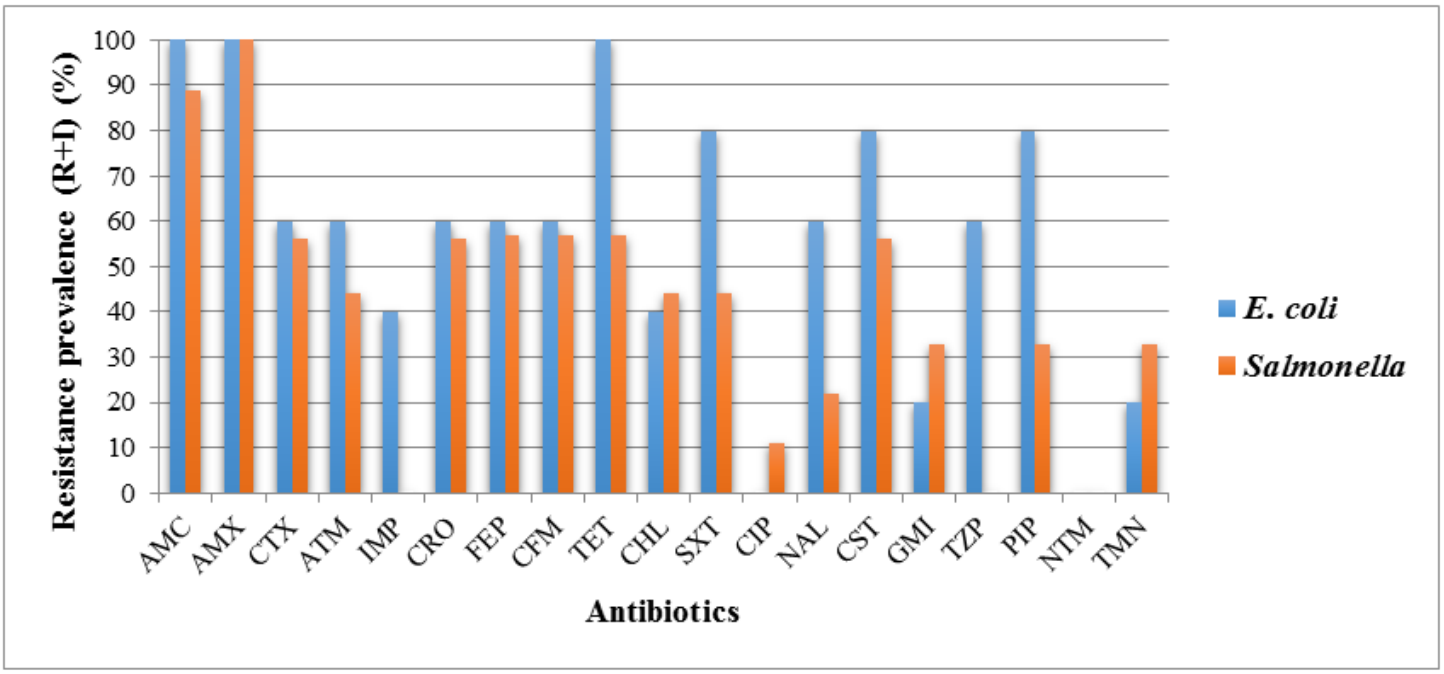

Figure 2

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-sulfametoxazole CIP = Ciprofloxacine, NAL = nalidixic acid, CST = Colistin sulfate, GMI = Gentamicin, TZP = Piperacillin-tazobactam, PIP = Piperacillin, NTM = Netilmicin, TMN = Tobramycin, I = Intermediate, R = Resistant.

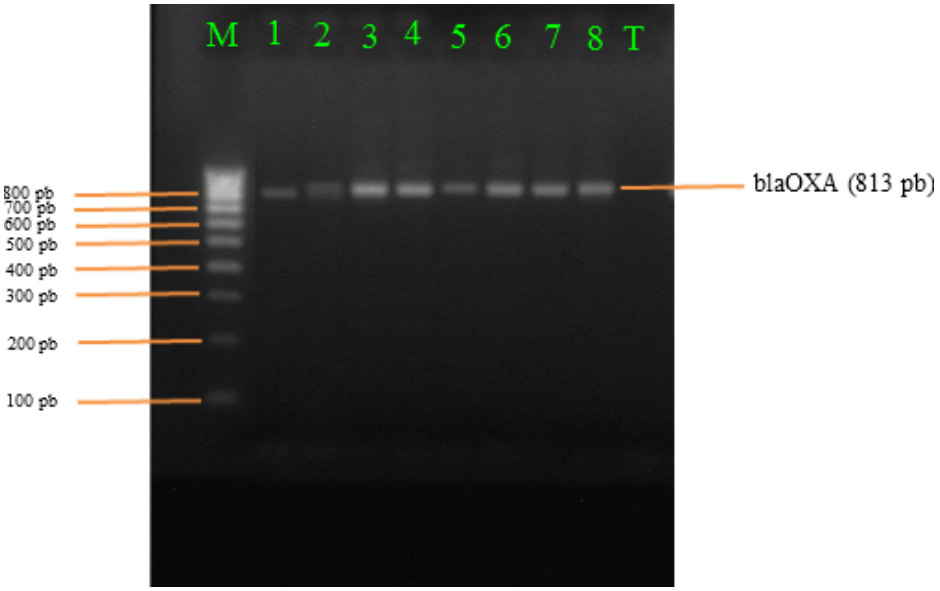

Figure 3

blaOXA gene detected in E. coli 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.

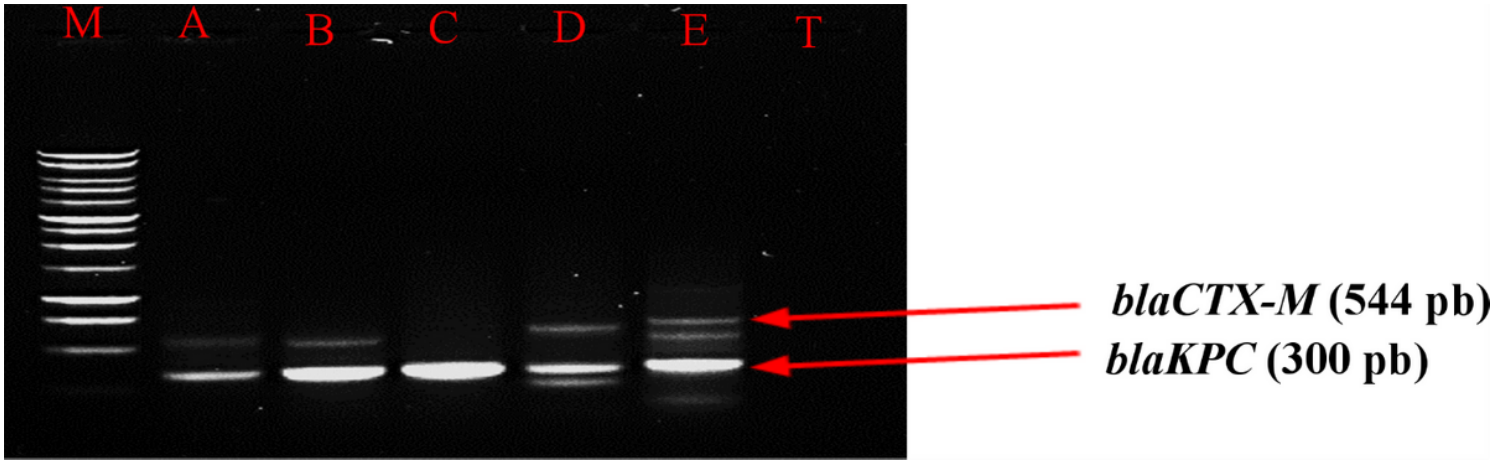

Figure 4

blaCTX-M and blaKPC genes detected in E. coli 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.