Epidemiology and molecular characterization of Enterobacteriaceae producing Extended spectrum β -lactamase in extensive and extensive breeding animals in Burkina Faso

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Abstract

Summary Background Antimicrobial resistance genes can be found in all ecosystems even in those where antibiotic selection pressure is less exerted. Extended spectrum β -lactamases (ESBL) determinants have been detected in clinical isolates and commensal bacteria from humans and animals. Here we investigated, for the first time, the ESBL-producing Enterobacteriaceae in stool from intensive and extensive breeding animals (cattle, pigs and poultry) in Burkina Faso. Results from March to June 2017 in the Bobo Dioulasso area, we investigated stool samples collected from healthy animals (cattle = 251; pigs = 250 and poultry = 397) in one (1) slaughterhouse, five (5) livestock farms and one (1) poultry market. The frequency of ESBL genes carriage was 41.03% among cattle, 69.60% among pigs, 0.8% among intensive farming and 19.1% among extensive poultry farming. Only all the poultry were fed with antibiotics. The bacterial strains carrying the ESBL were *E. coli* (278/315) and *K. pneumoniae* (36/315). The ESBL genes carried were CTX-M 15, TEM and Oxa-1-like. These three β -lactamase genes were associated in some bacterial strains. The *E. coli* strains belonged most commonly to the phylogroup A. **Conclusion** This study showed the high resistance of Enterobacteriaceae to antibiotics in livestock in Burkina Faso by production of ESBL. This high level of resistance was observed in animals that did not receive antibiotics. This situation could suggest an environmental contamination of the livestock with ESBL-producing bacteria.

Background

Antimicrobial resistance (AMR) is a global public health threat. The single most important factor leading to AMR is due to the selective pressure on microorganisms as a result of exposure to antimicrobials[1]. Indeed, antimicrobial use, misuse or overuse in clinical medicine is a major contributing factor in the development of AMR in human populations [2, 3, 4]. As in clinical medicine, antimicrobials are also widely used in domestic animals and livestock [5]. Sub-therapeutic doses of antimicrobials are used for growth-promotion in some countries [6]. This antimicrobial exposure is thought to be an important selective pressure for AMR in animals [7]. However, Antimicrobial resistance genes can be found in all ecosystems even in those where antibiotic selection pressure is less or never exerted like wildlife [8, 9]. Conversely, studies have also shown that various multidrug resistant bacteria lineages from animals also appear in humans [10, 11]. However, the direction of transmission (human-to-animal or animal-to-human) may be equivocal. Generally, the AMR bacteria colonize the gut of animals and might play an epidemiological role in the spread of resistance between Foods Producing Animals (FPA) and humans, either through direct contact or consumption of contaminated meat [12].

According to the Food and Agriculture Organization of the United Nations (FAO), worldwide, pork (36.3%) chicken (35.2%) and beef (22.2%) are the most common meat sources [13].

Production of extended-spectrum β -lactamases (ESBLs) is the most common mechanism of resistance to third-generation cephalosporins among Enterobacteriaceae [14, 15]. These resistant strains are

considered a significant public health issue due to the limited therapeutic options and increased morbidity and mortality associated with them [2].

The true prevalence of ESBL is not well-known in Africa and probably underestimated because of the paucity of studies in human health, animal health, and the food chain on the continent [16]. Currently there is little data regarding the prevalence of ARMs in animals raised without certain antibiotics such as cefotaxime [17]. Here, we investigate the epidemiology and molecular characterization of Enterobacteriaceae producing Extended spectrum β -lactamase in extensive and extensive breeding animals in Burkina Faso.

Results

Occurrence and distribution of Enterobacteriaceae producing Extended spectrum β -lactamase (ESBL)

Enterobacteriaceae producing ESBL were detected in 41.03% of cattle (103/251), 69.6% of pigs (174/250). Among the poultry, the enterobacteriaceae producing ESBL were detected in 0.8% of poultry in intensive breeding (02/244) and 19.6% of poultry in extensive breeding (30/153) (p value $\leq 10^{-3}$).

We identified three (3) enterobacterial species producing ESBL : *E. coli* (278/315), *K. pneumoniae* (36/315) and *C. amalonaticus* (1/315). (Table 1)

Antibiotic susceptibility patterns of Enterobacteriaceae isolates

The overall resistance of the isolates to antibiotics, in the figure 1, shows that resistance to amoxicillin (100%), Cefotaxim (99.7%), Ceftazidim (97.62%), and Cotrimoxazol (86.49%) was high while resistance to Chloramphenicol (5.67%), Netilmicin (2.1%) and Amikacin (0.3%) was low. All the isolates were susceptibles to Ertapenem and Imipenem.

The notion of antibiotic supplementaion

The notion of antibiotics supplementation could not be found in cattle and pigs at the abbatoir. It was found in poultry intensive breeding (100%) and in poultry extensive breeding (47.06%). The antibiotics used in supplementation were oxytetracyclin and colistin exclusively in poultry extensive breeding (100%) and majoritary in poultry intensive breeding (66.39%). In one site (Farakoba) six (6) antibiotics had been used in supplementation for poultry in intensive breeding (Neomycin, Doxycyclin, Erythromycin, Oxytetracyclin, Colistin and Streptomycin). In this site, among 100 poultry sampled, one (1) was ESBL producing.

Molecular characterization of ESBL and other β -lactamase genes

The PCR detected three resistance genes carried : one ESBL (Bla CTX-M15) and two beta lactamase gene (Bla TEM, Bla Oxa – 1 like).

E. coli phylogenetic groups

Phylogenetic groups could not be determined in all E. coli strains. The E. coli strains were distributed in nine phylogroup different. The phylogroup A was the most represented among all the animals :56.34% (40/71) among cattle, 58.50% (86/147) among pigs and 60.71% (17/28) among poultry.

Discussion

In this study we investigated for the first time in Burkina Faso, the prevalence of ESBL in extensive and intensive breeding animals. Our finding show high prevalence of ESBL in livestock in Burkina Faso (41.03% among cattle, 69.6% among pigs and 19.6% among poultry in extensive breeding). E. coli was the most common enterobacterial species producing ESBL (278/315). One ESBL gene, belonging to CTX-M-1 group, has been found in our study ; it was the blaCTX-M-15 gene. This blaCTX-M-15 gene was often associated with blaTEM gene and BlaOxa-1-like gene.

This study reiterates the finding in other studies worldwide, that have reported antibiotic resistance among bacteria especially E. coli isolated from animals is increasing at an alarming rate [20, 12, 21].

Earlier studies reported that Klebsiella species and Escherichia coli are the mostly species which produce ESBLs [22]. E. coli is considered an indicator, being a commensal bacterium ubiquitous in animals and capable of providing relevant hints on the spread of the antibiotic resistance [23].

During the last years, ESBLs belonging to the CTX-M family of enzymes have been reported worldwide from a variety of different food-producing animals, including cattle, chickens, and pigs [24, 25, 26, 12], and these animals are recognized as reservoirs of extended-spectrum β -lactamase producers [27].

Focusing on the diversity of ESBL enzymes among E. coli isolates from African livestock and worldwide, those belonging to CTX-M-1 group have demonstrated to be more abundant than other ESBL groups or types (SHV or TEM ESBLs). In the majority of the surveys, blaCTX-M-15 was the most common ESBL gene detected worldwide in animals and humans. [12, 28, 29] This result has been also found in Burkina Faso among human clinical samples and fecal carriage [30, 31, 32]. Then E. coli strains belonged majoritary to phylogroup A in our study. The majority of the african studies showed also a dominance of phylogroups A and B1 among healthy animals, derived meat, human clinical samples and human fecal carriage [12, 30, 31].

In our study, the prevalence of ESBLs was statistically higher among poultry extensive breeding than poultry intensive breeding and the notion of antibiotics supplementation was found among both. This notion of antibiotics supplementation could not be found among pigs and cattle. Cephalosporins belong to the β -lactam antibiotics, which can be hydrolyzed by extended-spectrum β -lactamases and thereby exert a selective pressure on ESBL-producing bacteria [14]. Despite in our study, like in several countries, cephalosporins are not used for animals, but high prevalence of ESBL-producing bacteria remains [33, 34, 17]. This suggests that there are additional sources for the contamination with ESBL-producing bacteria in livestock [35]. Our situation may be explained by an environmental contamination. Indeed, ESBL-producing bacteria may spread to environment by waste products from human activities and animal production [36].

In addition, large amounts of antibiotics are not metabolized, retaining their activities even after renal excretion [37]. Hence, the propagation of active antibiotics and metabolites represents another route of transmission from farms, hospital and human wastes (fecal peril) to the environment.

Large proportions of seemingly innocuous commensal bacteria continually exchange genes, completely unnoticed [38], mobilizing resistance genes. These bacteria are a largely ignored reservoir of resistance and provide many complex pathways by which the resistance genes present in animals may directly or more likely indirectly find a way to reach human pathogens through food, water, mud, and manure applied as fertilizers. It was observed that gut microbiota may be considered hubs for the transfer of resistance genes [39].

In this study, the antibiotics more used in supplementation were oxytetracyclin and colistin. Although nontherapeutic use in Europe and North America was forbidden, in many countries, the “antibiotic growth promoters” phenomenon (i.e., the addition of antibiotics to subtherapeutic concentrations in order to increase the growth rate, favoring spread and persistence of resistant bacteria and their genesis) has been spreading. In fact, the chronic use of a single antibiotic can enable resistance to more structurally unrelated antibiotics, linked on plasmid and transposon genes [40].

Conclusions

This study has revealed, for the first time, the high prevalence of fecal carriage of CTX – M -15 ESBL among pigs, cattle and poultry in breeding in Burkina Faso. This study, strongly indicate the urgent need to establish integrated national programs of surveillance of antimicrobial use and occurrence of ESBL-producing bacteria and other antimicrobial-resistant bacteria (with zoonotic potential) among people, livestock and environment in Burkina Faso.

Methods

Samples collection : from March to June 2017 in the Bobo Dioulasso area, we investigated stool samples collected from healthy animals (cattle = 251; pigs = 250 and poultry = 397) in one (1) slaughterhouse, five (5) livestock farms and one (1) poultry market (Figure 2). Cattles and pigs intended for slaughter at the

slaughterhouse are transported from urban and peri – urban farms and distant villages around Bobo Dioulasso area. For cattles and pigs, samples were collected with swab from the colon immediately after slaughtering the animal at the abattoirs aseptically. For poultry we made a cloacal swab.

Isolation and identification : The swab were immediately incubated in Broth Heart Brains at 37°C for 12h hours before being inoculated in Hektoen media supplemented with 4µl/ml cefotaxime at 37°C during 24h as previously described [17]. The strain *E. coli* ATCC 25922 was used as negative control and the strain *K. pneumoniae* ATCC 700603 was used as positive control. The strains were stored at -80°C until their identification by MALDI-TOF at Arnaud De Villeneuve teaching hospital at Montpellier (France).

Susceptibility test : Antimicrobial susceptibility was tested with the disk diffusion method on Müller-Hinton agar. The following antibiotics were tested: amoxicillin, amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, cefpirome, cefpodoxime, cefoxitin, ceftazidime, cephalotin, moxalactam, piperacillin, piperacillin-tazobactam, ticarcillin, ticarcillin-clavulanic acid, imipenem, nalidixic acid, ciprofloxacin, levofloxacin, ofloxacin, amikacin, gentamicin, netilmicin, tobramycin, fosfomycin, chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (Version 5.0) (http://www.eucast.org/clinical_breakpoints/).

ESBL production was detected by using the combined double-disk synergy method [18]. In case of high level production of cephalosporinase, the double – disk synergy was performed using cloxacillin supplemented medium (250 mg/L).

DNA was extracted from one single colony for each isolate in a final volume of 100 µL of distilled water by incubation at 95°C for 10 min followed by a centrifugation step. The presence of, blaCTX-M (CTX-M group 1, 2, 8, 9 and 25), blaTEM, blaSHV and blaOXA-1-like genes was assessed by multiplex PCR according to a previously published method [19]. DNA from reference blaCTX-M, blaTEM, blaSHV-positive strains was used as positive control. PCR products were visualized after electrophoresis on 1.5% agarose gels containing ethidium bromide at 100 V for 90 minutes. A 100 bp DNA ladder (Promega, USA) was used as a marker size. PCR products were purified using the ExoSAP-IT purification kit (GE Healthcare, Piscataway, NJ, USA) and sequenced bidirectionally on a 3100 ABI Prism Genetic Analyzer (Applied Biosystems). Nucleotide sequence alignment and analyses were performed online using the BLAST program available at the BLAST program available at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

Statistical analysis

Data were analysed with /IC version 13.0. Difference in the proportion of ESBL-producers between poultry groups were assessed using the Chi-square test. A p value < 0.05 was considered as statistically significant.

Abbreviations

ESBL = Extended spectrum beta-lactamases ; CTX-M = Cefotaximase Munich ; MALDI-TOF: matrix-assisted laser desorption ionization-time of flight ; TEM= Temoneira ; SHV= Sulfhydryl Variable

Ethics approval and consent to participate

For the conduct of this study we received the authorization of the provincial management of animals and halieutics resources of Bobo Dioulasso.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SS, ASO, SG, JPH, Conceived and designed the experiments

SS, AZ collected the samples and done the preliminary experiments

SS, ML, OOM realized the experiments

SS, AP, JZ, RT/O, ASO, GAO, CC, JPH, SG Contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: Distribution of enterobacteriaceae producing ESBL carried by animals

	Size of samples	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>C. amalonaticus</i>	Total
Cattle	251	88	17	00	105
Pig	250	159	18	01	178
Poultry intensive breeding	244	02	00	00	02
Poultry extensive breeding	153	29	01	00	30
Total	898	278	36	01	315

Table 2 : Analysis of antibiotic resistance genes carried

	<i>E. coli</i> (%)			<i>K. pneumoniae</i> (%)		
	Cattle	Pigs	Poultry	Cattle	Pigs	Poultry
Bla <i>CTX-M15</i>	27/88 (30.68)	50/159 (31.45)	7/32 (21.88)	1/17 (5.88)	5/18 (27.78)	0/1 (0)
Bla <i>TEM</i>	3/88 (3.41)	2/159 (1.26)	0/32 (0)	0/17 (0)	0	0/1 (0)
Bla <i>Oxa - 1 like</i>	1/88 (1.14)	0	0/32	0/17	0	0/1 (0)
Bla <i>CTX-M15+TEM</i>	45/88 (51.14)	76/159 (47.80)	9/32 (28.13)	9/17 (52.94)	8/18 (44.44)	0/1 (0)
Bla <i>CTX-M15+ Oxa - 1 like</i>	5/88 (5.68)	13/159 (8.18)	4/32 (12.50)	0/17 (0)	1/18 (5.55)	0/1 (0)
Bla <i>CTX-M15+TEM+Oxa-1 like</i>	6/88 (6.82)	16/159 (10.06)	12/32 (37.50)	5/17 (29.41)	3/18 (16.67)	1/1 (100)

Table 3 : Distribution of *Escherichia coli* phylogenetic group among the animals

	A	B1	B2	C	Clade I	D	E	F	Unknown
Cattle	40	10	0	4	5	1	0	3	8
Pigs	86	15	2	27	4	2	1	4	6
Poultry	17	1	0	8	0	0	0	2	0
Total	143	26	2	39	9	3	1	9	14

Figures

Figure 1

Susceptibility patterns of Enterobacteriaceae isolates of animals on the classes of antibiotics tested.

Figure 2

Mapping of sample collection sites.