



Research



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Epidemiology and molecular characterization of *Enterobacteriaceae* producing extendedspectrum β-lactamase in intensive and extensive breeding animals in Burkina Faso

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Abstract

Introduction: extended-spectrum & lactamases (ESBL) determinants have been detected in clinical isolates and commensal bacteria from humans and animals. We investigated, the ESBL-producing Enterobacteriaceae in stool from intensive and extensive breeding animals (cattle, pigs, and poultry) in Burkina Faso. Methods: we identified our bacterial strains by MALDI-TOF. Antimicrobial susceptibility was tested with Kirby Bauer method and ESBL genes identified by conventional Polymerase Chain Reaction (PCR). 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 gene 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. pneumonia (36/315). The ESBL genes carried were CTX-M 15, TEM, and Oxa-1-like. These three 6-lactamase genes were associated in some bacterial strains. The E. coli strains belonged most commonly to the phylogroup A.

Conclusion: this high level of resistance of Enterobacteriaceae to antibiotics in livestock in Burkina Faso by the production of ESBL, could suggest environmental contamination of the livestock with ESBL-producing bacteria.

Introduction

Antimicrobial resistance (AMR) is a global public health threat. The first global surveillance report on antibiotic resistance (ABR) published by WHO in 2014, shows that 45% of deaths in both Africa and South-East Asia were due to multi-drug resistant (MDR) bacteria [1]. Antimicrobial use, misuse, or overuse in clinical medicine is a major contributing factor in the development of AMR in human populations [2-4]. 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]. Conversely, studies have also shown that various multidrug-resistant bacteria lineages from animals also appear in humans [8,9]. 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 [10].

According to the Food Agriculture and Organization of the United Nations (FAO), worldwide, pork (36.3%), chicken (35.2%), and beef (22.2%) are the most common meat sources [11]. Production of extended-spectrum βlactamases (ESBLs) is the most common mechanism of resistance to third-generation cephalosporins among Enterobacteriaceae [12,13]. These resistant strains are considered a significant public health issue due to the limited therapeutic [1] options and increased morbidity and mortality associated with them [2]. The true prevalence of ESBL is not well-known in Africa and is probably underestimated because of the paucity





of studies in human health, animal health, and the food chain on the continent [14]. Here, we investigate the epidemiology and molecular characterization of *Enterobacteriaceae* producing extended-spectrum β -lactamase in intensive and extensive breeding animals in Burkina Faso.

Methods

A descriptive cross-sectional study was carried out for four (4) months from March to June 2017 in the Bobo-Dioulasso area.

Samples collection: we investigated stool samples collected from healthy animals (cattle = 251; pigs = 250, local poultry = 153 and exotic poultry = 244) in one (1) slaughterhouse (for cattle and pigs), five (5) livestock farms, and one (1) poultry market (for poultry) (Figure 1). For cattles and pigs, samples were collected aseptically with a swab from the colon immediately after slaughtering the animal at the abattoirs. For poultry, we made a cloacal swab. The concept of antibiotic supplementation was investigated through a questionnaire addressed to farm staff.

Isolation and identification: the swab were immediately incubated in broth heart brains. After 12-hours of incubation at 37°C, broths were subcultured on Hektoen media supplemented with 4µg/ml cefotaxim at 37°C for 24h as previously described [15,16]. Each type of colony has been purified on Hektoen media supplemented with 4µg/ml cefotaxim at 37°C for 24h and the strains were stored at -80°C until their identification by MALDI-TOF at Arnaud De Villeneuve teaching hospital at Montpellier (France). The strain *E. coli* ATCC 25922 was used as negative control and the strain *K. pneumonia* ATCC 700603 was used as a positive control.

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, cefalexin, cefepim, cefotaxim, cefpodoxim, cefoxitin, ceftazidim, piperacillin, piperacillin-

tazobactam, ticarcillin, ticarcillin-clavulanic acid, imipenem, ertapenem, nalidixic acid, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, fosfomycin, chloramphenicol, and trimethoprimsulfamethoxazol. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (Version 5.0) [17]. Extendedspectrum β-lactamase production was detected by the combined double-disk using synergy method [18]. For this purpose, Cefepim disc and Ticarcillin + Clavulanic acid disc were used because of the possibility of associated cephalosporinase production. Deoxyribonucleic acid (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, bla_{CTX-M} (CTX-M group 1, 2, 8, 9, and 25), bla_{TEM} , bla_{SHV} , and bla_{OXA-1-like} genes was assessed by multiplex PCR according to a previously published method [19]. Deoxyribonucleic acid from reference *bla*_{CTX-M}, bla_{TEM} , bla_{SHV} -positive strains were used as positives controls. E. coli phylogenetic grouping was performed using the PCR-based method described by Clermont et al. [20]. Polymerase chain reaction products were visualized after electrophoresis on 1.5% agarose gels containing ethidium bromide at 100 V for 90 minutes. A 100 bp Deoxyribonucleic acid ladder (Promega, USA) was used as a marker size. Polymerase chain reaction products were purified using the ExoSAP-IT purification kit (GE Healthcare, Piscataway, NJ, USA) and sequenced bi-directionally on a 3100 ABI Prism Genetic Analyzer (Applied Biosystems). Nucleotide sequence alignment and analyses were performed online using the BLAST program available at the National Center for Biotechnology Information [21].

Statistical analysis: data were analyzed with Stata/IC version 13.0. The difference in the proportion of ESBL-producers between poultry groups was assessed using the chi-square test. A p-value <0.05 was considered statistically significant.





Ethics approval and consent to participate: Burkina Faso does not have an ethics committee for animal studies, so we obtained written permission from the provincial department of animal resources of Houet (covering our study area) to conduct our study.

Results

We identified three (3) enterobacterial species producing ESBL: E. coli (278/315), K. pneumonia (36/315), and Citrobacter amalonaticus (1/315). Enterobacteriaceae producing ESBL was detected in 41.03% of cattle (103/251), 69.6% of pigs (174/250).Among poultry, the the Enterobacteriaceae producing ESBL was detected in 0.8% of poultry in intensive breeding (02/244) and 19.6% of poultry in extensive breeding (30/153) (p-value Ë,10-3) (Table 1). The PCR detected three resistance genes carried: one ESBL _{CTX-M15}) and two beta-lactamase genes (Bla (Bla TEM, Bla Oxa - 1 like) (Table 2). Phylogenetic groups could not be determined in all E. colistrains. The E. coli strains were distributed in nine phylogroup differents. 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 (Table 2).

The notion of antibiotics supplementation: this notion was found among poultry extensive and intensive breeding.

Antibiotic susceptibility patterns of *Enterobacteriaceae* isolates: the overall resistance of the isolates to antibiotics, (Figure 2), shows that resistance to amoxicillin (100%), ceftazidim (99.05%), and cotrimoxazol (86.39%) 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.

Discussion

In this study, we investigated for the first time in Burkina Faso, the prevalence of ESBL in extensive and intensive breeding animals. Our findings show a 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 the 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 the blaTEM gene and the 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 [12,22,23]. Earlier studies reported that Klebsiella species and E. coli are the most species that produce ESBLs [24]. E. coli is considered an indicator, being a commensal bacterium ubiquitous in animals and capable of providing relevant hints on the spread of antibiotic resistance [25].

The ESBLs belonging to the CTX-M family of enzymes have been reported worldwide from of different food-producing а variety animals [12,26-28], and these animals are recognized as reservoirs of ESBLs producers [27]. In the majority of the surveys, blaCTX-M-15 was the most common ESBL gene detected worldwide in animals and humans [12,29,30]. This result has been also found in Burkina Faso among human clinical samples and fecal carriage [31,32]. Then E. coli strains belonged majority to phylogroup A in our study. The majority of African studies showed also a dominance of phylogroups A and B1 among healthy animals, human clinical samples, and human fecal carriage [12,31,32].

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, cattle (at slaughterhouse), and poultry (in the poultry market). Indeed, the animals intended for slaughter come from the urban livestock farms of Bobo-Dioulasso (38%) and the villages near and far on the periphery of Bobo-Dioulasso (62%) [33]. There are two main poultry production systems in Burkina Faso, the extensive and the intensive system. The former, using local poultry, is well spread throughout the country. The intensive system, restricted to some peri-urban areas, uses exotic breeds. Rural poultry production is widely distributed important and among smallholder farms [34]. Despite in our study, like in several countries, cephalosporins are not used for animals, but the high prevalence of ESBLproducing bacteria remains [35,36]. This suggests that there are additional sources for the contamination with ESBL-producing bacteria in livestock [37]. Our situation may be explained by environmental contamination. Indeed, ESBLbacteria may spread producing to the environment by waste products from human activities and animal production [38]. In Burkina Faso, extensive livestock farming is mainly practiced. In this extensive system, animals reared in partial confinement with supplementation, feed in the environment during pasturage (cattle) or their divagation (pigs, poultry) [33,34,39,40].

In this study, the antibiotics more used in supplementation were oxytetracyclin (Cyclin) and colistin. In Burkina Faso, antimicrobials are used in livestock in four areas: therapeutics, metaphylaxis, prophylaxis or prevention, and as a growth factor. A study conducted in 2016 found that 93.65% farms used antimicrobials as of growth promoters [41]. This practice, forbidden in Europe and North America, is also widespread in Africa [42-44]. In addition to their use as antibiotics supplements, are used as self-medication by 74.60% of livestock farmers in Burkina Faso [41]. And according to the evolution of clinical signs, 69.84% of farmers then use technical agents for veterinary care of the animals [41]. Gram positive bacteria, yeast, and fungi were not considered in this study. We were unable to determine the multi locus sequencing typing (MLST), conjugation experiment, and the supports of resistance genes.

Conclusion

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 indicates 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 the environment in Burkina Faso.

What is known about this topic

- Multidrug resistant bacteria are circulating among livestock in Africa;
- E. coli and K. pneumonia are the main multidrug resistant bacteria found in livestock;
- Antibiotics are widely used in livestock in Africa without respect for standards and regulations.

What this study adds

- The prevalence of MDR bacteria carriage in livestock in Burkina Faso is determined for the first time;
- The source of contamination of African livestock with MDR bacteria is probably the environment.

Competing interests

The authors declare no competing interests.

Authors' contributions

SS, ASO, SG and JPH, conceived and designed the experiments. SS and AZ collected the samples and





done the preliminary experiments. SS, ML and OOM realized the experiments. SS, AP, JZ, RT/O, ASO, GAO, JPH and 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 Enterobacteriaceaeproducing ESBL carried by animals

Table 2: analysis of antibiotic resistance genescarried and distribution of *E. coli* phylogroups

Figure 1: mapping of samples

Figure2:susceptibilitypatternsofEnterobacteriaceaeisolatesofanimalsontheclasses of antibiotics tested

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Table 1: distribution of Enterobacteriaceae producing ESBL carried by animals								
	E. coli	K. pneumoniae	C. amalonaticus	Total Enterobacteriaceae producing ESBL				
Cattle (N=251)	88	17	0	105				
Pig (N=250)	159	18	1	178				
Poultry intensive breeding (N=244)	2	0	0	2				
Poultry extensive breeding (N=153)	29	1	0	30				
Total	278	36	1	315				



Table 2: analysis of antibiotic resistance genes carried and distribution of <i>E. coli</i> phylogroups									
		E. coli (%)		K. pneumoniae (%)					
		Cattle	Pigs	Poultry	Cattle	Pigs	Poultry		
Genotype ESBL	Bla CTX-M15	27/88	50/159	7/32	1/17	5/18	0/1 (0)		
		(30.68)	(31.45)	(21.88)	(5.88)	(27.78)			
	Bla TEM	3/88 (3.41)	2/159 (1.26)	0/32 (0)	0/17 (0)	0	0/1 (0)		
	Bla Oxa-1 like	1/88 (1.14)	0	0/32	0/17	0	0/1 (0)		
	Bla CTX-M15+TEM	45/88 (51.14)	76/159 (47.80)	9/32 (28.13)	9/17 (52.94)	8/18 (44.44)	0/1 (0)		
	Bla CTX-M15+ Oxa-1 like	5/88 (5.68)	13/159 (8.18)	4/32 (12.50)	0/17 (0)	1/18 (5.55)	0/1 (0)		
	Bla CTX- M15+TEM+Oxa-1 like	6/88 (6.82)	16/159 (10.06)	12/32 (37.50)	5/17 (29.41)	3/18 (16.67)	1/1 (100)		
Phylogroups	A	40/143	86/143	17/143	-	-	-		
	B1	10/26	15/26	1/26	-	-	-		
	В2	0/2	2/2	0/2	-	-	-		
	С	4/39	27/39	8/39	-	-	-		
	Cladel	5/9	4/9	0/9	-	-	-		
	D	1/3	2/3	0/3	-	-	-		
	E	0/1	1/1	0/1	-	-	-		
	F	3/9	4/9	2/9	-	-	-		
	Unknown	8/14	6/14	0/14	-	-	-		





Figure 1: mapping of samples

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Figure 2: susceptibility patterns of *Enterobacteriaceae* isolates of animals on the classes of antibiotics tested