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Original Article

Characterization of extended-spectrum beta-lactamase and carbapenemase genes in bacteria from environment in Burkina Faso

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Abstract

Introduction: This study aimed to characterize extended-spectrum beta-lactamase (ESBL) and carbapenemase genes in bacteria from the environment in Bobo-Dioulasso, Burkina Faso.

Methodology: This study was conducted from January 18 to December 31, 2019. Environmental samples were collected from the effluents of Souro Sanou University Hospital Center and the wastewater treatment plant at Bobo-Dioulasso. MacConkey agar media supplemented with 4 µg/mL cefotaxime was used for bacterial growth, and identification of bacteria was performed using API 20E system (BioMerieux SA, Lyon, France). Antibiotic susceptibility testing, synergy test, carbapenem inactivation method and molecular characterization were performed.

Results: A total of 180 bacterial isolates were identified from the different sites with a predominance of *Klebsiella oxytoca* and *Klebsiella pneumoniae* (27.5%). All 180 bacterial isolates were ESBL producers and 18 (10.0%) of them produced carbapenemases. Out of the 180 bacterial isolates, DNAs of 98.9% (178/180) bacterial isolates were extracted and tested through polymerase chain reaction (PCR) for characterization of resistant genes. The study showed that 89.8% (160/178) carried the *bla-$_{CTX-M}$* genes including 54.4 (87/160) from hospital effluents and 45.6 (73/160) from the wastewater treatment plant. Regarding the carriage of carbapenemase genes, 7.9 (14/178) *bla$_{NDM-1}$* was found in all the sites including 71.4% (10/14) from hospital effluents and 28.6 (4/14) from the wastewater treatment plant. *bla$_{OXA-48}$*-like was only found in bacteria from hospital effluents and represented 2.2% (4/178).

Conclusions: This study highlights the need to build hospital effluent treatment plants to reduce the load of resistant bacteria before discharging the effluents into the urban wastewater system.

Key words: antimicrobial resistance; ESBL; carbapenemase; genes; wastewater; Burkina Faso.

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Introduction

The emergence and spread of multidrug resistant (MDR) bacteria represent a major public health threat. MDR bacteria have acquired resistance to several families of antibiotics and high burden of antibiotic resistance are reported in both high-income and low-income countries [1]. Resistant bacterial pathogens are considered to be the main cause of community and nosocomial infections and associated with high mortality and morbidity rates [2]. These bacteria have developed different mechanisms that confer resistance to several antibiotics. The resistance mechanisms include extended-spectrum beta-lactamases (ESBLs) and carbapenemases, which confer resistance to third-generation cephalosporins and carbapenems, respectively [3].

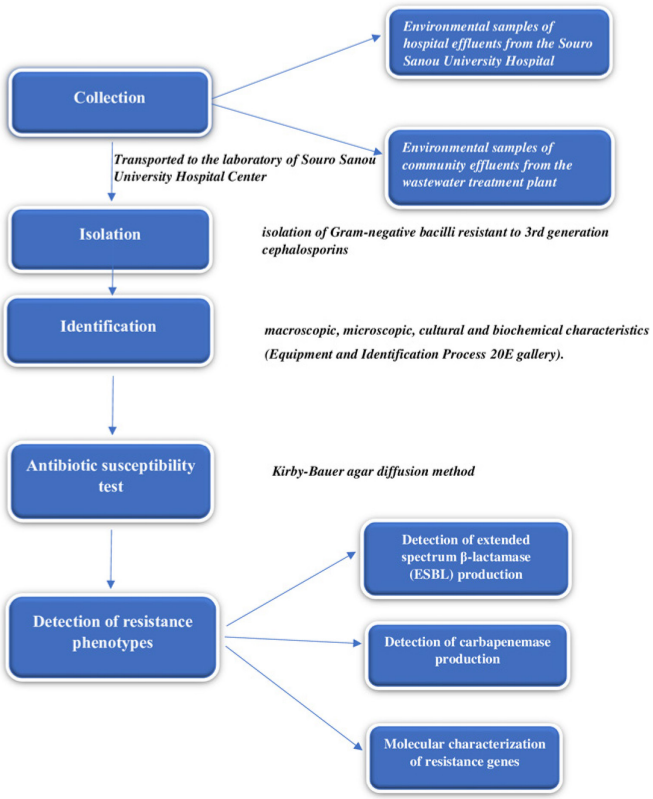
The ESBLs represent the most widespread mechanism of multidrug resistance. ESBLs are enzymes capable of hydrolyzing all beta-lactam antibiotics except cephamycins, the new "5th generation" cephalosporins and carbapenems. The first ESBL enzymes, derived from temoneira (TEM) and sulfhydral variable (SHV) penicillinases, were isolated from a strain of *Klebsiella ozaenae* in Germany in 1983

[4], and then from *Klebsiella pneumoniae* in France in 1985 [5]. Four years later, in 1989, Bauernfeind *et al.* again characterized an ESBL enzyme in *E. coli* in Germany, which they named CefoTaXimase Munich (CTX-M) because of its marked preferential activity on cefotaxime compared with ceftazidime [6]. The enzymes are mainly found in Gram-negative bacteria belonging to the family Enterobacterales which carry beta-lactam and carbapenem resistance genes [7]. Most of these resistance genes are carried by mobile genetic elements (MGEs) such as plasmids (e.g., IncF, IncX3), transposons or integrons [8]. The latter facilitate the circulation and exchange of resistance genes not only between different isolates and bacterial species but also between different ecosystems (human, animal and environment), thus explaining their rapid spread and dissemination [9]. A coordinated and multisectoral approach, referred to as "One Health" strategy, is required to address this problem of resistance because of its multidimensional and multifactorial complexity (human, animal and environment) [1].

In low-income countries such as Burkina Faso, there are several factors such as poverty, unfavorable economic conditions associated with malnutrition, inaccessibility to drinking water and difficult living conditions that contribute to an increase in antimicrobial resistance (AMR) [10]. Beyond these unfavorable social and economic conditions, the abusive use of antimicrobials in the animal and food industry also represents an important MDR risk within an environment [11].

The effluents from hospital activities could be potentially harmful not only to humans but also to their environment since they could contain noxious substances such as drug residues, chemical reagents, antiseptics, detergents, heavy metals (especially mercury and silver) and x-ray fixatives [12]. Hospital effluents with traces of antibiotic agents could represent a reservoir of multidrug-resistant bacteria as well as viruses and fungi [12]. Despite the importance of the problem and its health and economic consequences, studies focusing on the distribution of MDR bacteria within different ecosystems are scarcely carried out in Burkina Faso. Indeed, the contribution of AMR research in ecology is an essential research area, which could improve the different control strategies of antibiotic resistance. This study aimed to characterize extended-spectrum beta-lactamase (ESBL) and carbapenemase genes in bacteria from environment in Bobo-Dioulasso, Burkina Faso.

Figure 1. Flowchart of the methods used in the study.



Methodology

The following flowchart described all the techniques used in this study (Figure 1)

Study design and sites

Souro Sanou University Hospital Center (CHUSS) and the wastewater treatment plant (WWTP) were the two sites for sample collection. CHUSS is a 3rd level National University Hospital Center and referral hospital for health centers located in the Hauts-Bassins, Cascades, Boucle du Mouhoun and South-West regions of Burkina Faso. The WWTP in Bobo-Dioulasso consisted of two anaerobic ponds, one optional pond, pre-treatment settling tanks, one Parshall channel for flow measurements, 1 sludge depositor of length, one service building and one guard's lodge (Figure 2).

Sample collection

Wastewater and effluent samples were collected during the rainy season (September) and dry season (December) in 2019. A total of 50 effluent samples were collected from the exit of the sewer of CHUSS. For sample collection at the WWTP, 25 wastewater samples were obtained at the inlet of the treatment plant and 25 wastewater samples at the outlet of the WWTP. Approximately, 500 mL of wastewater was collected into a clean sterile bottle at each site. After sampling,

the samples were immediately kept at 4 °C and transported to CHUSS for bacteriological analyses.

Isolation and identification

Each sample was diluted with physiological water using a 10-fold serial dilution (10-1 to 10-3). Quantification of third-generation cephalosporin-resistant Gram-negative bacilli was performed by spreading 100 µL of the 10-2 dilution of each sample on MacConckey agar medium supplemented with 4 µg/mL cefotaxime. All media were incubated at 35 ± 37 °C in the incubator for 24 h. API 20E (BioMerieux SA, Lyon, France) system was used for identification of bacterial isolates.

Antibiotic susceptibility test

Antibiotic susceptibility test was performed as per the Kirby-Bauer agar diffusion method and the results were interpreted according to the recommendations of the French Society of Microbiology (CA-SFM) [13]. The susceptibility profile of the bacterial strains was determined using 30 µg cefotaxime (CTX), 20/10 µg amoxicillin/clavulanic (AMC), 10 µg imipenem (IPM), 10 µg gentamicin (GEN), 5 µg ciprofloxacin (CIP) and 30 µg amikacin (AK) (Liofilchem diagnostic, Roseto degli Abruzzi, Italy). The results were interpreted according to the CAS-SFM 2019 guidelines [14].

Identification of resistance phenotypes

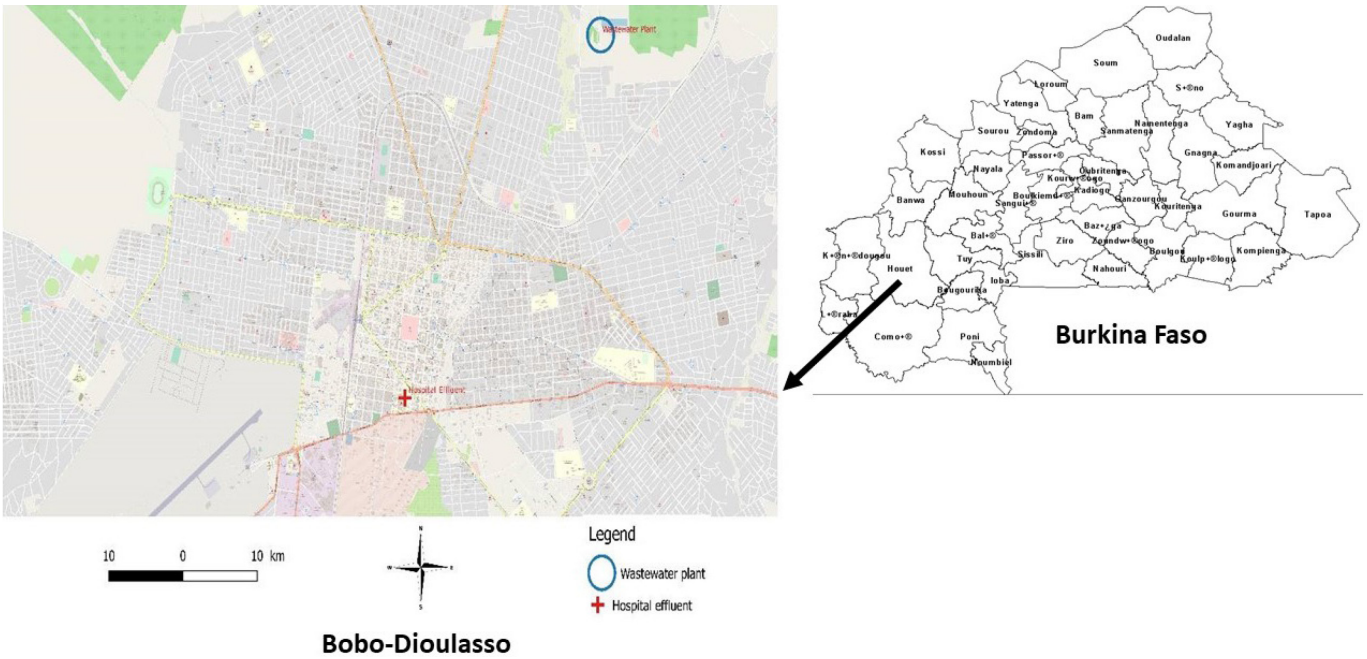
Extended spectrum β-lactamase (ESBL) production

ESBL detection was performed phenotypically through a double disk diffusion method by determining presence of a synergy zone between AMC and a third-generation cephalosporin disc cefotaxime (CTX), which is referred to as ‘’champagne cork’’ [13]. In addition, synergy zone between a disk of 75 µg ticarcillin associated with clavulanic acid (TTC) and 30 µg cefepime (FEP) was also investigated on the suspected ESBL-producing bacterial strains for which "champagne cork" could not be observed.

Carbapenemase production

Carbapenem inactivation method (CIM) was performed on all strains with reduced or resistant susceptibility to imipenem [15]. A few colonies of the strain with reduced or resistant susceptibility to 10 µg imipenem (IMP) were inoculated into 200 µL physiological water. Subsequently, a 10 µg meropenem disc (MER) was immersed in the suspension and incubated for at least 4 hours at 37 °C. After incubation, the disk was removed from the suspension using a sterile inoculating loop, placed on a Mueller-Hinton agar plate (Liofilchem Diagnostic, Roseto Degli Abruzzi, Italy), previously inoculated with a susceptible *E. coli* indicator strain (ATCC 29522), and the plate was

Figure 2. The study sampling sites.



The red cross represents the site for collection hospital wastewater samples while the blue circle corresponds to the site for collection of waste water from the wastewater treatment plant in Bobo-Dioulasso (Source: DIVA-GIS 7.5.0).

incubated at 37 °C for 24 h. Resistance to meropenem was considered as the production of carbapenemase.

Control quality

K. pneumoniae ATCC 700603 and *E. coli* ATCC 25922 were used as reference strains in this study for the detection of *E. coli* and *K. pneumoniae* ESBL producers.

Molecular characterization of resistance genes

After DNA extraction, the amplification of ESBL and carbapenemase genes was performed according to the conditions described by Dallenne *et al.* [16]. Further details are provided in the Supplementary Method. The group-specific primers used in this experiment are described in Table 1.

Results

Isolation of 3rd generation cephalosporin resistant isolates

A total of 100 wastewater samples were collected during this study. One hundred and eighty-three (183) 3rd generation cephalosporin resistant bacterial strains were isolated from the two sites. The prevalence of strains was high at CHUSS with 53.5% (Table 2).

Identification of bacterial isolates

Out of the 183 bacterial isolates identified in this study, a predominance of *Klebsiella oxytoca* and *Klebsiella pneumoniae* isolates was found at the two study sites (Table 3). *K. pneumoniae* and *K. oxytoca* isolates represented the same proportion (27.5%) in the hospital effluents while they accounted for 14 and 16%, respectively in the wastewater treatment plant (WWTP).

Resistance profile of the bacterial isolates

All strains of the 180 isolates were resistant to amoxicillin, cefotaxime and amoxicillin/clavulanic acid combination with 100% prevalence in both the hospital and WWTP. Low rates of resistance to imipenem ranging from 11 to 50% were observed in strains isolated at the hospital and from 4 to 44% for strains isolated at the WWTP. Ciprofloxacin resistance ranged

Table 1. Group-specific primers used for the assays [16].

Gene	Primer	Sequences (5'-3')	Amplicon size
CTX-M Gp1and 9	CTX-M-1 group for[†]	TTAGGAARTGTGCCGCTGYA	688
	CTX-M-1 group rev[ɛ]	CGATATCGTTGGTGGTRCCAT	
	CTX-M-9 group for	TCAAGCCTGCCGATCTGGT	561
	CTX-M-9 group rev	TGATTCTCGCCGCTGAAG	
NDM-1	NDM-1 for	CATTTCCGTGTCGCCCTTATTC	178
	NDM-1 rev	CGTTCATCCATAGTTGCCTGAC	
OXA-48-like	OXA-48_for	GCTTGATCGCCCTCGATT	281
	OXA-48_rev	GATTTGCTCCGTGGCCGAAA	

[†]Forward primer; [ɛ]Reverse primer.

Table 2. Prevalence of 3rd generation cephalosporin resistant bacterial isolates by sampling site.

Sample site	Total samples	Total number of bacterial isolates	Prevalence (%)
CHUSS[ɛ]	50	98	53,6
WWTP [ϕ] inlet	25	50	27,3
WWTP [ϕ] outlet	25	35	19,1
Total	100	183	100

[ɛ] Souro Sanou University Hospital Center; [ϕ] wastewater treatment plant.

Table 3. Distribution of 3rd generation cephalosporin resistant bacteria identified by sampling site.

Bacterial isolates	CHUSS[ɛ]		WWTP[ϕ] inlet		WWTP[ϕ] outlet	
	n[ǂ]	%[β]	*size*	%	*size*	%
Escherichia coli	19,0	19,3	06	12	07	20
Klebsiella pneumoniae	27,0	27,5	10	20	04	11,4
Klebsiella oxytoca	27,0	27,5	11	22	05	14,2
Enterobacter cloacae	02	2,0	07	14	01	2,9
Citrobacter diversus	18	18,4	16	32	07	20
Citrobacter freundii	02	2,0	_	_	01	2,9
Proteus vulgaris	_	00	_	_	09	25,7
Proteus mirabilis	01	1,2	_	_	01	2,9
Pseudomonas aeruginosa	02	2,0	_	_	_	_
Total	98	100	50	100	35	100

[ɛ] Souro Sanou University Hospital Center; [ϕ] wastewater treatment plant; [ǂ] number of bacterial isolates; [β] prevalence in percentage.

from 89 to 100% in hospital isolates and from 46 to 100% in WWTP isolates. Gentamicin resistance ranged from 47 to 100% in hospital isolates and from 23 to 100% (Table 4).

Prevalence of ESBL and carbapenemase producers

This study showed that all MDR strains were ESBL producers both at the hospital and at the WWTP (Table 5). Regarding carbapenemase production, the CIM test

Table 4. Resistance profile of the bacterial isolates by sampling site.

Species	N[a]	AX[b] (%)		CTX[c] (%)		AMC[d] (%)		IMP[e] (%)		AK[f] (%)		GEN[g] (%)		CIP[h] (%)	
		CHUSS[ǂ]	WWPT[ϕ]	CHUSS	WWPT	CHUSS	WWPT	CHUSS	WWPT	CHUSS	WWPT	CHUSS	WWPT	CHUSS	WWPT
Escherichia coli	32	100	100	100	100	100	100	16	00	10	08	47	23	95	46
Klebsiella pneumoniae	41	100	100	100	100	100	100	11	00	07	07	63	43	89	86
Klebsiella oxytoca	43	100	100	100	100	100	100	18	44	11	06	63	62	97	100
Enterobacter cloacae	10	100	100	100	100	100	100	50	25	00	06	50	37	00	75
Citrobacter diversus	41	100	100	100	100	100	100	22	04	17	17	50	43	00	91
Citrobacter freundii	03	100	100	100	100	100	100	00	00	00	00	00	00	00	00
Proteus mirabilis	02	100	100	100	100	100	100	50	00	100	00	100	100	100	100
Proteus vulgaris	09	_	100	_	100	_	100	_	22	_	00	_	44	_	89
Pseudomonas aeruginosa	02	100	_	100	_	100	_	100	_	00	_	50	_	50	_

[a]number; [b]amoxicillin; [c]cefotaxime; [a]amoxicillin-acid clavulanic; [e]imipenem; [f]amikacin; [g]gentamicin; [h]ciprofloxacin; [ǂ] Souro Sanou University Hospital Center; [ϕ] wastewater treatment plant

Table 5. Prevalence of ESBL and carbapenemase producers by sampling site.

Species	CHUSS[ǂ]			WWTP[ϕ] inlet			WWTP[ϕ] outlet		
	n[1]	ESBL[α] (%)	Carbapenemase (%)	*n*[2]	ESBL (%)	Carbapenemase (%)	*n*[2]	ESBL (%)	Carbapenemase (%)
Escherichia coli	19,0	100	2,1	06,0	100	00	07,0	100	00
Klebsiella pneumoniae	27,0	100	10,4	10,0	100	00	04,0	100	00
Klebsiella oxytoca	27,0	100	2,1	11,0	100	4,7	05,0	100	4,7
Enterobacter cloacae	02	100	00	07,0	100	00	01,0	100	00
Citrobacter diversus	18	100	00	16,0	100	00	07,0	100	00
Citrobacter freundii	02	100	00	_	100	00	01,0	100	00
Proteus vulgaris	_	_	00	_	100	00	09,0	100	00
Proteus mirabilis	01	100	00	_	100	00	01,0	100	00
Pseudomonas aeruginosa	02	100	00	_	100	00	_	100	00
Total	98	100	14,6	50	100	4,7	35	100	4,7

[ǂ] Souro Sanou University Hospital Center; [ϕ] wastewater treatment plant; [α]extended-Spectrum beta-lactam; [1,2]isolates number

Table 6. Resistance genes profile in ESBL and Carbapenemase producers by sampling site.

Species	CHUSS[ǂ]			WWTP[ϕ] inlet			WWTP[ϕ] outlet		
	N[ɛ]	ESBL[#] resistant gene (n1) [¥]	Carbapenem resistant gene (n2) [ϕ]	N	ESBL resistant gene (n3) [ǂ]	Carbapenem resistant gene (n4) [β]	N	ESBL resistant gene (n3) [ǂ]	Carbapenem resistant gene (n4) [β]
Escherichia coli	19	CTX-M[ŧ]-1(19)	NDM[Ɲ]-1(2)	06	CTX-M -1(6)	_	07	CTX-M -1(7)	_
Klebsiella pneumoniae	27	CTX-M -1(26), CTX-M-9 (1)	NDM-1(6), OXA[ж]-48-like (4)	10	CTX-M -1(8)	_	04	_	_
Klebsiella oxytoca	27	CTX-M -1(27)	NDM-1(2)	11	CTX-M -1(9)	NDM-1(4)	05	CTX-M -1(5)	-
Enterobacter cloacae	02	_	_	07	CTX-M -1(7)	_	01	CTX-M -1(1)	_
Citrobacter diversus	18	CTX-M -1(11)	_	16	CTX-M -1(16)	_	07	CTX-M -1(7)	_
Citrobacter freundii	02	CTX-M -1(2)	_	_	_	_	01	_	_
Proteus vulgaris	00	_	_	_	_	_	09	CTX-M -1(5)	_
Proteus mirabilis	01	CTX-M -1(1)	_	_	_	_	01	CTX-M -1(1)	_
Total	96	87	14	50	46	4	35	27	0

[#]ESBL: Extended-Spectrum beta-lactam; [ɛ]isolates number; [¥]extended-spectrum beta-lactam resistant gene; [ϕ] carbapenem resistant gene; [ǂ] Souro Sanou University Hospital Center; [β] wastewater treatment plant; [ŧ] cefotaximase Munich group-1; [Ɲ] New Dehli metallo-beta-lactamase; [ж]oxacillinase-48-like.

showed carbapenemase production in 18 bacterial isolates (10%) produced by this enzyme, 14 (14.2%) of which were produced at the hospital and 4 (4.7%) at the WWTP.

Resistance genes profile of the bacterial isolates

Most ESBL-producing isolates were identified as *CTX-M* producers in group 1 and accounted for 99.14% (116/117) (Table 6). There were 87 (90.6%) *CTX-M* producing isolates at the hospital and 73 (86.9%) at WWTP. For carbapenemases, the overall rate of confirmation of carbapenem-resistant gene production was 18 (10%) (Table 6). There were 10 (10.6%) *NDM-1* producing isolates among hospital isolates and 4 (4.7%) among WWTP isolates. There were 2 (2%) *OXA 48-like* producing isolates among isolates from the hospital.

Statistical analysis

All data were collected with Excel version 2013 software, statistically analyzed using STATA SE 12 (64-bit). Qualitative data were expressed as proportions.

Discussion

The purpose of this study was to characterize ESBL and carbapenemase genes in bacteria collected from the environment in Bobo-Dioulasso, Burkina Faso. bla_{NDM-1} gene was identified in bacteria from wastewater for the first time in Burkina Faso [17].

MDR bacteria were more abundant in the hospital effluents (53.5%) than in wastewater (46.4%). Our findings are consistent with previous studies carried out in Africa that confirmed the presence and the role of hospital and urban effluents in the dissemination of MDR bacteria [18-23]. The presence of low concentrations of unmetabolized antibiotics discharged from these effluents could lead to selection pressure on bacteria and transform hospital effluents into hotspots for the dissemination of MDR bacteria [24].

Water samples collected at the entrance of the water treatment plant were more contaminated with MDR bacteria compared to water samples collected at the outlet with a prevalence of 27.3% and 19.1%, respectively. The high prevalence of MDR bacteria in the wastewater samples collected at the entrance of the WWTP is evident because this water was not subjected to any treatment while the water at the exit of the WWTP was considerably treated, and hence, would contain less amount of MDR bacteria. This suggests that WWTP has an effect on the reduction of MDR bacteria.

Klebsiella pneumoniae and *K. oxytoca* predominated among MDR bacteria isolated in both hospital and WWTP (inlet and outlet) in this study. Indeed, these bacterial isolates are generally considered as the most common species isolated in hospitals [25]. The presence of fecal coliforms often indicates contamination of fecal origin, and their density is proportional to the degree of pollution and reflects the density of carriage in the population [26]. The presence of these third-generation cephalosporin (C3G) resistant bacteria in WWTP (inlet and outlet) clearly points out the contamination of wastewater with multi-resistant coliforms, and therefore, the potential risk of their release into the environment without treatment.

The MDR bacteria were producers of extended-spectrum beta-lactamase (ESBL) and carbapenemase producers. Our findings are in line with the works conducted in East Africa in which the authors reported the presence of MDR *Enterobacteriaceae* species with different resistance profiles [27,28]. Regarding carbapenemase production, the low resistance rate among carbapenems is not surprising, as they are considered the antibiotics of last resort for treatment of severe cases of infectious diseases and are not easily affordable [3]. Moreover, the emergence of carbapenemase could be related to the limited antibiotics for the treatment of ESBL producers in hospital settings [3]. Previous studies reported presence of carbapenemase-producing clinical isolates in hospital effluents [17,22,29]. Resistance to the beta-lactams and carbapenems are also coupled with the resistance to fluoroquinolones and aminoglycosides in this study. Our results are consistent with those of Anssour *et al.* in Algeria [30] and Guessennd *et al.* in Ivory Coast [23]. Indeed, *CTX-M* genes are easily transmitted by plasmids that facilitate co-resistance [8]. In Burkina Faso, previous studies pointed out associated resistance to fluoroquinolones and aminoglycosides in humans [26,31]. As a result, we hypothesize that the bacteria isolated in this study could be related to those found in humans. This observation could indicate the transmission of MDR bacteria between human and environmental interfaces.

This study showed that the majority of the MDR bacteria (99.1%) carried the bla_{CTX-M} genes. This finding correlates with numerous studies carried out in Africa, which reported the presence of bla_{CTX-M} genes with a predominance of *CTX-M-1* isolated from wastewaters [17-19,22,30-35]. Nevertheless, ESBL genes identified in the bacteria present in wastewaters are variable in nature with *CTX-M*1 being predominant in the *CTX-M* group [36]. Their presence is often of

chromosomal origin and the gene is found in *Kluyvera* spp [9].

The resistance genes encoding ESBL production were detected alone or in association with one to two other ß-lactamase genes. The overall rate of carbapenemase production was 14 (7.8%) for bla_{NDM-1} gene and 4 (2.2%) for the bla_{OXA-48}-like gene. Our results are similar to previous studies in which bla_{CTX-M} 1,2,9; bla_{OXA-48}-*like*; bla_{CTX-M1}, bla_{NDM} and bla_{OXA-48} genes were found in carbapenemase-producing bacteria from effluents [19,29,30,37]. The carbapenem-resistant genes characterized in these studies have already been found in clinically relevant Gram-negative bacteria in the United States [38]. This may infer a strong possibility of dissemination and transmission of highly resistant pathogenic bacteria between humans and the environment.

Limitations

One of the main limitations of this study is the lack of sequencing and multilocus sequence typing of housekeeping gene that could enable to establish the relatedness between the microbial species from hospital effluents and wastewater.

Conclusions

This study highlighted the presence of ESBL and carbapenemase genes in the hospital effluents and municipal wastewater in Bobo-Dioulasso in Burkina Faso. It also showed the presence of bla_{CTX-M} and bla_{NDM-1} genes, which trigger co-resistance. This study underscores the need to build hospital effluent treatment plant in order to reduce the load of resistant microorganisms before discharging effluents into urban wastewater systems.

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Annex – Supplementary Items

Supplementary method. Details of molecular characterization of resistance genes.

DNA extraction was performed on a bacterial colony heated in a total volume of 100 mL of distilled water (9 °C for 10 min), followed by centrifugation of the bacterial cell suspension.

Total DNA extract (2 mL) of each bacterial isolate was subjected to multiplex polymerase chain reaction (PCR) in a 50-mL reaction mixture containing 1x PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200 mM of each deoxynucleotide triphosphate, a variable concentration of group-specific primers (Table 1) and 1 U of *Taq* polymerase (Sigma Aldrich, St Quentin Fallavier, France).

Amplification of extended-spectrum beta-lactamase (ESBL) was performed as follows: initial denaturation at 94 °C for 10 min, 30 cycles of 94 °C for 40 s, 60 °C for 40 s, and 72 °C for 1 min; and a final elongation step at 72 °C for 7 min performed by the MiniAmp thermal cycler (ThermoFisher Scientific, Waltham Massachusetts, USA). Regarding the amplification of carbapenemase genes, the annealing temperature was optimal at 55 °C and 57 °C for amplification of bla_{NDM} and bla_{OXA-48} genes, respectively [13]. Subsequently, the PCR products were separated through electrophoresis on a 2% agarose gel containing ethidium bromide for 1 h at 100 volts and visualized by ultra violet (UV) transillumination ((BiORAD, Hercules, USA).). A 100-bp DNA ladder (New England Biolabs, Ipswich, MA, USA) was used as a size marker [13].