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Spread and antibiotic resistance profile of pathogens isolated from human and hospital wastewater in Ouagadougou

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ARTICLEINFO ABSTRACT

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Background: The discharge of improperly treated hospital landfill presents an enormous public health risk. In Burkina Faso, the management of hospital's wastewaters and the current antibiotic susceptibility of clinically relevant isolates need to be determined, because the multi-drug resistant isolates have been previously described in hospital settings. The aim of this study was to determine antibiotic resistance profile of isolates circulating in Ouagadougou. Methods: The biochemical characterization of the isolates was carried out by tests from the API 20E test and completed with molecular characterization by simple polyemerase chain reaction (PCR). Antibiotic susceptibility of the isolates was determined using the recommendations of CA-SFM 2019. Results: The hospital wastewaters do not undergo any treatment before been discharged into the environment. A total of 171 presumed isolates of Salmonella spp, Pseudomonas spp, and Escherichia coli were identified in this study. These isolates derived from environment (n=19) and clinical (n=152). These isolates were resistant to amoxicillin + clavulanic acid (95.32%), cefoxitin (72.51%), ceftazidime (78.94%), cefepime (80.71%), tobramycin (59.64%), gentamicin (42.10%), nalidixic acid (68.42%), norfloxacin (59.06%), ciprofloxacin (56.14%), imipenem (0.00%), chloramphenicol (26.31%), and colistin (77.77%). Somewhere else, 46 presumptive Staphylococcus aureus were resistant to vancomycin (30.43%), oxacillin (13.04%), penicillin G (89.13%), ceftriaxone (15.21%), cefoxitin (2.73%),t (36.95%), kanamycin (30.43%), ciprofloxacin (15.21%), norfloxacin (43.47%), Tetracycline (56.52%), chloramphenicol (13.04%), and Fosfomycin (2.73%). Conclusion: The hospital wastewaters harbour a variety of pathogens, most of which are resistant to several families of antibiotics.

Introduction

The emergence and spread of bacterial resistance are increasingly becoming serious public health problems around the world [1]. Multi-drug resistant bacterial infections kill approximately 23.000 and 25.000 patients per year in the United States and Europe, respectively [2]. This toll is higher in countries with limited resources due to insufficient and neglected involvement of authorities in the management of environmental resources [2].

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The environment in developing countries is highly vulnerable to agricultural, industrial and especially hospital waste dumps [3, 4]. Indeed, hospitals generate large quantities of liquid effluents loaded with antibiotic, antiseptic, detergent, and pathogenic bacteria residues, which are treated in the same way like urban landfills [5-7]. Thus, the bacterial flora within these effluents could be reduced to multidrug resistant bacteria due to the longtime contact with antibiotics, while some bacteria can also acquire new resistance due to the pressure of nonmetabolized antibiotic residues [8-10]. These dynamic aquatic pathogenic bacterial populations, once in surface waters, are frequently contracted by humans and animals through the food chain [11-13]. However, the management of pathogenic agents remains problem for clinicians in most African countries. Despite the alarm sounded by WHO and its partners since 2011 by dedicating the World Health Day to the fight against multi-bio-resistance, huge countries such as Burkina Faso do not have national data on bacterial resistance. Nevertheless, in Burkina Faso partial investigations have been conducted, including the prevalence of extendedbeta-lactamase (ESBL)-producing spectrum Enterobacteriaceae (E-ESBL) in hospitalized patients which were 67.22% and 58%, respectively, according to Dabiré et al. [14] and Ouédraogo [15]. Besides, studies have been conducted on the resistance profiles of Salmonella isolated from salads and pathological products [11] and on the incidence due to Salmonella enterica (9%) in children under 5 years of age [16]. Regarding these worrying results, few measures are taken for the management of hospital liquid effluents to limit the spread of bacterial resistance. Thus, the aim of this study was to consolidate this antimicrobial resistance monitoring base and determined the resistance profile of Salmonella spp., Pseudomonas spp., Staphylococcus aureus and Escherichia coli from clinical and hospital effluents in the city of Ouagadougou.

Materials and Methods

Isolation of environmental pathogens

The system of wastewater was determined by process of National Office for Water and Sanitation after a preliminary survey. Fifteen samples of wastewater were collected during five campaigns visits to three sites of wastewater plants of three sites between October 2019 and February 2020. These sites were the wastewater collection of the University hospital center (UHC) of Yalgado, UHC of Bogodogo and wastewater plant of Kossodo. Salmonella spp., Pseudomonas spp., Staphylococcus aureus and Escherichia coli were isolated according to ISO 6579 (2017), NF EN ISO 16266 (2018), NF EN ISO 6888-1/A2 (2018) and NF ISO 4831(2006) respectively.

Isolation of clinical pathogens

Clinical pathogens including *Salmonella spp.*, *Pseudomonas spp.*, *Staphylococcus aureus* and *Escherichia coli* were collected in three hospitals (UHC of Tengadgo, UHC of Bogodogo and district of Schiphra) from August 2019 to February 2020. These isolated from human origin were purified and stocked. These isolates were distributed according to pathological products, age and gender of patients.

Identification of isolates

API 20 NE kit (bioMereux[®], France) and API 20E kit (bioMereux[®], France) were used for identification of isolates. Only, *Staphylococcus aureus* were confirmed by using biochemical tests including Gram, catalase, DNase and coagulase test.

Molecular identification

The molecular identifications were realized by rep-PCR. GTG₅ (5'GTG GTG GTG GTG GTG-3 ') universal primer was used according to Ouoba et al. (2019). The PCR was carried out with a total volume of 25 µL containing 12.5 µL of Master Mix (FIREPol®), 1.5 µL of GTG₅, 6 µL of nuclease-free water and 5 µL of DNA extract. The amplification was monitored following program PCR: Initial denaturation (94° C/4 min), Denaturation (30 cycles/95° C/30 sec), Hybridization (30 cycles/45° C/1 min), Elongation (30 cycles/65° C/16 min), final elongation (4° C) and boiling for 8 min. Escherichia coli ATCC 8739, Staphylococcus aureus ATCC 25923, Salmonella enteritidis ATCC 13076, Salmonella abony NCTC 6017 and Pseudomonas spp. ATCC 27853 were used as positive control for isolation and identification. Staphylococcus epidermidis ATCC 12228 and Klebsiella pneumoniae ATCC 13883 were used as positives controls for identification of Staphylococcus aureus and Pseudomonas spp. respectively.

Susceptibility of antibiotics

Susceptibility of antibiotics was tested according to CA-SFM (2019). A total of 19 antibiotics were used: amoxicillin + clavulanic acid (20/10 μ g), Cefoxitin (30 μ g), ceftazidime (10 μ g), cefepime (30 μ g), Tobramycin (10 μ g), gentamicin (10 μ g), nalidixic acid (30 μ g), norfloxacin (10 μ g), ciprofloxacin (5 μ g), chloramphenicol (30 μ g), colistin (50 μ g), imipenem (10 μ g), vancomycin (30 μ g), oxacillin (5 μ g), penicillin G (10 μ g), ceftriaxone (30 μ g), kanamycin (30 μ g), tetracycline (30 μ g) and Fosfomycin (200 μ g). *Escherichia coli* ATCC 25922 was used for the evaluation of antibiotics quality according to CA-SFM (2019).

Classification of isolates according to multidrugresistance of antibiotics

The isolates were classified into three groups according to multidrug resistance: MDR (Multidrug Resistant), XDR (Extensively Drug Resistant), and PDR (Pan Drug Resistant). The isolates were tested by seven antibiotics to determine their status: MDR, XDR or PDR. According to the European Center for Disease Prevention and Control (ECDC) in 2011 [17]: MDR-type multidrug-resistant bacteria are defined as being resistant to at least three different families of antibiotics. Those multidrug-resistant XDR are characterized by their sensitivity to a single family of antibiotics recommended for their treatment. Finally, multidrug-resistant PDR bacteria are resistant to all families of antibiotics available.

Statistical analysis

The data were analyzed with SPSS v.20 software. Antibiotic resistance rates of isolates were determined using descriptive variances.

Results

Hospital effluent management

The hospitals have open-air wastewater storage points. These effluents have very variable characteristics including: odor, suspended matter, color, etc. **Figure 1** present the draining system of hospital's wastewater of one center hospital in Ouagadougou.

Wastewater's isolates

A total of 24 isolates from wastewater were isolates from hospital's wastewaters and distributed as follows: 4 Salmonella spp, 6 E. coli, 9 Pseudomonas spp and 5 Staphylococcus aureus. Thus, depending on the collection site, these isolates originated from three hospital centers. These isolates are distributed as follows: Yalgado (2 Salmonella spp, 3 E. coli, 3 Pseudomonas spp and 2 Staphylococcus aureus), Bogodogo (2 Salmonella spp, 1 E. coli, 4 Pseudomonas spp and 2 Staphylococcus aureus) and Kossodo (2 E. coli, 2 Pseudomonas spp and 1 Staphylococcus aureus).

Prevalence of bacterial isolates from different pathological specimens

A total of 193 isolates from clinical origin were isolated and identified. The distribution was

according to the origin of the products; Urines (120 *E. coli*, 7 *Pseudomonas spp*, and 13 *Staphylococcus aureus*), Stools (3 *Salmonella spp*, 3 *E. coli*), Pus (7 *E. coli*, 5 *Pseudomonas spp*, and 22 *Staphylococcus aureus*), CVS (4 *E. coli*, 2 *Pseudomonas spp*, and 6 *Staphylococcus aureus*) and blood culture (1 *E. coli*).

Type of infection by age

The 193 clinics isolates in this study were distributed according to the age intervals of the patients. Thus, the patients aged 61 years and older and most were predominating (75/193) mainly for urines infections (73/140). The low rate (9/193) of isolates was observed to patients old to less 15 years.

Distribution of urinary tract infections by gender The urinary tract is highly vulnerable (140/193) to bacterial infections. However, these infections are disproportionately higher in the male sex (71%) for urinary tract infections encountered in this study according to the gender.

Identification of isolates by rep-PCR

A total of 224 isolates including 24 environmental isolates, 193 clinical isolates and 7 reference isolates were subjected to PCR analysis with the GTG_5 . The two gels pictures of **figure** (2) show the characteristics bands.

Susceptibility of isolates to antibiotics

Isolates resistance to antibiotics varied according to the family and type of antibiotic molecules. This resistance to antibiotics differed according to the origin of the isolates studied. Thus, of the 193 clinical isolates, 185 showed resistance to at least one antibiotic molecule, i. e a rate of 95.85%, unlike the 24 environmental isolates which showed a resistance rate of 87.50% resistance. However, this resistance to antibiotics varied from isolates to isolates in the different species studied. Thus, for E. coli resistance rates varied from 00% (to imipenem) to 97.77% (to amoxicillin + clavulanic acid) for clinical isolates and to 83.33% for environmental isolates. Resistance rates for Pseudomonas spp ranged from 00% (to imipenem) to 92.85% (to amoxicillin + clavulanic acid) for clinical isolates and 88.88% for environmental isolates. Isolates of Salmonella spp. showed resistance rates ranging from 00% (to imipenem and nalidixic acid) to 100% (to amoxicillin + clavulanic acid and colistin) for clinical isolates and from 00% (to imipenem) to 100% (Amoxic ciprofloxacin) for environmental isolates. Staphylococcus aureus showed resistance rates varying from 02.43% (to cefoxitin and fosfomycin) to 90.24% (to penicillin G) for clinical isolates and from 00% (to nine antibiotic molecules used) at 80% (penicillin G) for environmental isolates. Figure 3 shows the resistance of different isolates to antibiotic molecules. Tables 1, 2 show the comparison of antibiotic activities among environmental and clinical isolates. The statistical analysis showed that there were no significant differences between the activities of cefoxitin, ceftazidime, imipenem, tobramycin, gentamicin, nalidixic acid, ciprofloxacin, Norfloxacin and colistin on environmental and clinical isolates. However, there were significant differences between the activities of Amoxicillin + clavulanic acid, cefepime and chloramphenicol on both types of isolates (environmental and clinical isolates).

Bacterial multi-resistance

According to the types of resistance observed, the isolates were classified into three categories: MDR, XDR and PDR. Indeed, a rate of 30.87% of multi-resistant (MDR) was observed. Though, **Table 3** shows the rate of multi-resistant (MDR) isolates observed this study. Of all the isolates studied, a rate of 1.38% (3/217 *E. coli* isolated from urine) of PDR isolate was observed. These isolates were resistant to all of the antibiotic families used in this study except imipenem. A level of 2.76% (5 *E. coli* isolated and 1 *S. aureus* isolated from urine) of XDR isolate was observed.

 Table 1. Analysis of variances (ANOVA) based on comparisons of antibiotic activities among environmental and clinical isolates of *Escherichia coli*, *Pseudomonas spp., Salmonella spp.*

		Sum of squares	ddl	Average of squares	F	Signification
Amoxiclav	Inter-groups	0.292	1	0.292	5.868	0.016
	Intra-groups	8.421	169	0.050		
	Total	8.713	170			
	Inter-groups	0.264	1	0.264	0.500	0.481
Cefoxitin	Intra-groups	89.257	169	0.528		
	Total	89.520	170			
	Inter-groups	0.702	1	0.702	2.934	0.089
Ceftazidime	Intra-groups	40.467	169	0.239		
	Total	41.170	170			
	Inter-groups	1.229	1	1.229	4.783	0.030
Cefepime	Intra-groups	43.414	169	0.257		
	Total	44.643	170			
	Inter-groups	0.124	1	0.124	0.572	0.451
Imipenem	Intra-groups	36.520	169	0.216		
	Total	36.643	170			
	Inter-groups	0.573	1	0.573	0.976	0.325
Tobramycin	Intra-groups	99.263	169	0.587		
	Total	99.836	170			
	Inter-groups	0.211	1	0.211	0.321	0.572
Gentamicin	Intra-groups	111.204	169	0.658		
	Total	111.415	170			
	Inter-groups	0.059	1	0.059	0.087	0.768
Nalidixic Acid	Intra-groups	114.572	169	0.678		
	Total	114.632	170			
Ciprofloxacin	Inter-groups	0.018	1	0.018	0.024	0.877
	Intra-groups	128.941	169	0.763		
	Total	128.959	170			
	Inter-groups	0.164	1	0.164	0.252	0.616
Norfloxacin	Intra-groups	110.362	169	0.653		

	Total	110.526	170			
Chloramphenicol	Inter-groups	5.158	1	5.158	6.953	0.009
	Intra-groups	125.368	169	0.742		
	Total	130.526	170			
Colistin	Inter-groups	0.105	1	0.105	0.449	0.504
	Intra-groups	39.579	169	0.234		
	Total	39.684	170			

Legend: ddl: degree of freedom

Table 2: Analysis of variances (ANOVA) based on comparisons of antibiotic activities among environmental and clinical isolates of *Staphylococcus aureus*.

		Sum of squares	ddl Average of squares		F	Signification
				squares		
Vancomycin	Inter-groups	1.530	3	0.510	0.920	0.439
	Intra-groups	23.275	42	0.554		
	Total	24.804	45			
	Inter-groups	0.528	3	0.176	0.363	0.780
Oxacillin	Intra-groups	20.342	42	0.484		
	Total	20.870	45			
	Inter-groups	0.561	3	0.187	0.652	0.586
Pencillin G	Intra-groups	12.047	42	0.287		
	Total	12.609	45			
	Inter-groups	0.550	3	0.183	0.309	0.819
Ceftriaxone	Intra-groups	24.928	42	0.594		
	Total	25.478	45			
	Inter-groups	0.913	3	0.304	1.661	0.190
Cefoxitin	Intra-groups	7.696	42	0.183		
	Total	8.609	45			
	Inter-groups	2.190	3	0.730	0.964	0.419
Tobramycin	Intra-groups	31.810	42	0.757		
	Total	34.000	45			
Kanamycin	Inter-groups	1.371	3	0.457	0.602	0.617
	Intra-groups	31.847	42	0.758		
	Total	33.217	45			
	Inter-groups	3.301	3	1.100	2.100	0.115
Ciprofloxacine	Intra-groups	22.003	42	0.524		
	Total	25.304	45			
Norfloxacin	Inter-groups	1.431	3	0.477	0.759	0.523
	Intra-groups	26.395	42	0.628		
	Total	27.826	45			
	Inter-groups	6.734	3	2.245	4.289	0.010
Tetracycline	Intra-groups	21.983	42	0.523		
	Total	28.717	45			

Chloranphenicol	Inter-groups	0.902	3	0.301	0.606	0.615
	Intra-groups	20.837	42	0.496		
	Total	21.739	45			
Fosfomycin	Inter-groups	0.221	3	0.074	0.837	0.481
	Intra-groups	3.692	42	0.088		
	Total	3.913	45			

Legend: ddl: degree of freedom

Table 3: Distribution of multidrug-resistant isolates (MDR-type multidrug-resistant)

	Escherichia coli		Pseudomonas spp.		Staphylococcus aureus		Salmonella spp.	
Families of antibiotics	Env (n=06)	Clinic (n=135)	Env (n=09)	Clinic (n=14)	Env (n=05)	Clinic (n=41)	Env (n=04)	Clinic (n=03)
Ceph-Fluo-Peni	00	50	03	00	00	01	02	00
Ceph-Fluo-Amin	00	33	01	00	00	00	00	00
Ceph-Fluo-Autr	00	08	02	00	00	00	00	00
Ceph-Fluo-Tétr	00	00	00	00	00	01	00	00
Fluo-Peni-Amin	00	35	01	00	00	01	01	00
Fluo-Peni-Autr	00	11	03	00	00	01	00	00
Fluo-Peni-Tétr	00	00	00	00	00	01	00	00
Peni-Amin-Autr	00	11	01	01	00	01	00	00
Peni-Amin-Tétr	00	00	00	00	00	01	00	00
Fluo-Amin-Autr	00	08	01	00	00	01	00	00
Fluo-Amin-Tétr	00	00	00	00	00	01	00	00
Ceph-Peni-Amin	01	43	02	02	00	00	00	01
Ceph-Peni-Autr	00	17	03	01	00	00	00	00
Ceph-Amin-Autr	00	10	01	01	00	00	00	00
Ceph-Peni-Tétr	00	00	00	00	00	01	00	00

Legend : Env: Environmental; Ceph: Cephalosporin; Peni: Penicillin; Tetr: Tetracycline; Amin: Aminoside; Fluo: Fluoroquinolone; Autr: Other

Figure 1 Risk of dissemination of multi-resistant pathogenic bacteria and the vulnerability of public health to hospital effluents in Ouagadougou.



A: Drainage channel for wastewater from a university hospital center of Ouagadougou in a municipal gutter. B: Wastewater from the same channel (A) rushing towards a body of water. C: A wastewater source where the population practices fishing and gardening

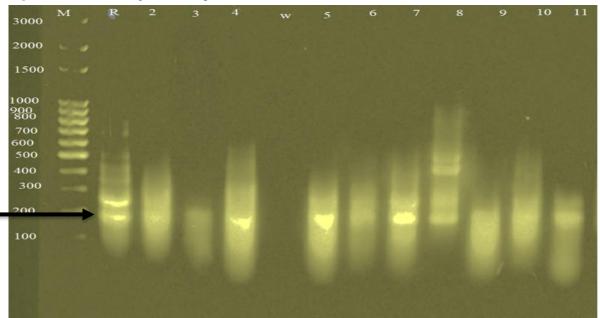
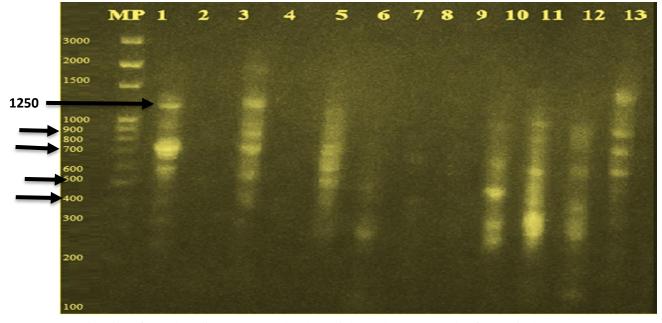


Figure 2 (A and B). Migration of replicons

A: Migration of *Pseudomonas* replicons: M (molecular weight marker), R (*Pseudomonas aeruginosa* strain ATCC 27853); 9 (*Klebsiella pneumoniae* ATCC 13883); 2, 3, 4, 8,10 and 11 (clinical *Pseudomonas spp*); 5, 6 and 7 (environmental *Pseudomonas spp*) and w (water PCR)

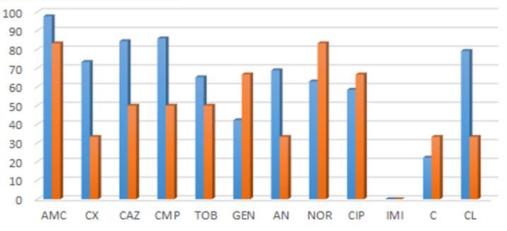
Pseudomonas strains have been identified with the characteristic bands of the *Pseudomonas aeruginosa* strain ATCC 27853. These bands have the following molecular weights: 180 bp.



B: Migration of *E. coli* replicons: MP (molecular weight marker), 3 (*E. coli* reference isolates ATCC 8739), 1; 5; 6 and 7 (clinical *E. coli*), 11; 12 and 13 (environmental *E. coli*), 2 and 9 (not tested), 4 and 8 (PCR water). The identity of the *E. coli* isolate was completed by referring to the different characteristic bands of the *E. coli* isolate ATCC 8739. These bands have the following molecular weights: 400 bp, 500 bp, 700 bp, 900 bp and 1250 bp

Figure 3 (A; B; C and D). Resistance profile of isolates clinicals and environments.

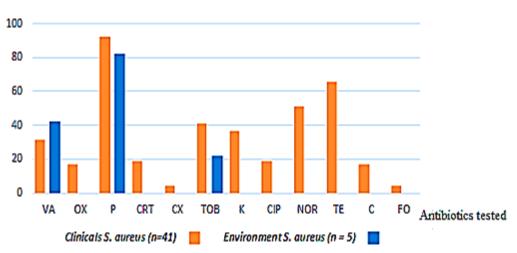
A: Resistance profile of E. coli



Rate of resistance to antibiotics

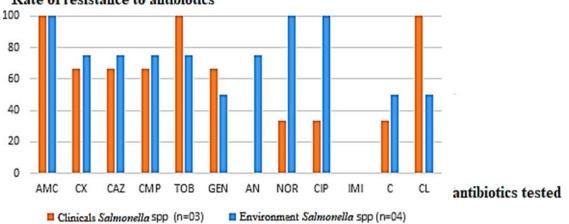
clinicals E. coli(n=135) Environments E. coli (n=06) antibiotics tested

B: Resistance profile of Pseudomonas spp.

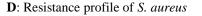


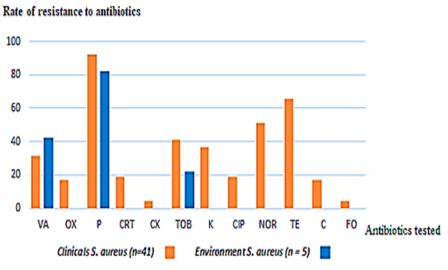
Rate of resistance to antibiotics

C: Resistance profile of Salmonella spp.



Rate of resistance to antibiotics





Discussion

Ouagadougou has the reference hospitals in Burkina Faso. These hospitals generate a variety of liquid effluents containing pathogens, antibiotic residues and other toxic substances. These effluents are released into nature without adequate treatment. [18] and [19] reported that liquid effluents are responsible for infections (100%), health toxicity (88%), ecotoxicity and carcinogenicity (82%). The characteristics of hospital liquid effluents observed in these studies are similar to those reported by other authors [19-22]. Previous studies on the microbiological quality of liquid effluents from the peripheral areas of the city of Ouagadougou had reported risks of infection in this exposed population [12,23]. Somewhere else, isolates of E. coli followed by S. aureus were more predominant than other bacterial species in the clinical samples. Previous studies have shown that these isolates ranked first as incriminated in different infections [4, 16, 24, 25]. This predominance can be explained by the fact that the isolates of E. coli and S. aureus are of commensal origin, which is an asset for the adhesion and colonization of the different organs of the organism [26]. This mechanism is more developed among E. coli with the expression of the K1 capsular antigen [27]. Their pathogenicity is caused by competition (with pathogens or other commensals) and poor food, body or medical hygiene. In contrast to these two groups, Pseudomonas and Salmonella had showed low rates of infection. Infections with the latter two isolates are occasional in the human body. However, most

Pseudomonas spp. infections are nosocomial and result from poor hygiene practices during therapy [28-30]. Infections caused by Salmonella spp. are frequently associated with poor food hygiene, especially with the consumption of meat from pigs or beef that are healthy carriers [11,31]. The incidence of urinary tract infections had been predominant in this study with a rate of 72.53%. These infections were more represented in subjects that are of 61 years of age and older with a sex ratio of 2.41. Previous studies reported that urinary tract infections affect more elderly male patients in Africa [32]. For example, in Antananarivo, urinary tract infections affect more elderly patients with a mean age of 49.05 years with extremes of 16 and 89 years [33]. In addition, in Lomé cases of urinary tract infections occur predominantly in males (58.82%) with a sex ratio of 1.43 and the average age of patients was 55.87 ± 12.48 years with extremes of 22 and 93 years [32]. Renal failure and diabetes have a high prevalence in the elderly, one of the most important common consequences of which is urinary tract infection [34-36]. Bacterial infections are however, a widespread problem in health systems worldwide, particularly in African countries. This situation appears to be worsened by the incidence of circulating multidrug-resistant bacteria [13].

Clinical isolates have shown resistance to all antibiotics used except imipenem. This same observation has been reported in isolates originating from hospital liquid effluents. **Dabiré et al.**, **Bonkoungou et al.**, **Traoré et al.**, **Ouédraogo et al.** and **Somda et al.** had reported the presence of circulating multidrug-resistant isolated in both hospitalized, non-hospitalized patients, food and urban wastewater in Burkina Faso. These resistances (MDR, PDR and XDR) in isolates circulating in the cities of Burkina Faso could be due by the expression of resistance genes of the *bla* and *mecA* types carried by mobile and transferable genetic elements between cells of the same or different species described previously by several authors [11,13,14,37,38]. The transfer of resistance genes between isolates depends on the type of ecosystem and the mode of transfer [39-43]. According to **Dubois-Brissonnet** [44], a bacterial isolate would have the ability to duplicate a gene of interest in order to transfer a copy and keep it.

Currently, the emergence of multidrug resistance is linked to the presence of genes encoding the production of carbapenemases [45-50]. In this study, the antibiotics showed less activity on isolates tested. Thus, these activity rates were 3.50%, 22.22%, 19.29% 21.05% and 27.48% from Amoxicillin + clavulanic acid, colistin, cefepime, ceftazidime and cefoxitin. Similar results on resistance to third generation cephalosporins have been provided.

Thus, in Niger, 77.5% of the isolates were resistant to cefotaxime [51]. This antibiotics resistance seems to be emerged year by year as demonstrated by some isolates, thus, the resistance of *Pseudomonas aeruginosa* to ceftazidime (CAZ) increased from 9.2% in 2012 to 53.5% in 2018 [51]. This problem of emergence of bacterial resistance are widespread but worse in Africa, which can be explained by several factors including poor hygiene in health centers, neglected management of hospital effluent, self-medication or poverty [13, 48].

Conclusion

This study revealed that most hospital's wastewaters do not undergo any treatment. The use of this water in market gardening represents a health risk for market gardeners and consumers. The presence of pathogenic bacteria multiresistant to antibiotics in these waters increases the health risk associated with their uses. Three types of bacterial resistance were reported in this study. In view of the above, the health authorities should draw the attention of health facilities to compliance with the rules for discharging hospital liquid effluents into nature.

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