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Phenotypic Detection of Carbapenemase and AmpC-β-Lactamase Production among Extended Spectrum β-Lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiella* spp., Isolated from Clinical Specimens

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Abstract: Background: In many African countries, clinical samples are not routinely tested for carbapenem-resistant bacteria, the resistance data remaining limited. **Material and methods:** In March 2020 –June 2022, we collected extended spectrum β-lactamase (ESBL) -producing Enterobacterales (ESBL-PE) isolates from five hospitals in Burkina Faso. The species were identified using API20E and ESBL production confirmed by double-disc synergy test. Production of carbapenemases and AmpC-β-lactamases and phenotypic co-resistance were determined. **Results:** Among the 473 ESBL-PE, 356 were ESBL- *E. coli* (ESBL-Ec) and 117 *Klebsiella* spp. (ESBL-K). Of the isolates, 5.3% were carbapenemase and 5.3% AmpC-β-lactamase positive. Three types of carbapenemases were identified: 19 NDM, 3 OXA-48-like and 1 VIM. Two isolates produced both NDM and OXA-48-like carbapenemases. Carbapenemase rates were highest among isolates in tertiary hospitals. Co-resistance rates were up to 85% for aminoglycosides, 90% for sulfonamides, 95% for fluoroquinolones and 25% for chloramphenicol, Fosfomycin resistance was 6% for ESBL-Ec and 49% for ESBL-K (49%). **Conclusion:** Many ESBL-Ec and ESBL-K co-produced carbapenemases and/or AmpC-β-lactamases, at all healthcare levels and in various samples, with high co-resistance rates to non-betalactams. Carbapenem resistance is no longer rare, calling for testing in routine diagnostics, vigorous resistance surveillance system, and infection control within healthcare.

Keywords: ESBL; carbapenemase; AmpC-β-lactamase; *E. coli*; *Klebsiella* spp; hospitals

Introduction

The emergence and spread of multidrug-resistant (MDR) bacteria are serious global public health threat. In 2019, an estimated number of 4.95 million deaths were associated with antimicrobial resistance (AMR), with 1.27 million directly attributable to MDR bacteria [1]. The highest burden is in Western sub-Saharan Africa, with 27.3/100 000 AMR-attributable and 114.8/100 000 AMR-associated deaths [1], the same region where AMR surveillance is minimal and resistance data limited. The health care costs of AMR reach nearly $1.2 Trillion in the "High-AMR" Case [2].

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According to the World Bank estimation/projection, by 2030, there will be an annual increase of $0.22 trillion of the extra health expenditure incurring in the low-AMR settings [2].

The emergence of carbapenem resistance substantially limits the therapeutic options at hospitals worldwide since carbapenems belong to our last resort antibiotics [3,4]. Regrettably, carbapenem resistance among Enterobacterales has experienced a dramatic increase and global spread over the past decade, with reports both from hospital and community settings [4]. Infections due to carbapenem resistant Enterobacterales (CRE) are associated with increased mortality rates [5–7]. Management of patients with CRE infections necessitates the use of combination therapies, which typically involve various types of antibiotics, such as tigecycline and colistin [8,9]. In low- and middle-income countries (LMICs) resistance data are scare due to the challenges in detecting carbapenemases in microbiology laboratories [10,11].

Two primary mechanism account for carbapenem resistance among bacteria: the first mechanism involves a reduction of membrane permeability by porin loss often associated with production of ESBL or AmpC-β-lactamase. The second mechanism entails the production of carbapenemase enzymes capable of hydrolyzing carbapenems [12]. However, although carbapenemase and AmpC-β-lactamase production are not routinely identified in clinical microbiology laboratories in LMICs, several recent studies across African hospitals have reported carbapenemase production among Enterobacterales [13–19]. Carbapenem resistance at rates of approximately 45% have been reported among *E. coli* and *Klebsiella pneumoniae* isolates from clinical specimens in two tertiary hospitals in Nigeria [14]. A phenotypic resistance rate of 23.3% and genotypic resistance rate of 43.1% to carbapenem were reported in Uganda [16]. Data on AmpC-β-lactamase are accumulating gradually also from Africa. In a recent study, a high rate of AmpC-β-lactamase was reported in five referral hospitals in Khartoum, with highest rates (49.3%) among *Acinetobacter baumannii* [20].

In Burkina Faso, data on prevalence of CRE or AmpC-β-lactamase production among clinical isolates are limited. One study conducted in a referral teaching hospital, identified 17 carbapenemase-producing strains, four in clinical samples and 13 fecal carriage isolates among 443 Gram negative bacteria [18]. Another reported NDM genes in clinical *E. coli* isolates [21]. Recent studies in the country have discovered carbapenemase-producing bacteria or the related genes to be prevalent in the hospital waste waters implying their likely presence in clinical samples as well [22,23]. In the current study, we assessed the prevalence of carbapenemase and AmpC-β-lactamase production among ESBL-producing *E. coli* (ESBL-Ec) and ESBL-producing *Klebsiella* spp. (ESBL-K) isolated from clinical samples and their co-resistance to non-betalactam anibiotics and distribution in hospitals of primary, secondary and tertiary health care in Burkina Faso.

Results

Bacterial Strains

A total of 473 clinical ESBL-PE isolates, comprising 356 ESBL-Ec and 117 ESBL-K, were collected from inpatients and outpatients at the five hospitals participating in the study: CHU-YO: (Centre Hospitalier Universitaire Yalgado Ouedraogo), CHR-KDG (Reginal Hospital of Koudougou), El Fateh Suka medical clinic, CMA Source de vie and CMA Saint Camille de Nanoro (Table 1).

Table 1. Prevalence of carbapenemase-producing isolates among extended-spectrum betalactamase (ESBL)-producing Enterobacterales: data provided by hospital and sample type.

	ESBL-*E. coli* total n= 356 n (%)	ESBL-*Klebsiella* spp. total n=117 n (%)	All total N=473	**Prevalence (%)**
Hospitals				
CHU-YO	18/211	4/82	22/293	7.5
CHR-KDG	1/44	0/13	1/57	1.8
El-Fateh Suka medical clinic	0/24	0/0	0/24	0

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CMA Saint Camille de Nanoro	0/58	2/14	2/74	2.7
CMA source de vie	0 /19	0/8	0/27	0
Sample Type				
Urine	16/228	4/71	20/299	6.7
Pus and	2/113	2/30	4/143	2.8
Blood culture	1/15	0/16	1/31	3.2
Overall prevalence	19/356 (5.3)	6 /117(5.1)	25/473	5.3

Prevalence of Carbapenemase-Producing Enterobacterales

In this study, the prevalence of carbapenemase production among the 473 ESBL-PE isolates was 5.3%. Of these, the 30 meropenem resistant isolates were tested for carbapenemase production: 25 of them, 19 (76%) *E. coli* and 6 (24%) *Klebsiella* spp. were found to produce carbapenemases (Table 1). Carbapenemase-producing isolates were identified across all the three levels of the healthcare system, with highest frequency among ESBL-PE isolates collected at the tertiary hospitals with a prevalence as high as 7.5% (22/293). Regarding the distribution of carbapenemase-producing isolates by sample type, the highest rates were found in urine samples with a prevalence of 6.7% (20/299 ESBL-PE) (Table 1). The rates among ESBL-Ec and -K were similar (5.3% and 5.2%, respectively) (Table 1).

Types of carbapenemase Produced

Among the carbapenamase-producing isolates, three types of carbapenemases were detected, with NDM (19/25; 76%) as the most frequent type, followed by OXA- 48 like (3/25; 12%), and VIM (1/25; 4%). Two ESBL-Ec isolates were found to produce both NDM and OXA- 48 like (2/25) carbapenemases. The rates of NDM were similar among ESBL-Ec (3.9%) and ESBL-K (4.3%) isolates. (Table 2)

Table 2. Carbapenemase findings among ESBL-PE isolates and proportions of each carbapenemase type.

	ESBL-*E. coli* total n= 356	ESBL-*Klebsiella* spp., total n=117	All isolates N=473 (%)	Isolates with carbapenemase n=25 (%)
Carbapenemases				
NDM	14 (3.9)	5 (4.3)	19 (4.0)	19/25 (76)
OXA-48-like	3 (0.8)	0 (0)	3 (0.6)	3/25 (12)
OXA-48like + NDM	2 (0.6)	0 (0)	2 (0.4)	2/25 (8)
VIM	0 (0)	1 (0.9)	1 (0.2)	1/25 (4)
Total	19 (5.3)	6 (5.1)	25 (5.3)	25 (100)

Prevalence and Distribution of AmpC-β-Lactamase Production

Among the 92 presumptive AmpC-β-lactamase-producing bacterial isolates (73 ESBL-Ec and 19 ESBL-K), twenty-five isolates (17 ESBL-Ec and 8 ESBL-K) were tested positive. The prevalence of AmpC -β-lactamase production among the ESBL-producing isolates was 5.3% (25/473), with somewhat higher rates among ESBL-K (6.8%) than ESBL-Ec (4.8%) isolates. The prevalence of AmpC-β-lactamase-producing isolates was high in all sample types (4.7% of ESBL-PE isolates in urine samples, 5.6% in pus and 9.7% in blood cultures). The frequency among ESBL-PE in the CHU-YO hospital was 6.5% (19/293) (Table 3).

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Table 3. Prevalence of AmpC-β-lactamase-producing isolates among ESBL-producing Enterobacterales; data provided by hospital and type of sample.

	ESBL-*E. coli* total n=*356* n (%)	ESBL-*Klebsiella* spp. total n=117 n (%)	All total N=473	Prevalence (%)
Hospitals				
CHU-YO	13/211	6 /82	19/293	6.5
CHR-KDG	0/44	0/13	0/57	0
El-Fateh Suka medical clinic	2 /24	0/0	2/24	8.3
CMA Saint Camille de Nanoro	1/58	1/14	2/74	2.7
CMA évangélique Source de vie	1/19	1/8	2/27	7.4
Sample Type				
Urines	8/228	6/71	14/299	4.7
Pus	7/113	1/30	8/143	5.6
Bloodculture	2/15	1/16	3/31	9.7
Overall prevalence	17/356 (4.8)	8/117 (6.8)	25/473	5.3

Antimicrobial Resistance Patterns

As the study explored ESBL-PE, the strains are, by definition, resistant to most penicillins and cephalosporins. We explored sensitivity to piperacillin + tazobactam and found 68.0% of ESBL-Ec and 76.79% ESBL-K as resistant.

As for carbapenem resistance evaluated with disc diffusion test, approximately 6% of all isolates displayed resistance to imipenem and meropenem and up to 19% of ESBL-Ec and 12.4% of ESBL-K to ertapenem.

The resistance rates proved high resistance also to fluoroquinolones (approximately 95%) and to SXT (approximately 90%) (Table 4).

Table 4. Resistance to various antibiotics among ESBL-producing *E. coli* and *Klebsiella* sp., isolates tested by disk diffusion method.

Antibiotics (concentration in µg)		*E. coli*	*Klebsiella spp.*
	N	Resistance n (%)	Resistance n (%)
Piperacillin + Tazobactam (110)	462	238 (68.0)	86 (76.8)
Meropenem (10)	472	22 (6.2)	8 (6.8)
Imipenem (10)	469	21 (5.9)	8 (6.9)
Ertapenem (10)	461	68 (19.5)	14 (12.4)
Gentamycin (10)	473	153 (43.0)	68 (58.1)
Amikacin (30)	466	64 (18.3)	11 (9.5)
Tobramycin (10)	473	215 (60.4)	69 (59.0)
Kanamycin (30)	468	300 (85.2)	100 (86.2)
Ciprofloxacin (5)	465	335 (95.7)	108 (93.9)
Sulfamethoxazole + trimethoprim (25)	449	297 (87.9)	100 (90.1)
Nitrofurantoin (300)	465	118 (33.9)	87 (74.36)
Fosfomycin (200)	466	20 (5.7)	56 (48.7)
Chloramphenicol	463	71 (20.4)	28 (24.6)

N= number of isolates tested; n= number of resistant isolates.

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In the aminoglycoside family, isolates showed high resistance rates to kanamycin (approximately 86%) and tobramycin (approximately 60%). Lowest resistance rates were recorded for amikacin: 9.5% among ESBL-K and 18,3% among ESBL-Ec isolates.

Less than 25% of all isolates were resistant to chloramphenicol. A total of 5% of ESBL-Ec and 51% of ESBL-K isolates were resistant to fosfomycin. (Table 4).

Discussion

Carbapenemase and AmpC-β-lactamase detection is not routine practice in microbiology laboratories in Africa, despite several studies reporting the presence of carbapenemase and AmpC-β-lactamase-producing Enterobacterales [14,20,24–29]. In our study, the prevalence of carbapenemase production was 5.3%. Twenty-five (25) isolates, primarily *E. coli* (76%), were carbapenemase producers, most of them originating in urine samples. Our results fall within the prevalence range of 2.6–6.7% reported in North Africa, but remain somewhat lower than the range of 9.0–60.0% reported in sub-Saharan Africa in a review from 2015 [30]. In Burkina Faso, a study conducted at Sanou Sourou teaching hospital in Bobo-Dioulasso reported a prevalence as low as 0.9% for carbapenemase producers among Gram-negative bacteria in clinical specimen, most of them urine samples [18], and another reported NDM, VIM, OXA-48 and KPC genes among E. coli isolated from clinical patient samples [21]. In contrast, a study conducted in two tertiary hospitals in northwestern of Nigeria reported a prevalence of 39% among 248 ESBL and non-ESBL *E. coli* and *Klebsiella pneumoniae* clinical isolates. These isolates were primarily obtained from urine samples [14]. These variations in prevalence could be attributed to several factors, including differences in sample types, methods employed for carbapenemase detection, and the geographical regions in which the study was conducted. Nonetheless, in all the studies, carbapenemase-producing isolates were predominantly urinary pathogens. This can be attributed to two evident reasons: first, urine samples are among the most common samples investigated in microbiological laboratories, and second, urinary tract infections constitute the most prevalent symptomatic manifestation of intestinal colonization by MDR bacteria.

Both Class B (NDM and VIM) and class D (OXA-48-like) carbapenemases were detected in our study. NDM carbapenemase-producing Enterobacterales were the most frequent findings (76%), consistent with results of several earlier studies from sub-Saharan Africa [16,21,30,31]. In our study, we identified carbapenemase-producing isolates in three hospitals, each representing different levels of the healthcare system. CHU-YO, a referral hospital at the highest level of specialized care facilities, showed substantial prevalence for both. This may be attributed to more abundant use of antibiotics, use of broader spectrum antibiotics, and prolonged duration of drug treatments as patients are referred from medical centers, regional hospitals, or medical clinics where they have already received antimicrobial treatment. Indeed, the misuse or overuse of antibiotics during hospitalization can contribute to the selection of MDR bacteria [32,33].

Production of AmpC-β-lactamases has been described in Africa at various rates [20,29,34] for example at a rate of 49.3% in Sudan [20], 15.2% in Nigeria [29], 2.5% in Ethiopia [24] and 36.5% in Uganda [35]. In our study, exploring 473 clinical ESBL-PE isolates, co-production of AmpC-β-lactamases was observed in 5.3% of isolates. Co-production of AmpC-β-lactamase at a rate of 5.2% was seen in a previous study from India [26] and 22% in Iran [36]. In a study from Ethiopia 3.6% of AmpC-β-lactamase positive isolates co-produced ESBL (5/139) [24]. Many other studies also report either separately ESBL and AmpC-β-lactamase production in various LMIC countries [24,26,34]: variations may be attributed to differences in sample sizes, types of study areas, and phenotypic methods used for AmpC-β-lactamase detection, which can yield differing results [29]. All these data unmistakably confirm the presence of AmpC-β-lactamase in clinical isolates. Consequently, AmpC-β-lactamase detection should be implemented in routines of hospitals also in Africa. This is of utmost importance since AmpC-β-lactamase production in bacteria can lead to challenges and failures of treatment, with increased morbidity and mortality [29].

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A total of 473 ESBL-isolates (356 *E. coli* and 117 *Klebsiella* spp.) were tested against various antibiotics. The resistance rates to regimen with potential efficacy against ESBL-producers, i.e. piperacillin + tazobactam, and amoxicillin + clavulanic acid, remained high.

ESBL-producing Enterobacterales have been investigated in numerous studies in Africa, revealing its generally high prevalences. For example, in a study in Kenya with rates of 42% versus 45% in urban versus rural communities and 70% and 63% in urban versus rural hospitals [38]. In 2017–2020 in Ghana, 50% of *E. coli* and 59% of *Klebsiella* sp in urinary samples were ESBL-producing [39], and in Tanzania approximately 20% [40]. A recent study from Burkina Faso showed ESBL-PE prevalences of 3.2% among *E. coli* isolates from urine in pregnant women versus 35.4% among clinical isolates [41]. Other reports from LMICs include for example those from Kano, northwest Nigeria [42], Khartum, Sudan [20], Ethiopia [24], and Algeria [17], and India [28] The high ESBL-PE rates in LMICs and their potential co-resistance should draw the attention of clinicians who often prescribe β-lactams in prophylaxis or infection management in health care facilities [43].

Although carbapenem-producing Enterobacterales often carry both ESBL and carbapenemase genes, not all CPE strains can be covered by a study on ESBL-PE, such as the present one. However, as most of the CPEs do, it was of interest to see in Burkina Faso, how large proportion of ESBL-PE actually are CPE strains. Most (76%) of the identified CPE were *E. coli*, with NDM as the most common carbapenemase type, reaching a prevalence of approximately 4% among our ESBL-PEs. The respective resistance rates with disc diffusion method proved somewhat higher, reaching almost 20 % for ertapenem among ESBL-Ec. Similar findings were reported in previous studies across Africa, resistance rates of 0 to 14.7% to imipenem and meropenem have been reported in recent studies in Togo, Nigeria, Ethiopia and Sudan among ESBL or AmpC-β-lactamase-producing isolates [20,24,42,44]. Our results were lower compared to 58.6% resistance against imipenem reported in India [28]. Earlier studies in Burkina Faso and Ghana did not find resistance to carbapenems [18,45], the differences potentially attributed to antibiotic consumption in various study areas [46]. For instance, in India carbapenem consumption is higher [47] than in Burkina Faso, where their use is more controlled and the drugs are very expensive. The presence of resistance could be attributed to the co-presence of genetic determinants of resistance to carbapenems with those of other antibiotics commonly used in our hospitals [22].

Among our ESBL-PE isolates, co-resistance was recorded particularly against aminoglycosides, fluoroquinolones, and sulfonamides. Among aminoglycosides, high resistance rates were recorded against all regimen tested, except amikacin. This finding is in line with those reported in similar studies in Burkina Faso [18], in Togo [44], in Sudan [20], in Ethiopia [24], in Algeria [17] and India [28,48]. Interestingly, our resistance rates appear high with respect to similar studies, which show wide variations in resistance level [42,45]. Our high rates could be associated with misuse or overuse of antibiotics in hospitals, communities or in farms since these antibiotics are routinely used and have easy access [49].

Amikacin, fosfomycin and chloramphenicol emerged as the most effective antibiotics in vitro. Our results align with previous research in Burkina Faso in 2021 [18], Algeria in 2019 [17], and Nigeria in 2014 [42], yet contrasting the higher resistance to amikacin observed in Ghana in 2013 [45]. Apart from having fosfomycin as one of the alternative drugs in cystitis (IDSA guideline), these three antibiotics are not the ones initially preferred for many infections but rather represent reserve antibiotics in current practice. Indeed, when treating infections caused by ESBL- or carbapenemase-producing Enterobacterales it is often necessary to resort to less effective reserve antibiotics.

Material and Methods

Study Design and Period

The prospective study was carried out in five hospitals in Burkina Faso from January 2020 to June 2022. ESBL-producing *E. coli* and *Klebsiella* spp. strains isolated from urine, blood and pus samples were collected from each hospital over a 12-month period. All isolates were characterized in the CRUN microbiology laboratory.

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Sampling and Sites Description

The health system in Burkina Faso comprises three levels. The first level encompasses peripheral health care facilities and primary hospitals. The second level comprises regional hospitals and certain medical clinics, which serve as reference facility for primary hospitals. The third level includes national and teaching hospitals, representing the highest level of referral care and offering specialized services [50].

Sampling was carried out in five hospitals, each selected to represent different levels of the health system: 1) Yalgado Ouédraogo teaching hospital (CHU-YO), a tertiary hospital located in the capital city, Ouagadougou; 2) The regional hospital of Koudougou (CHR-KDG); 3) the El-Fateh Suka medical clinic in Ouagadougou, both categorized as secondary hospitals, and two medical centers, 4) CMA Saint Camille de Nanoro a rural medical center and 5) CMA évangélique Source de vie in Ouagadougou, the last two representing primary health care. This diverse selection of healthcare units allowed a comprehensive sampling approach. A total of 473 clinical isolates were collected, comprising 356 ESBL-Ec and 117 ESBL-K strains. These isolates were collected from various clinical specimen, including urine (n=303), pus (n=140), and blood culture (n=30). The isolates were collected in tryptic soy agar tubes and kept at room temperature until transferred to the Clinical Research Unit of Nanoro (CRUN) microbiology laboratory for analysis.

Bacterial Isolation and Identification

At CRUN microbiology lab, isolates were plated on ESBL-selective chromogenic culture media (CHROMagar™ ESBL). Isolates Identification of the isolates was verified using API 20E (Biomérieux France) according to the manufacturer's instructions.

ESBL Production Test

All isolates identified were tested for ESBL production using the double disk synergy test between 3rd generation cephalosporins (ceftriaxone and ceftazidime) and 4th generation cephalosporin (cefepime) disks and amoxicillin-clavulanic acid disk according to the CLSI 2022 guidelines. The presence of ESBL production was indicated by the presence of synergistic inhibition zone between ceftazidime, ceftriaxone and/or cefepime and the amoxicillin-clavulanic acid disk.

Carbapenemase Production Test

A total of 30 isolates (22 *E. coli* and 8 *Klebsiella* spp.) that had meropenem inhibition zone diameter less than 22 mm in the AST, were investigated for production of the five main carbapenemases (OXA48-like, NDM, KPC, VIM and IMP) using the immunochromatographic test O.K.N.V.I. RESIST-5 (CORIS BioConcept, Belgium), according to the manufacturer's instructions.

AmpC-β-Lactamase Production

Bacterial isolates with cefoxitin inhibition zone diameter less than 18 mm were considered presumptive AmpC-β-lactamase producers. A total of 92 presumptive AmpC-β-lactamase producers bacterial isolates (73 ESBL-*E. coli* and 19 ESBL-*Klebsiella* spp.) were tested for AmpC-β-lactamase production using MH agar supplemented with cloxacillin at 4µg/l. A bacterial suspension prepared with fresh colonies (McFarland 0.5) was inoculated on to the entire surface of the MH agar supplemented with cloxacillin at 4µg/l and a disk of cefoxitin was placed on the plate. The test was positive if the inhibition zone diameter around cefoxitin disk was ≥18 mm.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test (AST) was performed using the disk diffusion method on Mueller Hinton (MH) agar as described by Bauer et *al.*, 1966. In total, 473 ESBL-producing isolates (356 *E. coli* and 117 *Klebsiella* spp.) were tested. The results were interpreted according to the American

Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines. 15 antibiotics listed in Table 4 were tested.

Conclusion

Our study in Burkina Faso revealed that a substantial proportion of ESBL-producing *E. coli* and *Klebsiella* spp. can also produce carbapenemases and AmpC-βlactamases. These highly resistant pathogens were primarily isolated from urine samples, most frequently from patients at tertiary hospitals. Three types of carbapenemases, NDM, OXA-48-like and VIM, were detected, with NDM as the most frequent finding. The antibiotic resistance patterns reveled notable co-resistance to antibiotics commonly used for patient treatment. Implementing antibiotic stewardship in all levels of healthcare, establishing effective and reliable AMR surveillance systems are essential measures for containing resistant bacteria and preventing their dissemination within hospitals and into the broader communities.

Author Contributions: ZG, IOJB, KH, LS, and NB conceived and designed the study. ZG collected samples. ZG performed bacterial isolation and antimicrobial susceptibility test. TR performed statistical analysis. HT, KH and AK contributed for the reagents/materials/analysis tools. ZG, BK, KH, JPK, IOJB, HMN, NB, LS and AK were the major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement: All data generated or analyzed during this study are included in this published article.

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Conflict of Interest: The authors declare that they have no competing interests.

Ethical Aspects: This study is part of AMRIWA project which received approval from the health research committee of Burkina Faso (N°153-12-2018/ CERS). In addition, authorizations were obtained from each hospital directing staff.

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