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Spread of Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae* Clinical Isolates from Patients in Bobo-Dioulasso, Burkina Faso

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Abstract

Antimicrobial resistance (AMR) is one of the top 10 threats to global health and it is estimated around 10 millions of deaths per year are associated with AMR until 2050. Burkina Faso is also facing the emergence and spread of AMR of several bacteria resistant strains such as those of public health concerns under surveillance *Enterobacteriaceae*. The aim of this study was to assess the prevalence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) clinical isolates from patients attending the bacteriology laboratory of the Centre MURAZ in Bobo-Dioulasso, Burkina Faso. Clinical isolates from urine, pus, stool, and semen were collected from April to June 2017. Identification and antibiotic susceptibility testing were performed using the VITEK 2 compact automated system according to EUCAST version 2015 recommendations. ESBL detection was then performed on the Muller-Hinton medium using the combined disc method. One hundred (100) strains of *Enterobacteriaceae* were isolated from 100 patients, including 52% of ESBLS. Escherichia coli (*E. coli*) was the most commonly isolated ESBL [(84.62, 44/72) ESBL], followed by *Klebsiella spp.* [(40%, 06/15) ESBL], then *Enterobacter spp.* [(40%, 2/5) ESBL]. Risk factor analysis revealed that ESBL-PE infection was

frequently found in pus samples (P = 0.042; [OR] = 3.16; 95% [CI] = 1.04 - 9.61) and that *E. coli* was the strain most likely to harbour ESBL (P = 0.008; [OR] = 3.60; 95% [CI] = 1.40 - 9.31). This study reports a high prevalence of ESBL-PE associated with strong resistance to quinolones and cotrimoxazole (over 80%), which calls for increased surveillance of these superbugs, the adoption of a rational antibiotic prescription policy, and rigorous hygiene measures to prevent the spread of these multi-resistant bacteria.

Keywords

Enterobacteriaceae, ESBL, Resistance, Antibiogram, Phenotypes, Burkina Faso

1. Introduction

The emergence and spread of multidrug-resistant (MDR) bacteria remain a global public health threat [1]. Among the bacteria responsible for infectious diseases, enterobacteria are the most dangerous, since they secrete enzymes such as extended-spectrum beta-lactamases ESBL and carbapenemases, and produce resistance associated with numerous families of antibiotics [2] [3]. The high level of colonization of these bacteria in the digestive tract of humans and animals facilitates the exchange and transfer of resistance genes within the organism [1] [4]. The threat of antimicrobial resistance is real because it reduces the clinician's therapeutic options, prolongs the patient's hospital stay, and increases healthcare costs for the patient [5]. In Africa, and Burkina Faso in particular, the population's low level of education, inaccessibility to microbiological expertise, and lack of awareness of antibiotic use lead many patients to self-medicate and use antibiotics inappropriately, thanks to easy access to pharmacies or parallel markets [6]. These practices of self-medication with inappropriate drugs contribute to the selection of mutant's resistant to the antibiotics used.

Indeed, studies carried out in sub-Saharan Africa on clinical isolates have reported that the prevalence of ESBL producing *Enterobacteriaceae* was 17.6% in 2011 in Togo [7] and 49.3% in 2013 in Ghana [3]. Burkina Faso, like other sub-Saharan African countries, is experiencing the emergence of ESBL-producing *Enterobacteriaceae*. A study carried out in 2017 using clinical isolates from three hospitals in Ouagadougou showed that the prevalence of ESBL-producing *Enterobacteriaceae* was 38.5% [8]. In addition, a 2016 multicenter study of clinical samples in Burkina Faso revealed a high prevalence of ESBL-producing *Enterobacteriaceae* of up to 58% [1]. Based on these hospital-based studies, we note a prevalence of ESBL-producing by *Enterobacteriaceae* in the country, but it should be noted that there is little data on bacterial resistance in community settings in Burkina Faso. This study aims to strengthen the antimicrobial resistance surveillance network by documenting the frequencies of extended-spectrum beta-lactamase-producing by *Enterobacteriaceae* isolated in the community, and the

description of resistance associated with other families of antibiotics commonly used in medical settings.

2. Methods

2.1. Study Site and Period

The clinical biology laboratory at the Centre MURAZ in Bobo Dioulasso was used as the setting for this study. The Centre MURAZ is a medical research institute of the Institut National de Santé Publique (INSP) located in Bobo Dioulasso, western Burkina Faso. This center was chosen as the setting for the study because of its geographical position, allowing easy access, and it is very popular with communities. The center has a clinical biology laboratory, which treats outpatients from the urban commune of Bobo Dioulasso. This was a descriptive cross-sectional study over three months from April to June 2017. Patients came as outpatients to the clinical biology laboratory at the Centre MURAZ for cytobacteriological medical exams.

2.2. Sample Collection and Bacterial Isolation

During the collection period, 514 outpatients were received at the laboratory for cytobacteriological medical exams. Bacterial isolation on appropriate culture media yielded 146 positive cultures. Identification of these strains using Vitek 2 compact distinguished 118 enterobacterial isolates, of which 100 strains were included. The 18 strains were systematically eliminated, including 11 duplicates and 7 lost strains. Enterobacteria isolates were obtained from urine (n = 75), pus (n = 19), stool (n = 5), and a semen sample (n = 1). The remaining 28 bacterial strains consisted of Gram-positive cocci (*Staphylococcus spp*, *Streptococcus sp*) non-fermentative Gram-negative bacilli (*Pseudomonas sp*, *Acinetobacter sp*), and Gram-negative cocci (*Neisseria gonorrhoeae*). The 100 enterobacterial strains were stored at −80˚C. They were re-isolated on Muller Hinton medium for further analysis. This study was approved by the ethical committees of the Health Science Research, Burkina Faso No 2024-042/MSHP/MESRI/CERS.

2.3. Identification and Resistance Phenotypes Detection

Identification (GN card) and antibiotic susceptibility testing (AST-N233 card) of bacterial species were carried out simultaneously using the Vitek 2 automated system (Biomérieux, Marcy l'Etoile, France). The following antibiotics were tested: Ampicillin, amoxicillin/clavulanic acid, ticarcillin, piperacillin/tazobactam, cefalotin, cefoxitin, cefotaxime, ceftazidime, imipenem, ertapenem, amikacin, gentamicin, tobramycin, nalidixic acid, ciprofloxacin, ofloxacin, nitrofurantoin and trimethoprim/sulfamethoxazole. The Vitek 2 Compact is equipped with an Advanced Expert System (AES) containing a database with a compilation of tens of thousands of Minimum Inhibitory Concentrations (MICs) of multiple antibiotics for various species with diverse resistance mechanisms, enabling the detection of resistance phenotypes. Results were interpreted according to EUCAST 2015

recommendations. A reference strain of *Klebsiella pneumoniae* (*K. pneumoniae*) (ATCC 700603) and *Escherichia coli* (*E. coli*) (ATCC 25922) were used during the analysis to ensure the good quality of vitek cards, media, and antibiotic discs. ESBL detection was performed on Muller Hinton media using the combined disc method (Figure 1) to confirm the results of the automated system. In the case of the production of hyperproduced cephalosporinases, the disc matching method was performed to detect the presence of ESBL.

Figure 1. Antibiotic susceptibility testing of E. coli on Muller-Hinton agar. Synergy was observed between a disk containing cefotaxime, ceftazidime, and aztreonam and a disk containing clavulanic acid (Amoxicillin + clavulanic acid: AMC, Ticarcillin + clavulanic acid: TCC). The presence of ESBL was expressed by the appearance of a "champagne cork" or "keyhole" synergy.

2.4. Data Analysis

Recording, graphics, and tables were produced using Microsoft Excel version 10.0 (Microsoft®, New York, USA). Statistical analysis was performed using STATA version 15.1 (STATA CORP, College Station Texas, USA). Associations between demographic variables (gender, age, sources of infection), identified germ, and infection with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) were analyzed using Odds ratios and the logistic regression model. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Socio-Demographic Characteristics

A total of 100 strains of Enterobacteriaceae were included from patients visiting the MURAZ Centre laboratory as outpatients. The median age of patients was 54 years, with males predominating at 62% (a sex ratio of 1.63 was found).

3.2. Distribution of *Enterobacteria* Strains by Type of Biological Product

75% (75/100) of the *Enterobacteriaceae* strains were isolated from urine, 19% (19/100) from pus, 5% (5/100) from stool, and 1% (1/100) from semen (Table 1). Of these strains, *Escherichia coli* was the most common pathogenic bacterium isolated from urine at 80% (60/75), pus at 14.66% (11/75), followed by *Klebsiella pneumoniae* from urine at 12% (9/75), and pus at 35.71% (5/14) (Table 1).

Table 1. Distribution of isolated *Enterobacteriaceae* strains by species and specimens.

Species	Specimens				
	Urine n, (%)	Pus n, (%)	Stool n, (%)	Semen n, (%)	Total n, (%)
Escherichia coli	**60 (60)**	**11 (11)**	0 **(0)**	1 **(1)**	72 **(72)**
Klebsiella pneumoniae	9 **(9)**	5 **(5)**	0 **(0)**	0 **(0)**	14 **(14)**
Enterobacter cloacae	2 **(2)**	0 **(0)**	0 **(0)**	0 **(0)**	2 **(2)**
Enterobacter aerogenes	2 **(60)**	1 **(1)**	0 **(0)**	0 **(0)**	3 **(3)**
Klebsiella oxytoca	1 **(1)**	0 **(0)**	0 **(0)**	0 **(0)**	1 **(1)**
Proteus mirabilis	1 **(1)**	2 **(2)**	0 **(0)**	0 **(0)**	3 **(3)**
Salmonella sp	0	0 **(0)**	3 **(3)**	0 **(0)**	3 **(3)**
Total	75 **(75)**	19 **(19)**	3 **(3)**	1 **(1)**	100 (100)

3.3. Resistance Phenotypes of Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae*

Of the 100 strains tested, 52% (52/100) were ESBL -producing. Table 2 shows the distribution of ESBL species according to the nature of the biological product.

Table 2. Distribution of ESBL clinical isolates by sex, age, and biological product.

Variable (socio-demographic criteria ou caracteristics)	Collection on site (ESBL strains all sites combined, (n = 52))		Total (n = 100)	
	ESBL + (n)	ESBL − (n)	ESBL + [n (%)]	ESBL [n (%)]
Sex				
Females	38	17	38 (38%)	17 (17%)
Males	14	31	14 (14%)	14 (14%)
Total	52	48	**52 (52%)**	48 (48%)
Ages (years)				
0 - 15	3	6	03 (03%)	06 (06%)
16 - 30	7	3	07 (7%)	03 (3%)
30 - 45	10	8	10 (10%)	08 (8%)
45 - 60	11	13	11 (11%)	13 (13%)
60 - 75	15	15	15 (15%)	15 (15%)
>75 ans	6	3	06 (6%)	03 (3%)
Total	52	48	52 (52%)	48 (48%)

Continued

Biological Product				
Urine	39	36	29 (39%)	36 (36%)
Pus	13	6	13 (13%)	06 (6%)
Stool	0	5	00 (0%)	5 (05%)
Semen	0	1	00 (00%)	01 (01%)
Total	52	48	52 (52%)	48 (48%)

3.4. Extended-Spectrum *β*-Lactamase Phenotypes Associated with Resistance to Other Antibiotic Families

These results report that amikacin was the most active molecule on all strains producing beta-lactam resistance phenotypes. Strains producing ESBL had associated resistance to gentamicin 38.46% and tobramycin 44.23%. In contrast, the other antibiotic families showed considerable resistance rates, with cotrimoxazole 88.46%, nalidixic acid 96.15%, ciprofloxacin 90.38%, and ofloxacin 84.61% (Figure 2).

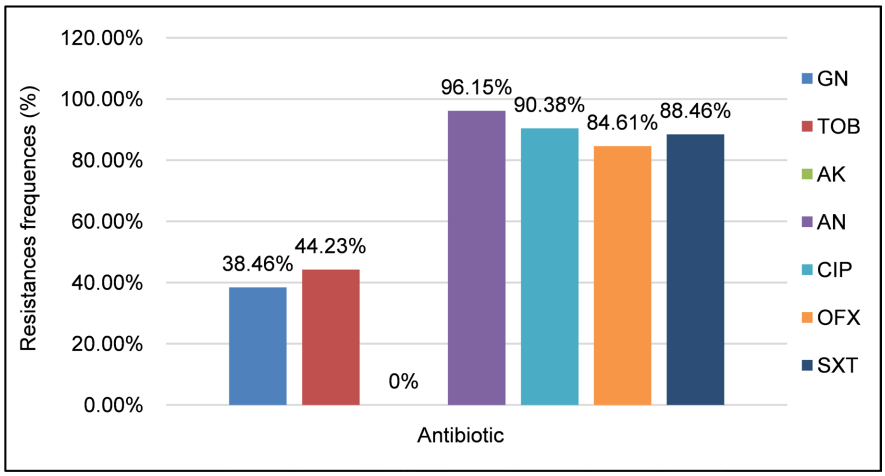

Figure 2. Beta-lactamase phenotypes associated with resistance to other antibiotic families. GN: gentamicin, TOB: tobramycin, AK: amikacin, AN: nalidixic acid, CIP: ciprofloxacin, OFX: ofloxacin, SXT: cotrimoxazole.

3.5. Risk Factors Associated with Resistance to Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae*

Depending on the nature of the pathological product, the risk of isolating ESBL-producing *Enterobacteriaceae* in a pus sample was 3.16 times higher [(P = 0.042, Odds Ratio [OR] = 3.16; 95% Confidence Interval [CI] = 1.04 - 9.61)] compared with other pathological products received in the laboratory. However, when considering hospitalization history, the risk of undergoing a previous hospitalization was not associated with harboring ESBL-PE strains (P > 0.88). Still, the presence

of ESBL was 3.60 times higher in *E. coli* strains compared with other Enterobacteria species isolated [(P = 0.008, Odds Ratio [OR] = 3.60; 95% Confidence Interval [CI] = 1.40 - 9.31)] (Table 3).

Table 3. Risk factors associated with ESBL production.

Variables	ESBL		Relative risk (95% CI)	P-value
	Numbers	%		
Sex				
Females	29	36.54	1	
Males	33	63.46	1.13 (0.50 - 2.55)	0.75
Previous hospitalization				
Not hospitalized	45	86.54	1	
Hospitalization < 6 months	7	13.4	1.08 (0.33 - 3.50)	0.88
Antibiotic therapy				
Yes	29	53.86	1	
No	22	43.14	1.70 (0.76 - 3.75)	1.30
Not specified	1			
Age (years)				
Other	38	73.07	1	
>65	14	26.93	1.00 (0.98 - 1.02)	0.745
Sources of infection				
Other	38	73.08	1	
Pus	14	26.92	3.16 (1.04 - 9.61)	0.042
Identified germs				
Other	8	15.38	1	
E. coli	44	84.62	3.60 (1.40 - 9.31)	0.008

Table 4. Multivariate analyses of factors associated with the production of ESBL-producing *Enterobacteria.*

ESBL	Odds Ratio	(95% I)	P-value
Other pathological products	1		
pus	3.87	1.06 - 14.3	0.040
Other germs	1		
E. coli	4.77	1.66 - 13.70	0.004
Antibiotic therapy	1.44	0.57 - 3.60	0.43

On multivariate analysis, we find that taking antibiotic exposure into account, the risk of isolating enterobacteria in pus was 3.87 times higher than in other pathological products. In addition, the risk of isolating ESBL-producing *E. coli* was 4.77 times higher than other *Enterobacteriaceae* species isolated (**Table 4**).

4. Discussion

In this study, the frequency of ESBL-producing *Enterobacteriaceae* and their associated resistance to other families of antibiotics commonly used in the therapy of bacterial infections was determined. *Enterobacteriaceae* are the main pathogenic bacteria commonly encountered in community and hospital infections, including gastrointestinal tract infections, urogenital tract infections, sepsis, meningitis, and device-associated infections [4]. Among the enterobacteria isolated, *E. coli* was the most frequently isolated species, with a frequency of 72%, followed by *K. pneumoniae*. These results show that *E. coli* and *K. pneumoniae* strains are the most involved in bacterial infections, as they possess adhesins and capsules that enable them to adhere to the host cell and escape phagocytosis.

Indeed, this work reveals a high frequency of 52% of ESBL producing strains of *Enterobacteriaceae*. These results differ from those of other hospital-based studies carried out in West Africa, notably in Ghana, where prevalences of 37, 96% in 2016 at Korle-Bu [9] and Ouédraogo *et al.,* in 2016 [1], who found a prevalence of 58% in clinical isolates from three hospitals in Burkina Faso. This difference in results could be explained by the size of the samples collected, the short duration, the region where the studies were carried out, and the ambulatory status of the patients. The high frequency of ESBL observed in our study could be attributable to the selection pressure induced by the consumption of broad-spectrum antibiotics such as cephalosporins [6], inappropriate treatments, and self-medication. Also, the use of antibiotics in the animal sector for therapeutic or prophylactic purposes, or as feed additives, could contribute to the emergence of antibiotic resistance in both animals and humans [6].

In this study, the majority of ESBL had resistance associated with multiple antibiotics, in particular, fluoroquinolones, aminoglycosides, and cotrimoxazole as reported by some authors [1] [8] (Ouedraogo *et al.* 2016 [1] [8] [10]. This stage of resistance could lead to a treatment impasse for the patient. More than a third of ESBL-PE strains were resistant to aminoglycosides. These results are superior to those obtained in Spain by Morosini *et al.*, in 2006, who found that 27.4% of ESBL-PE strains were resistant to gentamicin and tobramycin [11]. On the other hand, below those found by Kpoda *et al.* in 2017 in Burkina Faso, who recorded rates of 71.7% resistance associated with gentamicin and 81.3% with tobramycin [8] This difference in results could be explained by the small size of the study and the fact that it was conducted in a community setting. Despite this rate of associated resistance, amikacin was the most active molecule on ESBLPE strains, as demonstrated in previous studies [1] [12]. Furthermore, the aminoglycosides commonly used in human therapeutics are gentamicin, tobramycin, amikacin, and

netilmicin. Their use has contributed to the selection of resistant strains. The spread of these resistance phenotypes could be due to the localization of resistance genes from different families present on the same plasmid, leading simultaneously to the spread of other aminoglycoside resistance mechanisms such as aminoglycoside-modifying enzymes, reduced membrane permeability, structural alteration of the ribosomal target and expulsion of the antibiotic by the efflux system.

In this study, more than four-fifths of ESBL-PE strains were also resistant to nalidixic acid, ciprofloxacin, ofloxacin, and cotrimoxazole. The same results were reported by Kpoda *et al.* in 2017 in Burkina Faso, where ESBL-PE strains had developed resistance to nalidixic acid, ciprofloxacin, and cotrimoxazole [8]. The reduced sensitivity of this class of antibiotics could be due to a chromosomal mutation (gyr A, by C), an efflux mechanism, impermeability, or an enzyme (aac (6')-Ib-cr) [13]. This associated resistance found in our study could be explained by the fact that ESBL- PE genes, generally encoded by plasmids, are associated with resistance genes to other antibiotics. These worrying levels of fluoroquinolone-associated resistance are probably linked first and foremost to the massive prescription of this family of antibiotics, to self-medication and the often abusive use of these antibiotics in the urology department and the community, and the emergence of three plasmid resistance mechanisms to fluoroquinolones: the Quinolone resistance gene "Qnr", the genes coding respectively for an N-acetyltransferase, ACC-(6')-Ibcr and the genes coding for the efflux pump Qep A [14]. The high frequency of cotrimoxazole resistance could be the result of selection pressure due to inappropriate prescribing and its sometimes abusive use in hospital and community settings [15]. Over-the-counter dispensing and self-medication could also be the cause of this observed co-resistance. In addition, the impact of uncontrolled feeding, often with antibiotics used in agriculture and livestock breeding, could also explain this co-resistance [16]. In addition, cotrimoxazole resistance could also be due to resistance mechanisms emanating from the trimethoprim and sulfamethoxazole combination.

On univariate analysis, our results show that the chance of isolating ESBL-PE strains was significantly higher in pus samples (OR = 3.16; 95% CI = 1.04 - 9.61) P = 0.042) than in other pathological products. These results differ from those of Obeng *et al.* 2013 [3] who reported a higher risk of encountering ESBL in urine (OR = 1.69; 95% CI = 36 - 2.100; P = 0.001) than in other pathological products. This difference in results could be linked not only to our small sample size but also to the fact that the study took place in a community setting. The isolation of multi-resistant enterobacteria in pus reported in this study could be due to poor antibiotic prescription in health facilities, empirical probabilistic treatments, and self-medication. In addition, the frequency of ESBL-producing *E. coli* strains was significantly higher (OR = 3.60; 95% CI = 1.40 - 9.31; P = 0.008) compared with the other enterobacteria species isolated. The possible explanation is that the *E. coli* strain is a bacterium permanently resident in the digestive tract of humans and animals, which at some point may escape the digestive tract by migrating to

the urogenital mucosa and translocating from the digestive tract via the mesenteric lymph nodes into the bloodstream [17]. In both cases, it is responsible for major syndromes such as urinary tract infections, digestive tract infections, neonatal meningitis, and septicemia [18]. Added to this is its transmission in healthcare establishments and by hand.

The limitations of the present study were the lack of certain useful patient information on the examination form (the original department where the patient consulted, such as the hospital center or district hospitals, and the current probabilistic antibiotic therapy), the absence of certain antibiotic discs that could better refine the identification and confirmation of certain resistance mechanisms on the agar medium. Despite the shortcomings noted in the analysis, this in no way detracts from the quality of this work.

5. Conclusion

This study shows a high prevalence of ESBL-producing *Enterobacteriaceae* in Bobo Dioulasso. The spread of ESBL strains reduces the therapeutic arsenal of patients harboring this type of bacterial infection. However, ESBL-secreting bacteria were sensitive to amikacin, which is often the drug of choice for severe infections. This study points to the need for systematic detection of multidrug-resistant bacteria in Burkina Faso to inform the antimicrobial surveillance network and avoid therapeutic failures and the spread of multidrug-resistant bacteria.

Authors' Contributions

Kobo Gnada: Writing—Original draft, Methodology, Investigation; Michel Kiréopori Gomgnimbou: Conceptualization, Writing—Original draft, Validation; Louis Robert W. Belem: Writing-original draft; Arzouman Da: Methodology; Arnaud Quetin Sanou: Methodology; Armel Moumouni Sanou: Review & editing; Soufiane Sanou: Conceptualization, Validation, Writing—original draft.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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