RESEARCH ARTICLE
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Detection and molecular characterization of multiresistant *Enterobacteriaceae* carried by houseflies in the city of Bobo-Dioulasso, Burkina Faso

Sanou D. M. Soufiane[1], Yerbanga R. Serge[2,3], Bangre Tinlé[2], N'Do Sévérin[3], Kaboré N. Firmin[1], Traoré Isidore[1], Zoungrana Jacques[4], Yaméogo Inès[2], Nikiéma Félicité[2], Fofana Aminata[3], Batiéné Naomi[2], Ouédraogo Jean-Bosco[2], Sangaré Ibrahim[4], and Sanou Mahamoudou[5]

1. Centre MURAZ, Laboratoire de Biologie clinique, Bobo dioulaso, Burkina Faso; 2. Institut des Sciences et Technique (INSTech), Bacteriology Laboratory, Bobo-Dioulasso, Burkina Faso; 3. Department of Microbiology, Institute of Research in Health Sciences, Western Regional Direction, Bobo-Dioulasso, Burkina Faso; 4. Department of Pharmacy, Nazi BONI University, INSSA, Bobo-Dioulasso, Burkina Faso; 5. Department of Pharmacy, Joseph KI-ZERBO University, UFR/SDS, Ouagadougou, Burkina Faso.

Corresponding author: Sanou D. M. Soufiane, e-mail: domalick2000@yahoo.fr

Co-authors: YRS: yrserge@yahoo.fr, BT: bangret@yahoo.com, NS: severin.ndo@gmail.com, KNF: nongodo@yahoo.fr, TI: tiandiogo2002@yahoo.fr, ZJ: zojacques@yahoo.fr, YI: yameogoines@gmail.com, NF: nikiemaf66@gmail.com, FA: aminatafofana920@ymail.com, BN: batienenaomi@gmail.com, OJ: jbouedraogo@gmail.com, SI: babaibrasangare@yahoo.fr, SM: mahamoudsanou@hotmail.com

Received: 16-07-2023, **Accepted:** 12-12-2023, **Published online:** 12-01-2024

doi: www.doi.org/10.14202/IJOH.2024.12-19 **How to cite this article:** Soufiane SDM, Serge YR, Tinlé B, Séverin N, Firmin KN, Isidore T, Jacques Z, Inès Y, Félicité N, Aminata F, Naomie B, Jean-Bosco O, Ibrahim S, and Mahamoudou S (2024) Detection and molecular characterization of multiresistant *Enterobacteriaceae* carried by houseflies in the city of Bobo-Dioulasso, Burkina Faso, *Int. J. One Health*, 10(1): 12–19.

Abstract

Background: Houseflies (*Musca domestica*) are synanthropic insects that are vectors of a wide range of multidrug-resistant pathogens responsible for infectious diseases. The aim of this study was to detect and characterize multidrug-resistant *Enterobacteriaceae* carried by houseflies in Bobo-Dioulasso.

Materials and Methods: A total of 500 houseflies were captured in hospital and non-hospital environments in the city of Bobo-Dioulasso. For bacteriological analysis, they were divided into 125 batches of five flies each. Multidrug-resistant bacteria isolated on MacConkey agar supplemented with 4 µg/mL cefotaxime were identified on the basis of biochemical characteristics. Antibiotic susceptibility profiles were determined using the agar diffusion method. *blaCTX-M* resistance genes and quinolone resistance genes (plasmid-mediated quinolone resistance) were detected by conventional polymerase chain reaction.

Results: Among 115 bacterial strains obtained, 26 were extended-spectrum beta-lactamase (ESBL)-producing enterobacteria: *Escherichia coli* (15), *Klebsiella pneumoniae* (6), *Enterobacter cloacae* (4), and *Morganella morganii* (1). Carriers were statistically more important in hospitals (12/26, p = 0.03). No carbapenem-resistance strains were observed. We identified ESBL resistance genes (Cefotaximase Munich; *CTX-M* group 1) (25/26) and quinolone resistance genes (*QnrS*) (6/26).

Conclusion: Houseflies in the city of Bobo-Dioulasso are vectors for the transmission of multidrug-resistant enterobacteria. There is a need to monitor the associated risks for public health.

Keywords: Bobo-Dioulasso, extended-spectrum beta-lactamase, Gram-negative bacilli, housefly, multiresistant *Enterobacteriaceae.*

Introduction

Antimicrobial resistance (AMR) remains one of the biggest threats to public health despite decades of efforts to reduce the selection and spread of AMR through more appropriate use of antimicrobials [1]. An estimated 4.95 million (3.62–6.57) deaths were associated with bacterial AMR in 2019, including 1.27 million deaths attributable to bacterial AMR [2]. In total, antibiotic resistance is estimated to add USD 20 billion annually to direct healthcare costs in the USA, with additional costs to society resulting from lost productivity, which might be USD 35 billion a year [3].

Early detection of AMR in bacteria and ongoing surveillance are critical because they provide the information required to monitor and develop therapy guidelines, infection control policies, and public health interventions [4]. Both passive and active surveillance systems commonly exist [5, 6]. As regards passive surveillance, which is based on patients' clinical samples, active surveillance provides the opportunity to detect a wider range of resistance mechanisms [5]. The most widespread resistance mechanism in Enterobacterales is based on plasmid-mediated production of extended-spectrum beta-lactamases (ESBLs), which hydrolyze β-lactam rings, thereby reducing the efficacy of cephalosporins and monobactams [7].

Flies appear to be useful surveillance vectors for tracking AMR [8]. Many studies have reported on AMR genes in flies, reflecting the increasing awareness that these genes might play an important role in transmitting and maintaining resistance [9–11].

Houseflies are common and occur in large numbers in close association with human activity. These insects are attracted to human and animal wastes for feeding and reproduction [12]. Human (i.e., hospitals, food markets, and restaurants) and animal environments (i.e., farms, feedlots, and slaughterhouses) are the major points of focus and sampled areas for houseflies. Houseflies greatly amplify the risk of human exposure to a variety of pathogens, including bacteria, due to their diverse habitat preference, indiscriminate movement, the ability to fly long distances (until 7 km during adult life [13], and attraction to both decaying organic materials [14, 15]. Flies transmit pathogens through mechanical translocation from the exoskeleton, regurgitation, and defecation [16].

Flies are important reservoirs and vectors of antimicrobial-resistant bacteria (e.g., methicillin-resistant *Staphylococcus aureus* and ESBL-producing Enterobacterales [ESBL-E]). In a short report, antimicrobial-resistant bacteria were detected in flies (n = 25) in sub-Saharan Africa [17, 18]. However, the true burden of AMR in African flies is unknown, and the main vectors of ESBL-E and enteropathogenic bacteria are still unclear [19].

In Burkina Faso, passive surveillance of bacterial resistance to antibiotics is conducted through a network of sentinel sites. The aim of this study was to study the carriage of multiresistant *Enterobacteriaceae* on the exoskeleton of the housefly in the city of Bobo-Dioulasso.

Materials and Methods

Ethical approval

In Burkina Faso, there is no ethics committee for animal studies. We obtained written authorization from the municipal and health authorities of the city of Bobo-Dioulasso for this study.

Study period and location

This descriptive cross-sectional study was conducted from April to December 2021 in Bobo-Dioulasso. A total of 25 fly trapping sites with a global positioning system survey were selected (5 markets, 7 restaurants, 3 fish markets, 3 poultry markets, 6 health centers, and 1 slaughterhouse). These locations were selected on the basis of fly abundance, favorable conditions for their survival, and persistent human movement (Figure-1).

Sample collection and processing

We used Cochran's formula to determine the sample size:

$$n = (Z\alpha^2 * P * [1-P])/\Delta^2),$$

Where:

- $Z\alpha$ = the reduced deviation corresponding to an α error of 5% = 1.96
- P = fraction of the population carrying the germ (estimated at 50% to maximize sample size)
- Δ = the target absolute precision =0.05

A minimum sample size of 385 houseflies was necessary for our study; however, to divide the sample into five batches of four flies, we rounded it up to 500. The statistical unit used in the analyses was a batch of

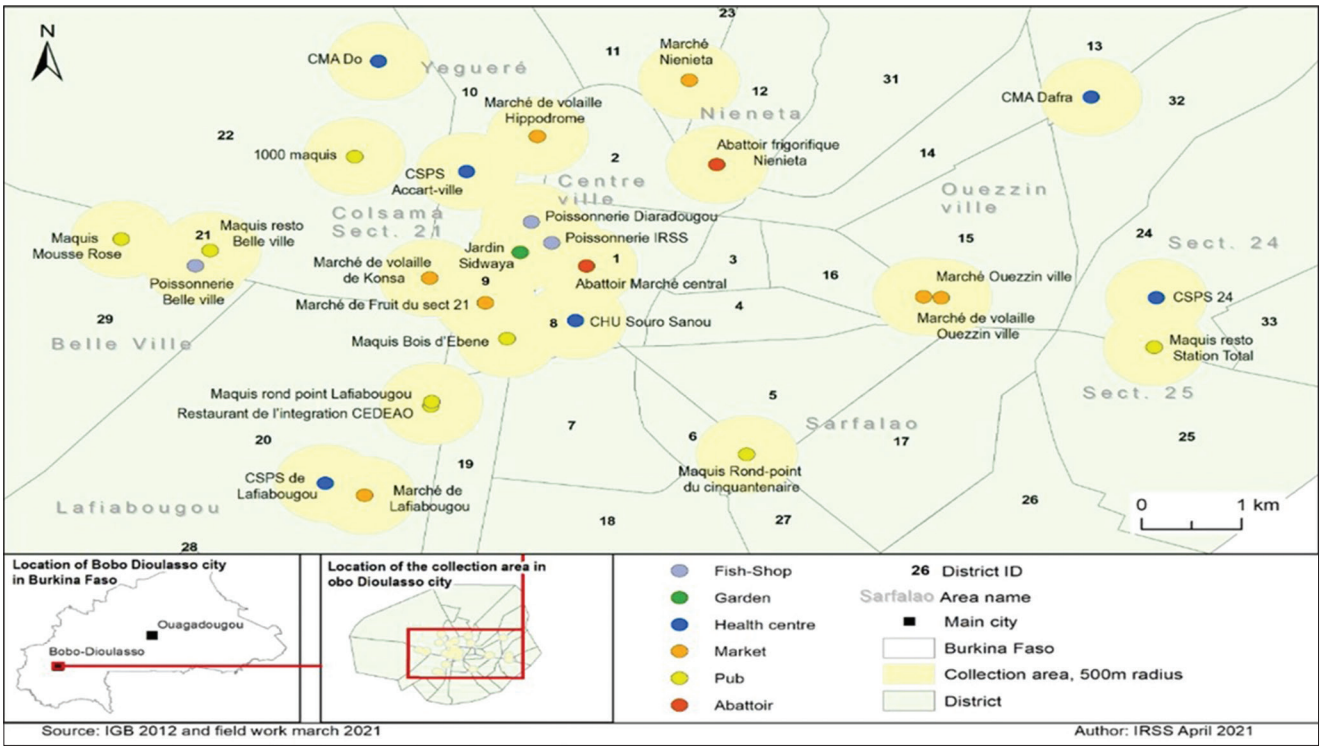

Figure-1: Location of collection sites in Bobo-Dioulasso.

4 flies [20]. Between April and December 2021, 125 batches of flies were obtained, of which 30 batches were obtained from health centers.

Flies were caught during the flight using sterile transparent bags. Fecal and blood baits from cattle and pigs, protected by a veil to avoid contact with the flies, were used to attract the flies to a large number of them. The flies in the bags were transported to the entomology laboratory at 8°C within 1 h of capture, where they were killed and stored at −20°C. Morphologically, they were identified by entomologists using a stereomicroscope to ensure that they were *Musca domestica* [21].

After identification, the 20 flies from each collection site were divided into 5 batches of 4 in sterile dry tubes. Then, 1 mL of physiological water was added to each tube (batch) and which was vigorously shaken with a vortex for at least 1 min to obtain a sample of fly exoskeleton homogenate. Then, 500 µL of each homogenate sample was transferred into 10 mL of brain heart nutrient broth (BHB) (BioMaxima, Lublin, Poland) and incubated for 18–24 h at 37°C.

Isolation and identification of bacteria

A 10-µL subculture of BHB was prepared on MacConkey (BioMaxima, Lublin, Poland) agar supplemented with 4 µg/mL of cefotaxime (CTX). Plates were incubated at 37°C for 18–24 h. Colonies recovered on MacConkey agar were identified according to biochemical characteristics (oxidase test and API20E) following Gram coloration.

Antimicrobial susceptibility test

AMR testing of the obtained isolates was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar based on the recommendations of European Committee on Antimicrobial Susceptibility Testing guidelines and clinical breakpoints (http://www.eucast.org/clinical_breakpoints/). ESBL production was detected with the combined double-disk synergy method [22] for *Enterobacteriaceae* isolates. The following antimicrobial agents were used: Ampicillin (10 µg), piperacillin (100 µg), amoxicillin + clavulanic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), cefepime (30 µg), cefoxitin (30 µg), cefadroxil (30 µg), ceftriaxone (30 µg), piperacillin + tazobactam (110 µg), imipenem (10 µg), ertapenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), amikacin (30 µg), fosfomycin (50 µg), colistin (10 µg), and trimethoprim + sulfamethoxazole (25 µg).

Molecular epidemiological typing of antibiotic resistance genes

All *Enterobacteriaceae* strains were, further, tested for the detection of antibiotic resistance genes: Cefotaximase Munich (*CTX-M*) group 1, *CTX-M* group 9, SHV, TEM, OXA-1-like [23], and plasmid-mediated quinolone resistance (PMQR: *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA*, *aac(6′)-Ib*138 *cr*, and *oqxAB*) [24].

The new polymerase chain reaction-based method described by Clermont *et al*. [25] was used to determine the phylogenetic group of the ESBL-*Escherichia coli* strain.

DNA samples from reference ESBL-E- and PMQR-positive strains were used as positive controls. Polymerase chain reaction products were visualized on 1.5% agarose gels containing ethidium bromide at 100 V for 90 min after electrophoresis. A 100-bp DNA ladder (Solis BioDyne, Estonia) was used as the marker size.

Conjugation experiments

Mating experiments were performed using rifampicin-resistant *E. coli* as recipient cells, as described previously by Touati *et al*. [26].

Statistical analysis

Statistical analyses were performed using Epi Info software version 7.2.3.0. Variables were compared at the 5% risk using the χ^2 test of independence and $p < 0.05$ was considered significant.

Results

A total of 26 *Enterobacteriaceae* ESBL-producing strains were isolated from 21 batches of flies captured at 15 sites (Figure-2). *Enterobacteriaceae* strains identified included *E. coli* (n = 15), *Klebsiella pneumoniae* (n = 6), *Enterobacter cloacae* (n = 4), and *Morganella morganii*. These ESBL-E were captured in all five types of collection sites (markets, restaurants, fish markets, poultry markets, and health centers) (Figure-2), but they were more statistically important in healthcare centers (12/26, p = 0.03) (Table-1).

Antimicrobial susceptibility

ESBL-E strains showed high levels of resistance to third-generation cephalosporins, cefepime, and cotrimoxazole (>50%). Carbapenems (Imipenem and Ertapenem) maintained good activity on the obtained strains (higher than 80%). Only amikacin remains active in all strains (Figure-3).

Molecular detection of antibiotic resistance genes and molecular epidemiology typing

The *CTX-M* gene was found in 95.16% (25/26) of the strains of group 1. In 80.77% (21/26) of the strains, this gene was associated with genes coding for the production of TEM, SHV, and/or Oxa-1-like beta-lactamases. It was associated with genes encoding PMQR in 19.23% (5/26) of the strains (Table-2). Conjugation experiments were performed on 11 ESBL-Es that were sensitive to rifampicin, and six transconjugants (Table-2) were obtained. The *E. coli* strains belonged to the B1 phylogroup (7/15) and the unknown phylogroup (6/15) (Table-2).

Discussion

Our research aimed to investigate the presence of ESBL-E in houseflies within the urban area of Bobo-Dioulasso. The findings of our study revealed

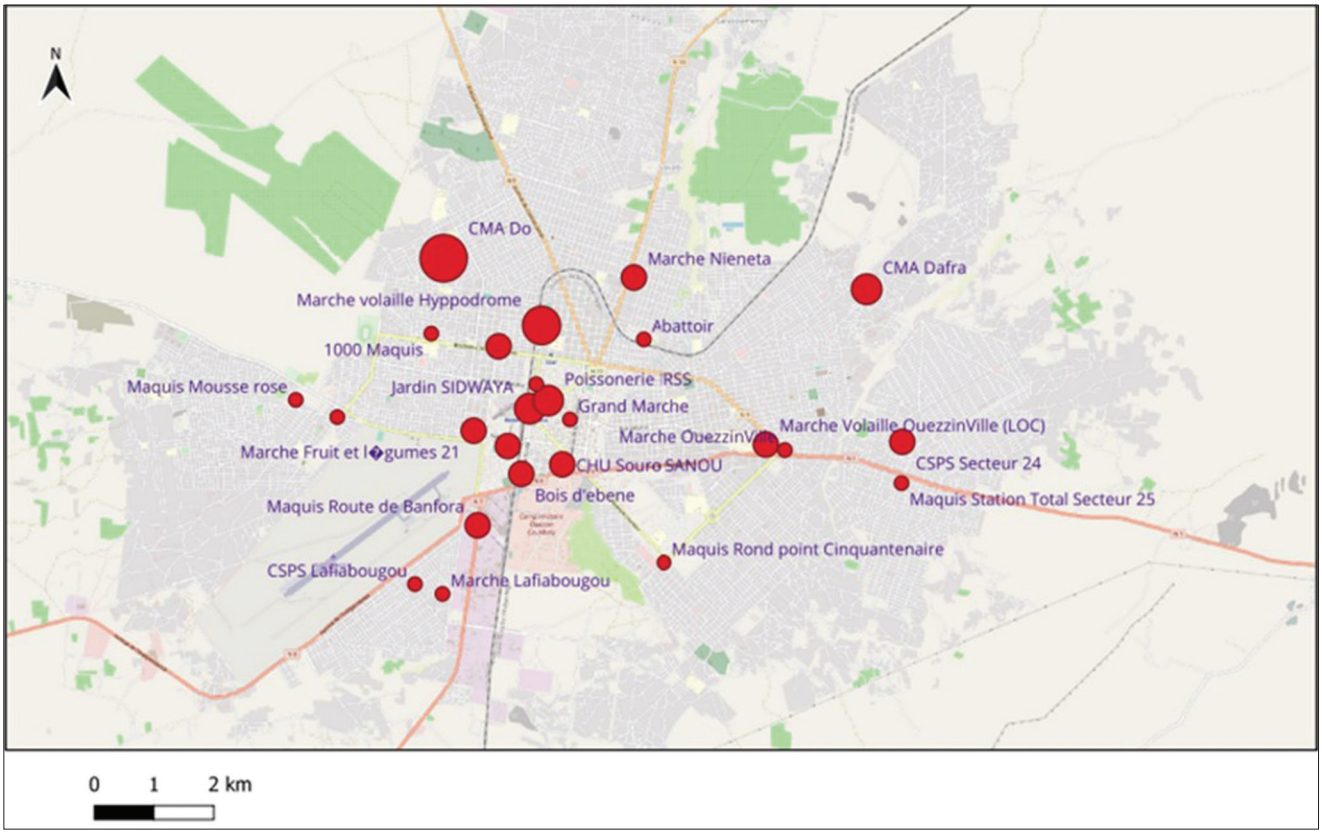

Figure-2: ESBL-producing *Enterobacteriaceae* carried on houseflies in the city of Bobo-Dioulasso.

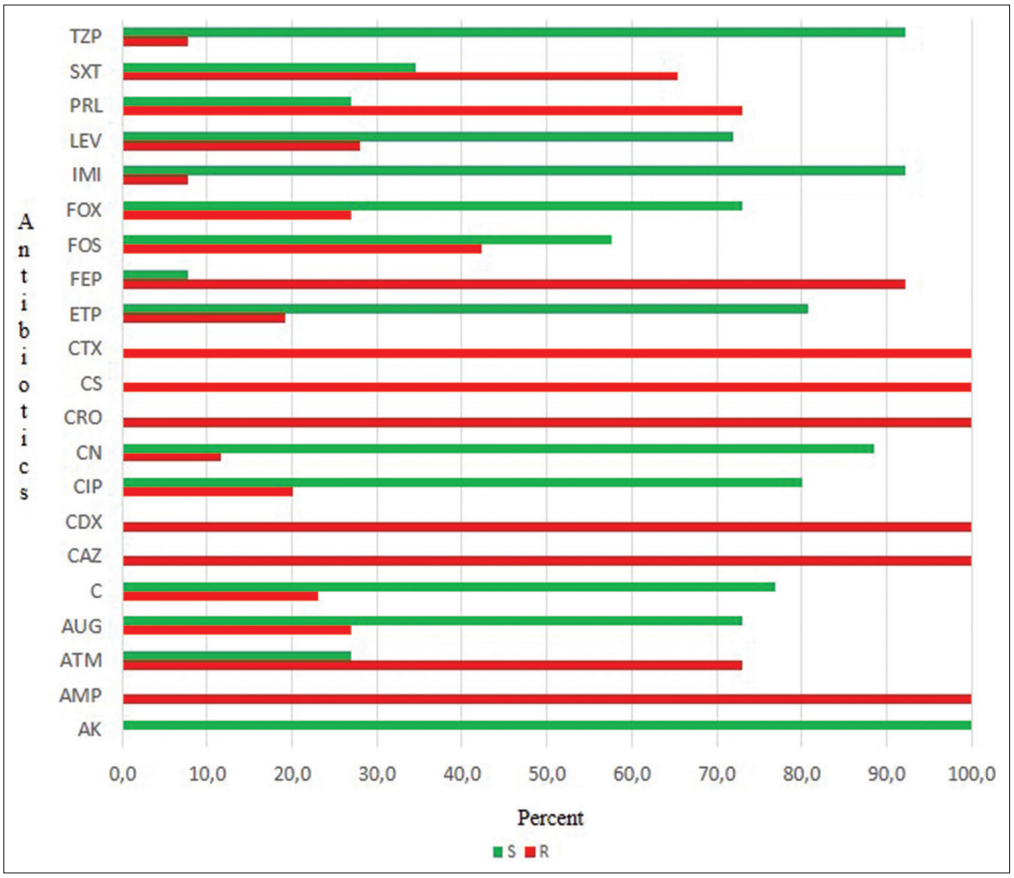

Figure-3: Susceptibility to antibiotics of *Enterobacteriaceae*-producting ESBL strains. AK: Amikacin, AMP: Ampicillin, AUG: Augmentin, ATM: Aztreonam, C: Chloramphenicol, CAZ: Ceftazidim, CDX: Cefadroxil, CIP: Ciprofloxacin, CN: Gentamicin, CRO: Ceftriaxon, CS: Colistin, CTX: Cefotaxim, ETP: Ertapenem, FEP: Cefepim, FOS: Fosfomycin, FOX: Cefoxitin, IMI: Imipenem, LEV: Levofloxacin, PRL: Piperacillin, SXT: Trimethoprim + Sulfametoxazol, TPZ: Piperacillin/ Tazobactam.

Table-1: Distribution of *Enterobacteriaceae* according to collection sites.

Bacteria	Collection sites					
	Markets	**Restaurants**	**Fish markets**	**Poultry markets**	**Health centers**	**Total**
Escherichia coli	1	2	0	5	7	15
Klebsiella pneumoniae	1	2	0	0	3	6
Enterobacter cloacae	0	0	2	1	1	4
Morganella morganii	0	0	0	0	1	1
Total, n (%)	2 (7.69)	4 (15.38)	2 (7.69)	6 (23.08)	12 (46.15) (p=0.03)	26

Table-2: Bacteriological and molecular characteristics of *Enterobacteriaceae*.

Number	Identifier	Strain isolated	Inactive antibiotics	Resistance genes	Conjugation experiment	Phylogroup
1	PIRSS L1a	*E. cloacae*	AUG, C3G, SXT, CIP, LEV	*CTX-M gp1, TEM, QnrS*	NP	-
2	PIRSSL1b	*E. cloacae*	AUG, C3G, SXT, CIP, LEV	*CTX-M gp1, TEM QnrS*	Positive	-
3	MRB L5	*E. coli*	AUG, C3G, ETP SXT, CIP, LEV	*CTX-M gp1, TEM Oxa-1-like, QnrS, QnrB, AAC (6')*	NP	B1
4	JSIDW L1c	*K.pneumoniae*	C3G, ATM	*CTX-M gp1, TEM SHV*	Positive	-
5	JSIDW L5a	*K. pneumoniae*	ATM, C3G, CIP, LEV, IMI	*CTX-M gp1, TEM SHV, QnrS, OqxAB*	NP	-
6	HYPPO L1b	*E. coli*	C3G, SXT	*CTX-M gp1, TEM*	NP	B1
7	HYPPO L2a	*E. cloacae*	C3G, ETP	*CTX-M gp1, TEM*	NP	
8	HYPPO L5a	*E. coli*	AUG, C3G, ETP	*CTX-M gp1, TEM*	NP	Unknown
9	HYPPO L5b	*E. coli*	C3G, SXT	*CTX-M gp1, TEM*	NP	Unknown
10	OZVMV L5a	*E. coli*	C3G	*CTX-M gp1*	NP	B1
11	MVK L5b	*E. coli*	C3G	*CTX-M gp1, TEM*	Negative	B1
12	BOEB L5a	*E. coli*	C3G, SXT	*CTX-M gp1, TEM*	NR	Unknown
13	MFL21 L4b	*E. coli*	C3G, SXT	*CTX-M gp1, TEM*	Negative	D/E
14	MNIET L1a	*K.pneumoniae*	C3G, CIP, LEV	*CTX-M gp1, TEM SHV QnrS*	Negative	-
15	CSACV L3	*E. cloacae*	AUG, C3G, ETP	*CTX-M gp9, TEM SHV, Oxa-1-like*	NP	-
16	CMADO L1b	*K. pneumoniae*	C3G, SXT	*CTX-M gp1, TEM Oxa-1-like*	Negative	-
17	CMADO L1c	*E. coli*	C3G, SXT	*CTX-M gp1, TEM SHV, Oxa-1-like*	Positive	Unknown
18	CMADO L2b	*E. coli*	C3G, SXT	*CTX-M gp1, TEM SHV, Oxa-1-like*	Positive	D/E
19	CMADO L2c	*K. pneumoniae*	C3G, SXT	*CTX-M gp1, TEM*	Positive	-
20	CMADO L3	*E. coli*	C3G, SXT	*CTX-M gp1, Oxa-1-like*	NP	B1
21	CMADO L5a	*K. pneumoniae*	C3G, SXT	*CTX-M gp1*	Negative	-
22	CMADO L5b	*E. coli*	C3G	*CTX-M gp1*	NP	B1
23	CS24 L5b	*E. coli*	C3G, AUG, ETP, SXT	*CTX-M gp1*	NP	B1
24	CMADAF L2	*M. morganii*	C3G, AUG, SXT	*CTX-M gp1, TEM*	NP	-
25	CMADAF L3b	*E. coli*	C3G, SXT	*CTX-M gp1*	NP	Unknown
26	CHUSS L3a	*E. coli*	C3G, SXT	*CTX-M gp1, TEM*	Positive	Unknown

AUG=Augmentin, ATM=Aztreonam, C3G=Third generation cephalosporins, CIP=Ciprofloxacin, CTX-M gp1=Cefotaximase Munich group 1, ETP=Ertapenem, *E. cloacae=Enterobacter cloacae, E. coli=Escherichia coli, K. pneumoniae=Klebsiella pneumoniae*, LEV=Levofloxacin, *M. morganii=Morganella morganii*, Oxa-1-like=Oxacillinase type 1 like, Qnr=Quinolone resistant, SHV=Sulfhydryl variable, SXT=Cotrimoxazole, TEM=Temoneira, NP=Not performed

the existence of ESBL-E strains harbored by flies in various public settings within urban regions. These areas included markets, restaurants, poultry markets, fish markets, and health centers where the caught flies were obtained. The bacteria in question are commonly found on the exoskeleton of flies, in particular on the mouth, wings and legs. Houseflies are commonly observed in close proximity to human activities, such as residential neighborhoods, food court establishments, medical facilities, and livestock farms [27]. These bacteria were transported from the environment to animals and humans during the feeding process [9, 28]. The presence of these pathogens is linked to inadequate hygiene and hygiene practices in the environment where the flies are captured.

In this study, ESBL-E identified in carriage exhibited greater numerical significance within the healthcare context (12 out of 26 cases, p = 0.03). The current findings are similar to the results of previous studies conducted by Nazari *et al*. [29] in Hamadan, Iran, and Akter *et al*. [30] in Bangladesh. These studies reported a greater prevalence of bacterial strains isolated from flies in hospital settings. In a study conducted by Boulesteix in Senegal, an investigation was conducted in an intensive care unit. The findings revealed that 82.5% (99 out of 120 flies) of the captured flies were

carriers of several infectious diseases. Of the total 120 flies, 17 were found to carry BMR, accounting for approximately 14% of the captured flies, as reported in reference [31]. In a study carried out by Stefan in a tertiary hospital in Rwanda, antibiotic-resistant bacteria were found in 48% (20 out of 42) of flies. A previous study by Heiden *et al*. [32] showed that 36% (15/42) of the samples carried ESBL-E. In addition, *E. cloacae*, *Klebsiella oxytoca*, *Citrobacter freundii*, and *Raoultella ornithinolytica* were present in 19% (8/42), 9% (4/42), 7% (3/42), and 4% (2/42) of the samples, respectively. Our findings, similar to those of other studies, indicate a likely association between *M. domestica* and nosocomial transmission of multidrug resistant bacteria in hospital wards, particularly in sub-Saharan Africa.

The *blaCTX-M* group 1 gene was predominantly present in the *Enterobacteriaceae* strains in our study with a carriage rate of 95.16%. The CTX-M-G1 gene was associated with TEM and SHV penicillinase genes in 73.07% and 15.38% of the isolated strains, respectively. The combination of these enzymes would neutralize the effect of beta-lactamase inhibitors and thus extend the resistance spectrum of ESBL-producing bacteria.

The CTX-M family of enzymes, specifically ESBLs, has been widely documented to have a global pandemic distribution. Furthermore, dissemination process has been linked to the proliferation of epidemic clones characterized by certain enzymes, such as CTX-M-15 [33]. *bla*CTX-M-15 emerged as the prevailing ESBL gene identified globally in both animal and human populations [34–36]. The aforementioned finding has also been observed in Burkina Faso across several human clinical samples, fecal carriage and animal fecal transport [37–39].

Conjugation experiments were limited to a subset of 11 strains, representing approximately 42% of the overall population of 26 strains. Elimination of the 15 donor strains (ESBL-E) can be attributed to their resistance to rifampicin. The introduction of an alternative chemical, such as sodium azide, would facilitate the execution of the experiment on a larger number of strains. However, it should be noted that a significant proportion of the ESBL-E isolates, specifically 54% (6 out of 11), had the capacity to transfer the *blaCTX-M* resistance gene from group 1 by conjugation.

In our study, *E. coli* is the most common enterobacterial species producing ESBL, followed by *K. pneumoniae*. *Klebsiella* species and *E. coli* are the predominant producers of ESBLs [40]. *E. coli* is a well-known indicator organism due to its commensal nature and widespread presence in animals, the environment, and humans. Its ability to offer valuable insights into the dissemination of antibiotic resistance has been well documented [41]. *E. coli* strains belonged to the majority of phylogroup B1 in our analysis. Numerous studies on African populations have consistently indicated the prevalence of phylogroups A and B1 in healthy animals, human clinical samples, and human fecal carriage [34].

These findings indicate that flies can serve as perfect vectors and sentinels for monitoring bacterial infections and antibiotic resistance in Burkina Faso [8].

Conclusion

Our study showed that houseflies represent a risk for the transmission of multiresistant bacteria in the city of Bobo-Dioulasso. ESBL-E was found on the exoskeletons of flies caught in various public places (e.g., the community and hospital). No carbapenemase-producing bacteria were isolated in this study. These results confirm the need for active surveillance of AMR. The housefly would be an ideal sentinel for active surveillance of antibiotic resistance in low-resource countries.

Authors' Contributions

SDMS, YRS, FA, and ZJ: Conceptualization and writing–original draft preparation. SDMS conducted the study with BT, YI, and BN for formal (bacteriological and molecular) analysis. BT, YI, NF, and BN: Formal analysis. NS: Conceptualization and reviewed the manuscript. KNF and TI: Methodology and reviewed the manuscript. OJ, SI, and SM: Supervision and reviewed the manuscript. All authors have read, reviewed, and approved the final manuscript.

Acknowledgments

This study was partly funded by the FAO Regional Office for Africa (FAORAF) in Accra, Ghana (Protocol of agreement N° 041/2021/FRBKF of July 16, 2021). The authors would like to thank Mr. Assogba Sessinou Benoît for reviewing this document.

Competing Interests

The authors declare that they have no competing interests.

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