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


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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, multidrug-resistant 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 Enterobacteriales 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 Enterobacteriales [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:

\[
\text{n} = \left(\frac{Z_{\alpha}^2 \cdot \text{P} \cdot [1-\text{P}]}{\Delta^2}\right),
\]

Where:
- \(Z_{\alpha}\) = the reduced deviation corresponding to an \(\alpha\) error of 5% = 1.96
- \(\text{P}\) = fraction of the population carrying the germ (estimated at 50% to maximize sample size)
- \(\Delta\) = 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
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 the 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 χ² 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-lactamas. 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.

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) 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, Enterobacteriaceae, 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]. blaCTX-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 scale. An alternative chemical, such as sodium azide, would facilitate the execution of the experiment on a larger scale. 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.

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Competing Interests

The authors declare that they have no competing interests.

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References

Musca domestica,


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