

DISTRIBUTION OF *bla*TEM GENE IN *ESCHERICHIA COLI* ISOLATED FROM HOSPITAL ENVIRONMENT

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Abstract

Extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* may represent hospital reservoirs for antimicrobial-resistant bacteria. Houseflies (*Musca domestica*) can help spread these organisms from contaminated locations to areas where patients and health care workers might be. In the present study, *bla*TEM gene was determined in the *E. coli* collected from houseflies present in the environment of a tertiary care hospital in Lahore, Pakistan, and was their antimicrobial susceptibility profile described. 200 houseflies were collected from the patient wards, Hospital Corridor, Surgical wards, Waste Disposal Areas and surrounding areas. A total of 50 *E. coli* strains were successfully isolated and tested for antimicrobial susceptibility using the disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Genomic DNA was extracted by heat lysis and molecular identification of *bla*TEM was done by polymerase chain reaction. Of the 50 *E. coli* isolates, 23 (46%) were positive for *bla*TEM and 27 (54%) negative. There was significant variation in the antimicrobial susceptibility of the isolates. Sulfamethoxazole (42%) and colistin (42%) had the next highest resistance rates, with ceftazidime (100%) having the highest resistance rate. Fosfomycin, levofloxacin, tigecycline, amikacin and meropenem resistance rates were 12%, 10%, 10%, 8% and 0%, respectively. The results indicate that a significant portion of the *E. coli* that was recovered from hospital houseflies possessed *bla*TEM and that the different isolates exhibited different resistance profiles. The above observations reinforce the need for environmental monitoring, good dissemination controls, infection controls, sanitation and drug utilization in hospitals and other healthcare facilities. Retracing the transmission of antimicrobial resistance in Pakistan must be further explored by larger scale multicenter studies, along with the molecular research of further resistance determinants.

1. Introduction

One of the significant public health threats global now is antimicrobial resistance. It is noteworthy that extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* has become

widespread and can diminish the effectiveness of commonly used β -lactam therapeutics, making them a challenge to treat with infections. This same behavior is not limited to clinical isolates. Resistant bacteria and

resistance genes are part of environmental reservoirs, and can be spread from patients to healthcare workers, surfaces, wastewater, and waste materials, to other parts of the hospital environment (Facciola et al., 2019; Partridge et al., 2018).

Hospitals create situations to allow resistant organisms to be sustained, spread and transmitted. Opportunities for the survival and spread of resistant bacteria may exist through continuous exposure to antimicrobial products and contaminated surfaces, patient faeces, wastes or wastewater, medical equipment and inappropriate sanitation. Environmental surveillance is thus a valuable addition to understanding antimicrobial resistance where clinical surveillance might not provide complete understanding of its transmission pathway, especially as this provides a window into other potential sources of resistance or opportunities to cut transmission (Neidhofer et al., 2023; Aleem et al., 2021).

Houseflies are of special interest among the environmental vectors. This species is often seen near human residences, hospitals, and other health care centers and can pick up microorganisms from garbage, fecal material, rotting organic matter, refuse containers, contaminated surfaces and other sources. Mechanically transport microorganisms to other hosts via their body surface, legs, adhesive structures and mouthparts. Studies have previously demonstrated that houseflies can be a vector for enteric and antimicrobial-resistant bacteria (AMR) potentially linking humans and people to contaminated environmental sites (Khamesipour et al., 2018; Onwugamba et al., 2018; Wiktorczyk-Kapischke et al., 2022).

E. coli is the most common bacteria present in the intestinal tract of humans and animals and are also gram-negative, members of Enterobacteriaceae. While most strains are commensal, pathogenic strains can cause gastrointestinal disease, urinary tract infection, sepsis and other infections. Since *E. coli* is also present in fecal sources and widely used as an indicator of fecal contamination, its presence in the environment may also be indicative of sanitation and contamination pathways (Jang et al., 2017; Tawfick et al., 2022).

Production of β -lactamases is one of the important mechanisms of resistance in *E. coli*. ESBLs can break down a variety of β -lactam antibiotics, such as third-generation cephalosporins. *bla*TEM type genes are located in the class A β -lactamase family and are commonly found in Gram-negative resistant bacteria. They are related to mobile genetic elements that can promote the spread of resistance genes from one population of bacteria to another (Bush, 2018; Lee et al., 2012; Oduro-Mensah et al., 2016).

While clinical, food, animal, wastewater, and environmental sources of *bla*TEM producing *E. coli* have been reported, the contribution of housefly to the spread of *bla*TEM in the hospital environment is less studied than in other settings, especially in Pakistan. The focus of the existing literature has been toward more clinical, agricultural environments, but less attention has been given to vector mediated transmission in hospitals, e.g., responses to the lepidopterous larvae associated with the involvement of mosquitoes in the transmission of diseases from insects to humans. The present study was thus conducted in the context of finding out the distribution of *bla*TEM gene in *E. coli* isolated from hospital houseflies and the pattern of antimicrobial susceptibility of the recovered *E. coli*.

2. Literature Review

2.1 Antimicrobial resistance and the hospital environment

The antimicrobial resistance results from the acquisition or expression of mechanisms that have the ability to inhibit the action of the antimicrobial drug. They can be generated by genetic mutation, gain resistance genes or horizontal gene transfer. Regardless, resistance determinants can be shared between bacteria via mobile genetic elements, like plasmids or transposons, which can move resistance from one bacterial species to another within populations, as well as between populations (Partridge et al., 2018; von Wintersdorff et al., 2016).

Health centers are essential in this process since the use of antibiotics, close contacts between patients and workers and waste, water, surfaces

are a feature of the environment. Resistant bacteria can survive in hospital settings and can lie dormant in biofilms or be re-introduced and spread throughout the environment by people. It is therefore important to use environmental surveillance in addition to patient-based surveillance because environmental contamination can also be a cause for healthcare associated transmission.

2.2 Houseflies as potential vectors of resistant bacteria

Bacteria can pick-up from contaminated material and be mechanically transported by houseflies. They can be vectors for the sparing transfer of the microorganisms between the waste and the food, the surfaces as well as the human-related areas. A systematic review showed that houseflies can transport a large number of human pathogens and recent investigations revealed the ability of flies to transport antimicrobial-resistant *Enterobacter* (AMRE) and/or ESBL-producing organisms (Epic) from both hospital and environmental settings (Khamesipour et al., 2018; Tufa et al., 2020; Obeng-Nkrumah et al., 2025).

E. coli and genes that code for *BlaTEM*, resistance genes found in flies at food markets, farms, poultry environments and elsewhere have also been reported in studies out of hospitals. The results suggest that flies can contribute to environmental reservoir or mechanical bear of antimicrobial resistance determinant. But data on antimicrobial resistance in agricultural and community settings cannot be automatically extrapolated to a hospital setting, as there may be significant variation in microbial pressure, waste management practices and sanitation systems.

2.3 ESBLs and *blaTEM*

ESBLs are enzymes capable of hydrolysing a wide variety of β -lactam antibiotics. Some of the most common ESBL producers are in major class A families, such as TEM, SHV, and CTX-M enzymes. *blaTEM* genes are seen commonly and have been frequently found in *E. coli* and other Enterobacterales. Their presence has been linked to resistance to β -lactam antibiotics and have been co-located with other resistance determinants resulting in

multidrug resistant phenotype (Paterson & Bonomo, 2005; Feizabadi et al., 2010; Bajpai et al., 2017). *blaTEM* is present in different frequencies in various geographic locations, bacterial populations, host cases and environmental sources. *blaTEM* has been reported in *E. coli* strains from clinical samples, farms (livestock and poultry), food, wastewater and flies. Studies conducted in in Zambia, ESBL-associated genes have also been found in houseflies and studies conducted in Indonesia and elsewhere have shown *E. coli* carrying *blaTEM*-associated resistance genes are carried by flies and other environmental reservoirs. Recent hospital research described above reinforces worrying reports of ESBL-producing *Enterobacter* being carried by flies in healthcare settings.

2.4 Research gap

Although there is abundant literature available regarding the presence of *blaTEM* positive *E. coli* in both clinical and environmental reservoirs, hospital associated houseflies in Pakistan have limited information. This space represents a key gap as flies may gain access from contaminated environmental areas to human-associated areas. If a hospital fly is found carrying *E. coli* that contain *blaTEM*, it may be useful to have baseline data to aid in environmental surveillance and infection-control plans.

3. Materials and Methods

3.1 Study area and design

The study was conducted in an environment of tertiary care hospital in Lahore Pakistan. Houseflies (*Musca domestica*) were captured in patient rooms, hospital corridors, surgical wards, waste disposal sites, and exterior sites where fly activity was frequently noted. The study aimed at isolating *E. coli* from hospital flies, and to assess the antimicrobial susceptibility profile of the isolates and the presence of the *blaTEM* gene.

3.2 Sample size and selection criteria

200 houseflies were collected from the public setting within the hospital. Of those, 50 samples were successfully isolated as *E. coli*, and

were chosen for further experimental analysis. The individual fly was used as an individual sample and the likelihood of cross-contamination was reduced. The houseflies that were trapped in the hospital units and *E. coli* isolates that were confirmed from culture media were added on. Samples and isolates that were damaged, contaminated, collected in an improper way and unconfirmed as *E. coli* were not included.

3.3 Sample collection and processing

Houseflies were collected using bright yellow sticky adhesive traps. Traps placed in various settings in hospital. The captured flies were gently extracted, and each individual fly was transferred to sterile 1.5-mL Eppendorf tubes. The data on collection sites and dates were noted and the samples were taken to the laboratory for processing. Homogenates of houseflies were prepared by adding sterile phosphate buffered saline (PBS) to each fly, and then by crushing them under sterile conditions. In order to get a homogenized sample, the suspension was thoroughly mixed up and shaken. The homogenate solution was divided into two aliquots, one containing vancomycin and incubated at 37°C for 24 hours. After enrichment, cultures were streaked onto MACCA and incubated at 37 °C for 18–24 hours. The colonies that produced pink lactose were presumptive *E. coli* and were chosen for additional testing.

3.4 Identification of *E. coli*

Presumptive isolates were sub-cultured on solid Media (agars) - Eosin Methylene Blue (EMB) and incubated aerobically under 37°C for 18-24 hours. Any colonies with the characteristic dark appearance with metallic green shade were thought to represent *E. coli*. Isolates for antimicrobial susceptibility testing, and molecular analysis were selected from the culture findings.

3.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out with Disk Diffusion (DD) method on Mueller-Hinton agar (MHA) according to Clinical and Laboratory Standards Institute (CLSI). Bacterial lawns were prepared by

inoculating fresh colonies isolated in well. The eight antimicrobial agents tested were ceftazidime, meropenem, levofloxacin, amikacin, tigecycline, fosfomycin, sulfamethoxazole and colistin. Antibiotic disks were used for inoculated plates which were incubated for 18–24 hours at 37°C. The ZOI was determined and based on the CLSI breakpoints found at acceptable levels. Zones of inhibition were measured in millimeters with the applied CLSI breakpoints interpreted as susceptible, intermediate, and resistant.

3.6 DNA extraction

Thus, genomic DNA was isolated from cultured colonies of *E. coli* in accordance with the protocol by heat lysis. A bacterial colony was suspended in nuclease-free water and heated for 10 minutes at 95°C. The lysate was cooled and then centrifuged at 14,000 rpm for 5-10 minutes. This extracted DNA was transferred to a sterile microtube, and transferred to be used in PCR, or conserved for further study.

3.7 PCR detection of *blaTEM*

The *blaTEM* gene was detected using PCR. The total reaction volume was 25 µL in which 22 µL of PCR master mix, forward and reverse primers 0.5 µL each and 2 µL of DNA template were added. The thermal cycling parameters were as follows: 35 cycles: 95°C for 45 s for denaturation, 57°C for 1 minutes for annealing, 72°C for 1 minutes for extension followed by final extension: 72°C for 5 minutes, with final hold: 4°C.

Primers for the detection of *blaTEM* were *blaTEM-F* (ATCAGCAATAAACCAGC) and *blaTEM-R* (CCCCGAAGAACGTTTTC) to obtain a 516 bp amplicon was reported. Agrograms of PCR products were analyzed by 1.5% agarose gel electrophoresis. If the band at ~516 bp was present it was considered positive for *blaTEM*, and if the band was not present it was considered negative.

4. Results

4.1 Isolation and identification of *E. coli*

Two hundred houseflies were collected in hospital environment. Forty-five samples

yielded *E. coli*, of which 50 were successfully isolated and used for the further antimicrobial susceptibility and molecular analysis. The isolates produced pink lactose fermenting

colonies on Mac Conkey. Colonies on EMB agar were observed to have a green metallic sheen, which is helpful in the identification of *E. coli*.

4.2 Antimicrobial susceptibility pattern

Table 1. Antimicrobial susceptibility profile of *E. coli* isolates (n=50).

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Ceftazidime	0	0	100
Fosfomycin	78	10	12
Meropenem	100	0	0
Amikacin	78	14	8
Levofloxacin	86	4	10
Colistin	58	0	42
Sulfamethoxazole	58	0	42
Tigecycline	80	10	10

The susceptibility pattern of the isolates was found to be variable. All tested isolates were reported as resistant to ceftazidime, with resistance highest of all. Forty-two percent of them were colistin resistant and 42% were sulfamethoxazole resistant. The following drugs were found with lower resistance levels: fosfomycin (12%), levofloxacin (10%), tigecycline (10%) and amikacin (8%). The study dataset did not contain any reports of resistance to meropenem. Meropenem (100%), levofloxacin (86%), tigecycline (80%), fosfomycin (78), amikacin (78) and colistin (58)

and sulfamethoxazole (58) had the best sensitivity results.

4.3 Molecular detection of *bla*TEM

The presence of *bla*TEM in isolates was confirmed using PCR and agarose gel electrophoresis, showing a distinct band ~516 bp. Of the 50 *E. coli* isolates, 23 of which were positive and 27 negative for *bla*TEM. Thus, 46% of the *E. coli* isolates were positive for the presence of *bla*TEM.

Table 2. Frequency and percentage distribution of *bla*TEM among *E. coli* isolates.

<i>bla</i> TEM status	Frequency (n)	Percentage (%)
Positive	23	46
Negative	27	54
Total	50	100

5. Discussion

The present study aimed to find the prevalence and distribution of *bla*TEM in *E. coli* respectively recovered from houseflies collected from a tertiary care hospital in Lahore, Pakistan. A total of 50 *E. coli* isolates were successfully obtained from the 200 houseflies that were collected for further analysis. A molecular test was used to detect the *bla*TEM gene and established an overall prevalence of 46% (23/49 isolates). The results of this study suggest that a significant fraction of the environmental *E. coli* strains was representative

of a resistance determinant, and therefore lead us to believe that the hospital-associated flies may also contribute to the environmental spread of antimicrobial-resistant bacteria.

In the present study, the prevalence of *bla*TEM was 46% in accordance with the wide range found for flies or other environmental sources of these. Previous studies have documented TEM resistant *E. coli* in fly samples collected from food markets, farms, and poultry settings as well as other polluted areas. The paper from Zambia isolated *bla*TEM in *E. coli* in houseflies, with other studies in other contexts reporting

E. coli carrying *bla*TEM, or blades of other genes, from the environment and animal-associated *E. coli*. Bacteria exposure and contamination may vary widely however, with sampling site and bacterial population, exposure to antimicrobials and laboratory methodology.

Another interesting finding of the present study is that the flies were collected from tertiary care hospital as opposed to a farm, food market or community setting. Microorganisms may be subjected to greater and greater constant effective antimicrobial selective pressure in hospital environments, and may be found on contaminated patient-associated materials, surfaces and waste and wastewater. Resistant organisms could be picked up by houseflies as they travel between these areas and spread mechanically to any area of the facility. The carriage of ESBL-producing Enterobacteriales by flies has been reported previously and their vector potential in environmental surveillance has to be taken into account in other hospital-based studies.

The results obtained on the sensitivity of the isolated bacteria to these agents served to further confirm the resistant isolates recovered. 100% of the tested isolates were ceftazidime resistant. Ceftazidime is a 3rd generation cephalosporin and ESBL-producing Gram-negative bacteria have been shown to be resistant to this class. It is important, even in the present data set to emphasize, that the complete resistance observed is an environmental finding, but if only *bla*TEM was examined, this resistance should not be concluded as being the sole resistance mechanism involved in the resistance phenotype, due to the fact that other resistance mechanisms were not examined in the present study.

The colistin and the sulfamethoxazole resistance rates were found to be 42%. Given the value of colistin for treating intractable infections due to Gram-negative bacteria, detection of colistin resistance in environmental isolates should be of interest. The results also revealed reduced resistance levels to fosfomycin, levofloxacin, tigecycline and amikacin. In the study dataset 100% of isolates were sensitive to meropenem. These

differences highlight that the susceptibility patterns of all the isolates were not the same and that considerable variation has the potential to occur in terms of antimicrobial resistance.

The level of susceptibility to meropenem observed is in line with observations of environmental *E. coli* isolates showing good susceptibility to carbapenems in reported cases. However, since both the *bla*TEM and the ceftazidime resistance rate are found in the environment, it is not justifiable to assume that agents considered environmental are harmless only because they are still susceptible to certain agents. There is the possibility of co-existence of resistance determinants with some other genes and spreading via mobile genetic elements. Therefore, it is crucial for continuous surveillance, especially in hospitals where resistant organisms can be transmitted from the environmental to the clinical settings.

Houseflies are potentially involved in the transmission of antimicrobial resistance, since they can come in contact with microorganisms that have been contaminated with resistance genes and subsequently spread to human-associated surfaces. Truly, it has been shown that flies can be vectors of ESBL and resistance genes such as *bla*TEM, tet genes and others. The local evidence in the present finding further confirms the significance of putting environmental vectors into a wider lens of One Health approaches to the surveillance of antimicrobial resistance, with that of Lahore.

The results also impact on infection-control practice. Resistant organisms may be less likely to survive and spread if sanitation and waste management measures are taken, fly control, environmental cleaning, antimicrobial stewardship, and routine surveillance are undertaken. The results should not be assumed to indicate flies exclusively as evidence for transmission. Instead, they suggest that houseflies could be one part of the environment where resistant *E. coli* can be transmitted within health care establishments.

5.1 Study limitations

Some limitations of this study are noted below. The sample size was not very large, and samples were obtained from one hospital, which

restricts extrapolating the findings to other hospitals or in other regions of Pakistan.

The molecular study only covered the *blaTEM*, but not important other resistance determinants like *blaSHV*, *blaCTX-M*, *blaOXA*, or carbapenem-resistance genes.

The study did not actually prove transmission of *blaTEM*-positive *E. coli* from houseflies to patients or healthcare workers. The results should be interpreted in light of these limitations.

6. Conclusion

The present study showed that houseflies collected from a tertiary care hospital, Lahore, were also found to be carrying *E. coli* with *blaTEM* β -lactamase amongst them. Twenty-three of 50 (46%) isolates of *E. coli* tested were positive for *blaTEM*, and 27 (54%) were negative. The isolates also exhibited high level of antimicrobial resistance pattern: 100% resistance against ceftazidime, 42% against colistin and sulfamethoxazole, and 100% sensitivity against meropenem. These infer that the housefly could be a mechanical vector of antimicrobial resistance *E. coli* and the hospital environment should be taken into antimicrobial resistance monitoring. Improving infection control, environmental hygiene, vector control and antimicrobial use practices can help to decrease the potential spread of resistant organisms.

7. Recommendations

Further research is needed on other resistance mechanisms and other genes like *blaSHV*, *blaOXA*, *blaCTX-M*, and carbapenem-resistance genes.

Multicentred surveillance of hospitals from various regions of Pakistan should be conducted to determined larger estimation of *blaTEM* distribution.

In future, the genetic diversity and the potential spreading of the virus may be described using Whole genome sequencing and phylogenetic analysis.

Comparisons of environmental samples from the hospital, wastewater, clinical samples, and housefly samples may aid in understanding the

connections between resistance found in the environment and in clinical samples.

Combating resistance should be viewed as a multifaceted problem that includes environmental sanitation, fly control, waste management, environmental surveillance and the prescription and proper use of antimicrobials.

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