

# Carriage of ESBL-producing Gram-negative bacteria by houseflies captured in a hospital and its suburban surroundings in Ethiopia

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# Abstract

**Background:** Local data from the Asella Referral and Teaching Hospital in the town of Asella, Ethiopia reveal a high prevalence of extended-spectrum  $\beta$ -lactamase- (ESBL) producing Gram-negative bacteria in clinical isolates. To investigate a possible route of transmission, we determined the colonization rate of houseflies with ESBL-producing Gram-negative bacteria in the hospital compound and in the town of Asella.

**Methods:** Houseflies were collected in August 2019 from the neonatal intensive care unit (NICU), the orthopedic ward, the hospital's waste disposal area, and from a butchery situated 1.5 km from the hospital. After trapping, the flies were macerated and suspended in sterile normal saline. The suspensions were inoculated on MacConkey agar and incubated overnight. Species identification and antimicrobial susceptibility testing were performed using Vitek<sup>®</sup>-MS and VITEK<sup>®</sup> 2.

**Results:** In total, 103 bacterial isolates were obtained from 85 flies (NICU: 11 isolates from 20 flies, orthopedic ward: 10 isolates from 20 flies, waste disposal area: 37 isolates from 26 flies, butchery: 45 isolates from 27 flies). The prevalence of ESBL-producing bacteria in collected flies was 82%, 90%, and 57% in NICU, orthopedic ward and the waste disposal area, respectively and 2% (1/45) in the butchery. The difference between flies trapped inside and outside the hospital compound was statistically significant ( $p \leq 0.001$ ). The frequency of ESBL was 67% (6/9) in *Raoultella* spp. 67% (4/6) in *Kluyvera* spp., 56% (5/9) in *Enterobacter* spp., 50% (5/10) in *E. coli* and *Citrobacter* spp., and 44% (8/18) in *Klebsiella* spp.. Of the 40 ESBL-genes detected, 85% were CTX-M-like, 83% TEM-like, 23% SHV-like, and 2% CTX-M-2-like. ESBL-producing bacteria showed higher rates of antimicrobial resistance against ciprofloxacin (66% vs. 5%), gentamicin (68% vs. 3%), piperacillin-tazobactam (78% vs. 5%), and trimethoprim-sulfamethoxazole (88% vs. 16%), compared to non-ESBL-producing bacteria.

**Conclusion:** A high proportion of the hospital's housefly population were colonized with pathogenic ESBL-producing bacteria, but not houseflies collected at a distance of 1.5 km from the hospital. Houseflies can be potential vectors for transmission of multidrug-resistant (MDR) bacteria within hospitals. Further studies are needed to determine the source of MDR colonization in houseflies and possible impact of the high rate of MDR for nosocomial infections.

## Introduction

The housefly, *Musca domestica* has been proposed to be a potential vector for communicable diseases and multidrug resistance (MDR) in hospitals, particularly in developing countries (1, 2). Houseflies can transmit MDR microorganisms in the three ways: mechanical translocation, regurgitation (bio-enhanced transmission) and defecation, in which MDR bacteria may become a part of the gut flora of houseflies thus carrying bacteria for the life span of the fly and contaminating their environment via feces and/or regurgitation. If houseflies play a role in AMR transmission, current hospital hygiene programs, focusing on patient isolation, hand hygiene, and antimicrobial stewardship programs, may not be sufficient to

address the expansion of antimicrobial resistance (AMR), especially in resource limited, and possibly fly-abundant, settings (1, 3).

The expansion of MDR due to extended spectrum  $\beta$ -lactamase- (ESBL) production in Gram-negative bacteria (GNB) has become an emerging threat to antibiotic treatment success in resource limited settings. ESBLs are enzymes, encoded by genes often found on mobile genetic elements, which mainly include class A  $\beta$ -lactamases, such as CTX-M-type, TEM, and SHV and they confer resistance to the penicillin and cephalosporin antibiotic classes. CTX-M-type  $\beta$ -lactamases are the most abundantly found ESBL enzymes worldwide (4).

Ceftriaxone and ceftazidime are the most commonly used antibiotic substances for the treatment of blood stream infections caused by GNB at the study site. Local data from the Asella Referral and Teaching Hospital (ARTH) reveal a high prevalence of ESBL-producing GNB in clinical isolates, hampering the efficacy of empiric antibiotic therapy (5). To our knowledge, there are no reports on the colonization rate of houseflies with ESBL-producing bacteria in Ethiopian hospitals and the possible implications for the spread of AMR. A prospective study was therefore initiated to investigate the colonization of houseflies with ESBL-producing GNB at the ARTH compound and in Asella town.

## Materials And Methods

The houseflies were collected in August 2019 in the ATRH's neonatal intensive care unit (NICU), the orthopedic ward, the hospital's waste disposal area, and in a butchery located 1.5 km from the hospital. The flies were trapped with non-toxic retail flycatchers (Profissimo® Giftfreier Fliegenfänger, Germany) and stored in 2 ml of sterile normal saline within the same day.

For further analysis in this study, only animals displaying essential taxonomic morphologic criteria of houseflies such as size, shape and color were selected.

After maceration in sterile saline the suspensions were inoculated on MacConkey agar and incubated at 37 °C for 18-24h. All colonies growing on MacConkey agar were isolated and sub-cultured for species identification, and the isolates were preserved at -81 °C in the Microbank® vials (Pro-Lab Diagnostics Inc., Toronto, Canada). The specimens were transported to Germany for identification using MALDI-ToF-MS (VITEK®-MS, bioMérieux, Marcy-l'Étoile, France) and antimicrobial susceptibility testing (AST) with VITEK® 2 (bioMérieux) and Kirby-Bauer for confirmation of some results by VITEK 2 (performed at the Institute of Medical Microbiology and Hospital Hygiene, Heinrich Heine University Düsseldorf, Germany). All results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 2018 V8.0. All bacteria positive in the VITEK ESBL phenotype screening were subjected to molecular detection using PCR as detailed below.

After identification, the bacterial DNA was prepared by producing a suspension of a pure colony from MacConkey agar in 200  $\mu$ L of Tris-EDTA pH 7.5. The suspension was then heated at 95 °C for 10 minutes,

followed by centrifugation at 10000 rpm for 2 minutes. Then 150 µl the supernatant was transferred to the new 1.5ml tube and was stored at -20 °C until PCR testing.

Identification of bacterial resistance genes was performed by PCR of ESBL-gene sequences common to groups of ESBL types. Bacterial strains with suspected production of ESBLs were investigated by PCR, following the protocols described by Strauß et al. for identification of the β-lactamase (*bla*) CTX-M, *bla*<sub>SHV</sub> and *bla*<sub>TEM</sub> genes (6).

The frequency of ESBL genes detected in houseflies was compared with the frequency of ESBL genes previously detected in clinical isolates from patients with acute infectious diseases or sepsis from the same hospital (unpublished data). *Escherichia coli* and *Klebsiella pneumonia* were the most common GNB isolated from blood, urine and wound swabs which were used as the clinical isolates to compare the proportion of ESBL frequency with GNB colonized house flies. The same method was followed for identification and AST result interpretation for the bacteria isolated from clinical samples and house flies.

IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA) was used for statistical analysis.

## Results

A total of 103 bacterial isolates were obtained from 85 flies (NICU: 11 isolates in 20 flies, orthopedic ward: 10 isolates in 20 flies, waste disposal area: 37 isolates in 26 flies, butchery: 45 isolates in 27 flies). *Klebsiella* spp., and *Proteus* spp. were among common pathogenic bacteria isolated in the butchery. However, nearly half of the bacteria isolated from flies caught at the butchery in Asella town were not commonly pathogenic for humans. The frequency of ESBL-production among isolated bacteria from flies caught at the different study sites was variable (see Table 1).

Table 1: Frequency of ESBL-producing Gram-negative bacteria in 85 houseflies caught in at ARTH and Asella town, Ethiopia.

Site	Isolates (n)	Rate of ESBL- n (%)
NICU	11	9 (82%)
Orthopedic ward	10	9 (90%)
Waste disposal area	37	21 (57%)
Butchery	45	1* (2%)

\*We found only a single *Escherichia coli* ESBL producing from the butchery.

The prevalence of colonization of investigated flies with ESBL producing bacteria was 9 (82%), 9 (90%), and 21 (57%) in NICU, orthopedic ward and waste disposal area in the hospital's compound, respectively, and 1 (2%) in the butchery. Overall, the colonization rate of houseflies with ESBL-producing bacteria in the

hospital compound was 39 (67%). The different colonization rates among flies trapped inside and outside the hospital compound was statistically significant ( $p \leq 0.001$ ) (see Figure. 1).

The frequency of ESBL expression was 67% (6/9) in *Raoultella* spp., and 67% (4/6) in *Kluyvera* spp., 56% (5/9) in *Enterobacter* spp., 50% (5/10) in *E. coli* and *Citrobacter* spp. and 44% (8/18) in *Klebsiella* spp., respectively (Table 2).

Table 2: Frequency of ESBL expression among the different Gram-negative bacteria isolated from houseflies trapped from hospital compound (wards and waste disposal area) and butchery.

Bacterial species	ESBL positive	ESBL negative	Total
	n (%)	n (%)	n
<i>Raoultella</i> spp.	6 (67%)	3 (33%)	9
<i>Kluyvera</i> spp.	4 (67%)	2 (33%)	6
<i>Enterobacter</i> spp.	5 (56%)	4 (44%)	9
<i>Escherichia coli</i>	5 (50%)	5 (50%)	10
<i>Citrobacter</i> spp.	5 (50%)	5 (50%)	10
<i>Klebsiella</i> spp.	8 (44%)	10 (56%)	18
<i>Providencia</i> spp.	2 (29%)	5 (71%)	7
<i>Acinetobacter</i> spp.	1 (25%)	3 (75%)	4
<i>Proteus</i> spp.	1 (13%)	7 (87%)	8
<i>Moellerella wisconsensis</i>	1 (10%)	9 (90%)	10
Others*	3 (30%)	7 (70%)	10

\*Others (one isolate each): *Comamonas testosteroni*, *Pantoea agglomerans*, and *Rahnella aquatilis* (ESBL expression); *Aeromonas hydrophila*, *Cedecea davisae*, *Hafnia alvei*, *Leclercia adecarboxylata*, *Lelliottia amnigena*, *Serratia liquefaciens*, and *Yokenella regensburgei* (no ESBL expression); Even though we found four isolates of *Acinetobacter* spp., their frequency of ESBL were not analyzed in this study. As described in (Figure.1) above, the frequency of ESBL was near to similar in GNB isolated from clinical samples and houseflies caught in hospital compound. However, it was extremely low 1 (2%) in house flies caught in butchery.

Among bacteria carrying ESBL isolated from flies in this investigation, 85% (n=34) carried CTX-M-like, 83% TEM-like (n=33), 23% SHV-like (n=9) and 1 CTX-M-2-like genes. CTX-M-9- and CTX-M-8/25-like genes were not detected. The comparison of the detection frequency of the different major ESBL genes of GNB isolated from blood, urine and wound swab samples between 2016 and 2019 from the same hospital (own data, not published) and of bacterial isolates from house flies shows clear similarities (Table 3).

However, CTX-M-9 was only detected from clinical specimens and CTX-M-2 was only detected from isolates from house flies.

Table 3: Comparison of frequency and characterization of ESBL genes from clinical isolates (n=32) and isolates from houseflies (n=40)

	ESBL genes					
	Total	CTX-M-1 n (%)	TEM n (%)	SHV n (%)	CTX-M-9 n (%)	CTX-M-2 n (%)
ESBL in clinical isolates	32	26 (81%)	22 (69%)	7 (22%)	2 (6%)	0
ESBL in isolates from flies	40	34 (85%)	33 (83%)	9 (23%)	0	1 (3%)

ESBL: extended spectrum  $\beta$ -lactamases; CTX-M: cefotaximase-Munich; SHV: Sulfhdryl variable; TEM: Temoniera

Among isolated bacteria, phenotypical AMR against non-  $\beta$ -lactam antibiotics used for treatment of other ESBL-producing bacteria was very high. ESBL-producing bacteria showed a higher rates of AMR against ciprofloxacin (66% vs. 5%,  $p < 0.001$ ), gentamicin (68% vs. 3%,  $p < 0.001$ ), piperacillin-tazobactam (78% vs. 5%,  $p < 0.001$ ), and trimethoprim-sulfamethoxazole (88% vs. 16%,  $p < 0.001$ ) compared to non ESBL-producing bacteria (Figure. 2).

## Discussion

Recently, houseflies were recognized as potential vectors for AMR in hospital and non-hospital environments (7, 8). In the study center, the frequency of ESBL-producing GNB isolated from clinical samples was very high (Figure. 1). We conducted this study in order to compare the colonization of houseflies with ESBL-producing GNB at various locations inside and one location with high density of house flies outside of the hospital compound. We found a high prevalence of ESBL-producing bacteria among flies collected inside the hospital compared to the near absence of ESBL genes in bacterial isolates from flies collected 1.5 km away from the hospital. The ESBL prevalence was highest at the NICU and at the orthopedic ward, and only slightly lower at the hospital waste disposal area (Table1). Our findings could partly be explained by exposition of bacteria to different antibiotics in the environment of the hospital or more likely by the accumulation of resistant bacteria in and on flies in the patients' environment. Similar to our findings, a study conducted in Iran shows that bacterial isolates from houseflies in a hospital compound had a significantly higher level of antimicrobial resistance against various antibiotics, than bacteria isolated from houseflies in non-hospital environment (7). A study conducted in Berlin, Germany showed that the prevalence of ESBL in flies trapped from two different residential areas differed (0% vs. 18%) (9). According to this study, the distribution of ESBL-producing bacteria among flies in certain geographical locations are not uniform.

In our study, the frequency of ESBL-producing GNB was very high among common pathogenic bacteria like *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Raoultella* spp. compared with opportunistic bacteria. *Kluyvera* spp. are opportunistic bacteria with the highest rate of ESBL-production (Table 2). Half of the bacteria isolated from the butchery in Asella town were not commonly pathogenic bacteria. This different distribution of bacteria colonizing the isolated flies might also influence the frequency of ESBL isolates from different sites based on pathogenicity of the bacteria and their exposure to cephalosporin antibiotics (10).

In this study, the most frequently detected resistance genes in confirmed ESBL-producing GNB colonized houseflies were CTX-M-1-like gene, followed by TEM-like gene and SHV-like gene, respectively. The frequency and characterization of ESBL genes of clinical samples and houseflies isolates showed similarities (Table 3) (11) and also similar findings reported by Boulesteix G. et al. in 2005 from Dakar, Senegal (12). This suggests that houseflies may acquire the bacteria from the hospital environment. Similar findings were reported by Fotedar R. et al. in 1992 (13); however, to clearly identify the source of the ESBL-producing bacteria on houseflies needs further investigation. On the basis of our results, no statement can be made on the question of whether houseflies can be considered as vectors for MDR bacteria. In this context, however, it is interesting to note that Rahuma N. et al (2005) reported earlier that houseflies may be potential vectors for the transmission of MDR bacteria from hospitals to surrounding communities.(14).houseflies colonized with ESBL-producing bacteria found in the hospital compound can possibly spread AMR to surrounding residential areas or restaurants, thereby endangering public health (15). As described in (Table 2), not only well-known pathogenic bacteria but also opportunistic bacteria can carry clinically relevant resistance genes and enhance the spread of AMR in the community.

Published investigations from Ethiopia demonstrate that MDR GNB commonly express the *bla*<sub>CTX-M-1</sub> gene encoded in ESBLs and the *bla*<sub>NDM-1</sub> gene in carbapenemase-producing bacteria (5, 16-19). For molecular detection of ESBL, *bla*<sub>CTX-M-1</sub> ESBL gene can be used as target gene by either conventional PCR or a loop-mediated isothermal amplification (LAMP) technique, which is rapid, effective and affordable to detect the presence of *bla*<sub>CTX-M-1</sub> ESBL gene in RLS like Ethiopia (20).

In this study, the susceptibility to non-β-lactam antibiotics such as ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole was significantly lower in ESBL-producing bacteria compared to ESBL-negative bacteria (p<0.001). This finding is an indicator for the limited options of appropriate antibiotic therapy regimen for ESBL-producing bacterial infection management. Poudel A, et al (2019) also reported high rates of resistance of *E. coli* and *K. pneumoniae* isolated from flies against tetracycline and ampicillin due to emergence of ESBL-producing strains (21). This might probably be caused by plasmid-mediated mobile resistance genes such as quinolone-resistance (*qnr*) genes, aminoglycoside acetyltransferase (*aac*), *dfp* (trimethoprim resistance) and *sul* (sulfamethoxazole resistance) genes, being more frequent in ESBL-producing bacteria compared to ESBL-negative bacteria (22-25). However, the identification of other resistance genes than the described ESBL genes was not part of this investigation.

In tropical regions where poor hospital hygiene is common, hand hygiene and patient isolation or implementation of antimicrobial stewardship programs may not be sufficient to control the expansion of AMR. Our findings can be considered as indicators for a possible dissemination of antimicrobial resistance inside and outside of hospital compounds and to the nearest environments by houseflies. Therefore, to tackle the expansion of ESBL-producing bacteria, fly-control measures in critical areas of the hospitals might be essential (7, 15). In order to inhibit further expansions of ESBL-carrying bacteria from hospitals to residential areas, environmental and health professionals and municipality administration should work together and strengthen a one health approach. Future AMR prevention and control protocols may consider screening of houseflies for AMR and eradication-measures to control the population density of houseflies at health care facilities in tropical regions (21). As distribution of ESBL genes in clinical samples and houseflies caught in the hospital show comparable results, houseflies might be used as an indicator organism for ESBL-prevalence in hospital facilities.

Our study has certain limitations. ESBL-colonization in the community was reported as low, but houseflies were sampled from a single butchery only. Even though we found high rates of ESBL-producing bacteria in houseflies at the hospital compound, the source of the ESBL was not addressed. The study design also fails to point out whether the external organs like legs and mouth or the gut of the flies are more involved in carrying ESBL-producing bacteria, a factor with possible impact for the transmission of the bacteria. The role of houseflies in transmission of nosocomial infections and the source of ESBL-producing bacteria in the hospital needs further investigation.

## Conclusions And Recommendations

A high proportion of houseflies trapped within the hospital compound were colonized with ESBL-producing bacteria, whereas ESBL-production was nearly absent among houseflies collected in a butchery 1.5 km away from the hospital. The houseflies may be a relevant factor in the spread of MDR microbes in hospitals or hospitals surroundings. Antibiotic susceptibility to ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole was lower in ESBL-producing bacteria compared with ESBL-negative bacteria which can limit treatment options. Antimicrobial resistance prevention and control protocols should consider the role of houseflies in hospitals in tropical regions. Our findings warrant the need of a one health approach to minimize the spreading of MDR strains to the environment. Further studies are needed to determine the role of houseflies as vectors for MDR nosocomial infections.

## Declarations

### Ethics approval and consent to participate

Local ethical clearance from Arsi University and national ethical clearance from Ethiopian Ministry Science and Technology was obtained for transportation of bacteria isolates from Ethiopia to Germany.

### Consent for publication

For bacteria isolated from clinical samples written informed consent of patients was granted.

#### Availability of data and material

All data generated or analyzed during this study are included in this published article.

#### Competing interests

The authors declare that they have no competing interests.

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There was no influence of the funding organization on analysis or interpretation of the described data.

## **Authors' contributions**

Tafese Beyene Tufa: Conception and design, acquisition and analysis of the data, interpretation of data, drafting of the manuscript and approval of the manuscript for publication

Andre Fuchs: interpretation of data, revision of the manuscript and approval of the manuscript for publication

Tobias Wienemann: Analysis of the data, performance of laboratory investigations and approval of the manuscript for publication

Yannik Eggert: Interpretation of data, drafting of the manuscript and approval of the manuscript for publication

Sileshi Abdissa: Performance of laboratory investigations and approval of the manuscript for publication

Marlen Schneider: Interpretation of data, revision of the manuscript and approval of the manuscript for publication

Björn-Erik Ole Jensen: Conception and design, critical revision of the manuscript and approval of the manuscript for publication

Johannes G. Bode: Interpretation of data, critical revision of manuscript and approval of the manuscript for publication

Klaus Pfeffer: Interpretation of data, critical revision of the manuscript and approval of the manuscript for publication

Dieter Häussinger: Interpretation of data, critical revision of manuscript and approval of the manuscript for publication

Colin Mackenzie: Interpretation of data, critical revision of the manuscript and approval of the manuscript for publication

Hans Martin Orth: Conception and design, drafting of the manuscript and approval of the manuscript for publication

Torsten Feldt: Interpretation of data, critical revision of the manuscript and approval of the manuscript for publication

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## **Abbreviations**

aac Aminoglycoside acetyltransferase

AMR Antimicrobial resistance

AST Antimicrobial susceptibility testing

ATRH Asella Teaching and Referral Hospital

CTX-M Cefotaximase-Munich

dfr dihydrofolate reductases

EDTA Ethylene diamine tetraacetic acid

ESBL Extended spectrum  $\beta$ -lactamase

EUCAST European Committee on Antimicrobial Susceptibility Testing

GNB Gram-negative bacteria

LAMP loop-mediated isothermal amplification

MALDI-TOF matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

MDR Multidrug-resistant

NDM New Delhi metallo- $\beta$ -lactamase

NICU Neonatal intensive care unit

PCR polymerase chain reaction

qnr quinolone-resistance

RLS Resource limited settings

sul sulfamethoxazole resistance gene

SHV Sulfhydryl variable

TEM Temoniera

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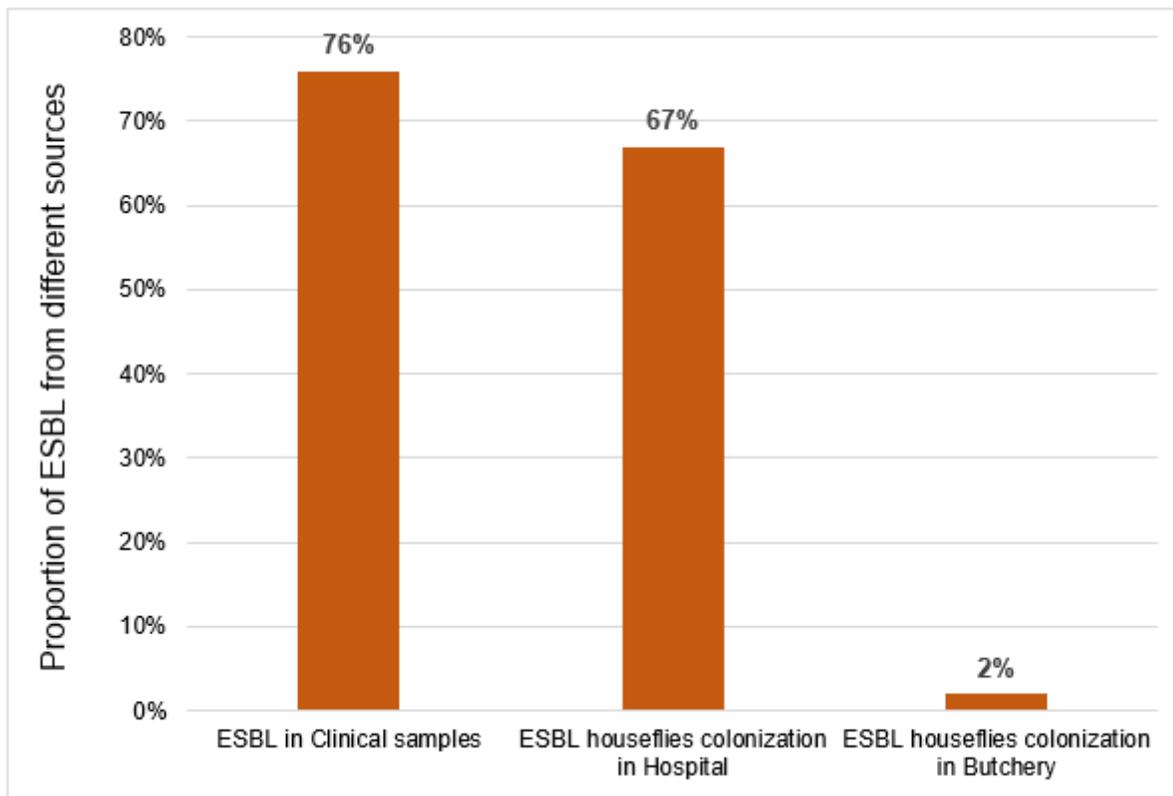
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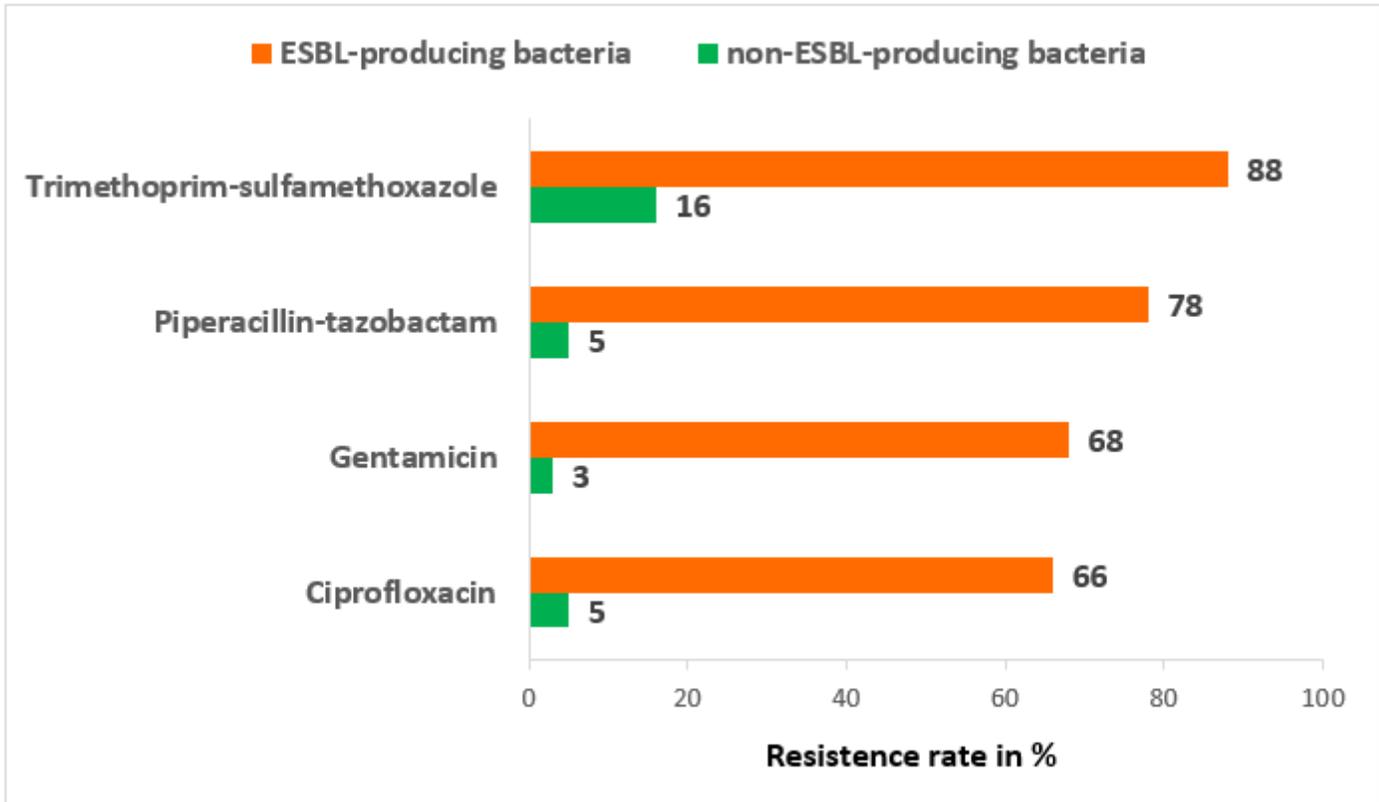
## Figures



**Figure 1**

Comparison of ESBL frequency in clinical samples and housefly colonization. Source of clinical samples data: ESBL in clinical isolates of Gram-negative bacilli at Asella teaching and referral hospital, central

Ethiopia (2016 to 2019). The proportion of ESBL houseflies colonization in hospital was significantly different compared with ESBL houseflies colonization in butchery (67% vs. 2%; P value < 0.001).



**Figure 2**

Resistance rate among ESBL- (n=40) and non-ESBL-producing (n=62) Gram-negative bacteria isolated from houseflies