The Haryana Veterinarian (June, 2023) **62(1),** 42-45 ISSN 0033-4359 (P) **Research Article**

OCCURRENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI* AND *SALMONELLA* SPP. IN HOUSEFLIES INFESTING FARM AND NON-FARM PREMISES IN WAYANAD, KERALA, INDIA

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ABSTRACT

The present study was designed to explore the antimicrobial resistance of *Escherichia coli* and *Salmonella* spp., isolated from house flies (*Musca domestica*) trapped on-pig farm and non-farm settings. A total of thirty pools of flies (15 pools from farm settings and 15 pools from non-farm settings) were evaluated from different farms and other units in and around Wayanad district. The recovery rate of *E. coli* from flies was 100% from both the sources and that of *Salmonella* spp. was 13.33 % from the farm settings and 20% flies in the non-farm settings. This study further revealed that the recovered *E. coli* and *Salmonella* spp. were drug-resistant strains and the *E. coli* isolates exhibited resistance to Ceftazidime (20%), and Cefotaxime (23.33%), whereas none of the *Salmonella* spp. isolates were resistant to Ceftazidime and 60% of isolate showed resistance to Cefotaxime. In this study, 50% of the isolates exhibited ESBL production among *E. coli*, while it was 40% among *Salmonella* spp. The occurrence of ESBL genes detected in the pools of *E. coli* were *bla*CTX-M (46.70%), *bla*SHV (40%), *bla*TEM (40%), whereas among *Salmonella* spp. isolates were *bla*CTX-M (40%), *bla*SHV (60%), *bla*TEM (40%). The present study suggested that the house flies can harbour multi-drug resistant bacteria and warrants vigilant monitoring of carriage of AMR genes to eliminate the risk of drug-resistant pathogens in food sources.

Keywords: Antimicrobial-resistant, ESBL, *E. coli*, Housefly, *Salmonella* spp.

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Antibiotic usage hastens the formation and development of resistance among organisms, with commensal organisms being the most vulnerable. Although antibiotics are used in clinical medicine and food animal production, there is growing evidence that commensal bacteria, environmental factors, and animal hosts play essential roles in the emergence, dissemination, and evolution of antibiotic resistance. Contaminating food, water, slurry and insects, among other things, speed up the transfer of resistance determinants between food animals and humans and is a matter of huge concern.

Insects comprise one of the most diverse taxa of life and include more than a million described species representing more than half of all known living organisms. Given its vast diversity of species and its close interaction with humans, it is surprising that only relatively limited information is available regarding the impact of insects and the implications of insect-associated bacteria on human health. Around 90% of insects in the human habitat are houseflies. As per the CDC, house flies were considered to be cleanliness indicators in public places. Flies from garbage and sewage pick up pathogenic organisms and transfer them to humans and animal food via their mouthparts, vomitus, faeces, and contaminated body parts. The movement of flies from animal or human faeces to food that will be eaten uncooked by humans is of particular concern (Olagunju, 2022). In addition, some pathogens can be harboured in the mouthparts or alimentary canal of flies for several days after being fed and then spread when the flies defecate or regurgitate. The role of a housefly in disseminating various diseases like malaria and cholera is already documented (Olagunju, 2022). House flies can be considered one health indicator of AMR transmission in humans, animals, and their environment due to their presence in all places. Several studies have demonstrated antibiotic resistance in houseflies and their function in food chain transmission. This study aims to evaluate the occurrence of extended-spectrum beta-lactamase-producing *E. coli* and *Salmonella* spp. in houseflies from pig farms and non-farm settings.

MATERIALS AND METHODS

Fly trapping and processing

During the period from December 2020 to November 2021, domestic flies were caught from the farm and non-farm premises using commercial and traditional fly traps. Plastic bottles filled 2:1 mix of dried fish, chicken powder, along with sugar syrup were used as a traditional fly trap. Traps were set up at various pig sheds, where the flies were plenty, and flies were collected. Three trapping attempts were carried out on each farm and yielded six pools of

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samples which were further processed for isolation of *E. coli* and *S.* Typhimurium. The number of flies obtained per catch was not less than 10. Flies were first washed in 1X Phosphate Buffer Saline (PBS), and their wing patterns were examined according to Sen and Fletcher guidelines at 10X magnification to identify the genus. Flies were pooled and washed three times in 1X PBS to eliminate surface pollutants and centrifuged at 12000 rpm for 10 minutes in 1.5 ml PBS. The flies settled down at the bottom were crushed using a sterile metal rod. The supernatant was used for bacterial isolation after 10-minute centrifugation at 6000 rpm.

Bacterial Isolation and Antimicrobial Susceptibility Testing

Gram-negative rods growing on Mac Conkey agar were plated on Eosin Methylene Blue agar to presemptively identify them as *E. coli*. Lactose non-fermenting colonies were further streaked onto Xylose Lysine Deoxycholate (XLD) agar to identify *Salmonella* spp. The isolates were finally confirmed by PCR using species-specific genes. Extended -spectrum beta-lactamase production was observed in isolates with reduced susceptibility to clavulanic acid and resistance to ceftazidime and cefotaxime. For each isolate, 18-24 h old bacterial suspension corresponding to a McFarland standard of 0.5 was plated as a uniform lawn on Mueller Hinton agar. After this, the antibiotic soaked discs were placed on the agar surface, and then the plates were incubated at 37°C for 18-24 h. Zones of inhibition were read as susceptible, intermediate, and resistant according to the standards of the Clinical and Laboratory Standards Institute.

Extraction of DNA from Bacterial Isolates of Flies and PCR

The genomic DNA extraction kit (Origin- India) was used to extract total nucleic acid from the bacterial isolates according to the manufacturer's protocol. Presumptive colonies of *E. coli* and *Salmonella* spp. were confirmed by PCR targeting the *uid*A gene and the *inv*A gene, respectively. The presence of the ESBL enzyme-encoding genes, namely; *bla*TEM, *bla*SHV and *bla*CTX-M were confirmed by PCR. The primers used in this study are provided in Table 1.

RESULTS AND DISCUSSION

Isolation of *E. coli* and *Salmonella* spp.

A total of 30 pools of flies (15 pools each from the farm and non-farm setting) were trapped (Table 2). *E. coli* and *Salmonella* spp. were isolated from 100% and 13.30% of flies collected from farm settings, respectively. The recovery rate of *E. coli* and *Salmonella* spp. collected from flies in non-farm settings were 100% and 20%, respectively. This result was in agreement with the colonization rates observed for *E. coli* and *Salmonella* spp. in flies from other studies (Rahuma *et al.*, 2005).

Antimicrobial Resistance of the Isolates

In the study, *E. coli* isolates obtained from farm settings revealed the resistance to cefotaxime (26.70%) and ceftazidime (20%). In the case of isolates obtained from flies in non-farm settings, 20% were resistant to both cefotaxime and ceftazidime.

Out of the *Salmonella* spp. isolates recovered from flies in a farm setting, 50% were resistant to cefotaxime and on the other side, out of three positive isolates from non-farm settings, 66.67% were resistant to cefotaxime and none were resistant to ceftazidime. This observation was similar to Dahms *et al.* (2015) and was lower as compared to Lay *et al.* (2021). Altogether, 80% of flies (24/30 pool) harboured antimicrobial-resistant bacterial strains.

Phenotypic assessment of ESBL production by *E. coli* and *Salmonella* spp. were assessed by Kirby-Bauer disc diffusion test. These isolates were typically resistant to cefotaxime or ceftazidime and the difference in zone of inhibition with their clavulanic acid ≥ 5 mm is considered an ESBL producer. The phenotypic assessment revealed that 60% of *E. coli* and 50% of *Salmonella* spp. isolates from the farm and 40% of *E. coli* and 33.3 % of *Salmonella* spp. isolates from non-farm settings were ESBL producers. In total, the study revealed that the recovered *E. coli* and *Salmonella* spp. were multi-drug resistant and the *E. coli* isolates exhibited resistance to Ceftazidime (28.6%), and Cefotaxime (49%), whereas, 39.9% *Salmonella* spp. isolates were resistant to Cefotaxime, respectively.

E. coli isolated from flies in farm and non-farm settings were found to harbour *bla*CTX-M gene in 60% and 33.3% of isolates, respectively, while *Salmonella* spp. isolates from these sources had *bla*CTX-M gene in 50% and 33.34% of the isolates. The presence of *bla*TEM gene in *E. coli* isolates in farm setting and non-farm setting is 46.7% and 33.3%, whereas *Salmonella* isolates encoded *bla*TEM as none and 66.67%, respectively. Higher level of *bla*SHV in farm settings (60%) and 20% in non-farm settings in the case of *E. coli*, while *Salmonella* isolates had *bla*SHV gene in 50% and 66.7%, respectively as detailed in Table 4.

Overall, out of 30 pools of house fly samples screened, the recovered *E. coli* and *Salmonella* spp. were multi-drug resistant strains and the *E. coli* isolates exhibited resistance to Ceftazidime (20%), and Cefotaxime (23.33%). In case

Table 1. Details of primer used in the study

Target organism	Gene	Gene Primer sequence (5'-3')	Amplicon size (bp)	Reference
E. coli	*uid*A	F: TGGTAATTACCGACGAAAACGGC R: ACGCGTGGTTACAGTCTTGCG	162	(Mian *et al.*, 2002)
Salmonella spp.	*inv*A	F: GTGAAATTATCGCCACGTTCGGGCA R: TCATCGCACCGTCAAAGGAAC	284	(Nambiar *et al.*, 2009)
ESBL producing *Enterobacte-riaceae*	*bla* CTX-M	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550	(Vinodh Kumar *et al.*, 2021)
	bla SHV	F: GATGAACGCTTTCCCCATGATG R: CGCTGTTATCGCTCATGGTAA	214	
	bla TEM	F: ATGAGTATTCAACATTTCCG R: GTCACAGTTACCAATGCTTA	847	

Table 2. Details of Housefly sampling

Sl. No.	Site	No. of trappings	Actual flies trapped per sampling			Total flies	Flies taken for processing
1.	Farm 1	3	28	22	18	68	60
2.	Farm 2	3	35	38	44	117	60
3.	Farm 3	3	40	38	45	123	60
4.	Farm 4	3	25	31	14	70	60
5.	Farm 5	3	21	12	16	49	49
	Total farm	15	149	141	137	427	289
6.	Non-farm setting	15	More than 10 flies from each pool			178	178
	Total					605	467

Table 3. Phenotypic assessment of ESBL production

Sample source	No. of isolates obtained	Antibiotics used										ESBL (%)
		CTX (in %)			CEC (in %)		CAZ (in %)			CAC (in %)		
		S	I	R	NS	S	S	I	R	NS	S	
Non-Farm setting	*E. coli* (15)	66.7	13.3	20.0	86.7	13.3	66.7	13.3	20	80%	20	40
	Salmonella (3)	0	33.33	66.7	100	0	33.33	66.7	0	100	0	33.34
Farm setting	*E. coli* (15)	40.0	33.3	26.7	100	0	66.7	13.3	20	100%	0	60
	Salmonella (2)	0	50	50	100	0	50	50	0	100	0	50

**CTX- Cefotaxime, CEC- Cefotaxime + Clavulanic acid, CAZ-Ceftazidime, CAC- Ceftazidime + Clavulanic acid combination.*

of *Salmonella* spp. isolates, none were resistant to Ceftazidime and 60% were resistant in case of cefotaxime. ESBL resistance (by phenotypic assays) was recorded more in *E. coli* (50%) isolates than in *Salmonella* spp. (40%). In the molecular study, ESBL genes could be detected in all 30 pools of the *E. coli* i.e., *bla*CTX-M (46.7%), *bla*SHV (40%), *bla*TEM (40%) and *Salmonella* isolates *bla*CTX-M (40%), *bla*SHV (60%), *bla*TEM (40%). Surveillance data indicated that AMR in *E. coli* is consistently higher in houseflies collected from farm settings than in non-farm settings. Further, we identified *bla*CTXM, *bla*TEM and *bla*SHV genes from flies trapped in farm and non-farm settings. While *bla*CTXM and *bla*SHV are the most identified beta-lactamases from flies, including farm and non-farm settings, *bla*SHV is highly prevalent in flies in this study, suggesting that flies may have transmitted to human or animal populations. Wadaskar *et al.* (2019) investigated the occurrence of ESBL producing *E. coli* and *Salmonella* spp. in houseflies trapped on different farm premises and pointed out the increasing number of ESBL producing genes in houseflies. Blaak *et al.* (2014) reported a higher number of *bla*SHV in

Table 4. *bla*CTX-M, *bla*TEM and *bla*SHV genes detected from *E. coli* and *Salmonella* isolates

Sl. No.	Taluk	No. of isolates obtained	ESBL					
			*bla*CTX-M		*bla*SHV		*bla*TEM	
			No. of +ve isolates	%	No. of +ve isolates	%	No. of +ve isolates	%
1	Non-Farm setting	*E. coli* (15)	5	33.3	3	20	5	33.3
		Salmonella (3)	1	33.34	2	66.7	2	66.7
2	Farm setting	*E. coli* (15)	9	60	9	60	7	46.7
		Salmonella (2)	1	50	1	50	0	00
	Total from farm and non farm settings	*E. coli* (15)	14	46.7	12	40	12	40
		Salmonella (5)	2	40	3	60	2	40

houseflies comparable to the present study. The higher level of antimicrobial determinants shows a possible chance of getting AMR pathogens from animal and environment. Since flies can travel more than 20 miles in their lifetime, it indicates AMR genes' dissemination.

CONCLUSION

The high levels of ESBL producing *Salmonella* spp. and *E. coli* in flies sampled in this study indicated a serious public health threat. It was noticed that bacteria isolated from farm settings carried more number of ESBL producing genes, indicating the urgent need to control house flies in the farm settings. In addition, house flies are a menace to farms and humans.

REFERENCES

Blaak, E., Rostalski, A., Harlizius, J., Gangl, A., Simoneit, C., Grobbel, M., Kollas, C., Tenhagen, B.A. and Kasbohrer,A. (2014). Antibiotic resistance in *Escherichia coli* from pigs from birth to slaughter and its association with antibiotic treatment. *Prev. Vet. Med.* **165**: 52-62.

Dahms, C., Hübner, N.O., Kossow, A., Mellmann, A., Dittmann, K. and Kramer, A. (2015). Occurrence of ESBL-Producing *Escherichia coli* in Livestock and Farm Workers in Mecklenburg-Western Pomerania, Germany. *PLOS ONE* **10(11)**: e0143326.

Ebenezer, A., Hanry, A.I. and Better, B.O. (2020). Parasite and Microbial Load of Housefly Collected from Selected Houses in Amassoma Community, Bayelsa State, Nigeria. *Res. J. Parasitol.* **15**: 14-19.

Jonge, N. de, Michaelsen, T.Y., Ejbye-Ernst, R., Jensen, A., Nielsen, M.E., Bahrndorff, S. and Nielsen, J.L. (2020). Housefly (*Musca domestica* L.) associated microbiota across different life stages. *Sci. Rep.* **10(1)**: 7842.

Khamesipour, F., Lankarani, K.B., Honarvar, B. and Kwenti, T.E. (2018). A systematic review of human pathogens carried by the housefly (*Musca domestica* L.). *BMC Public Health.* **18(1)**: 1049.

Lay, K.K., Jeamsripong, S., Sunn, K.P., Angkititrakul, S., Prathan, R., Srisanga, S. and Chuanchuen, R. (2021). Colistin Resistance and ESBL Production in *Salmonella* and *Escherichia coli* from Pigs and Pork in the Thailand, Cambodia, Lao PDR and Myanmar Border Area. *Antibiotics.* **10(6)**: 657.

Mian, L.S., Maag, H. and Tacal, J.V. (2002). Isolation of *Salmonella* from muscoid flies at commercial animal establishments in San Bernardino County, California. *J. Vector Ecol.* p. 4.

Olagunju, E.A. (2022). Housefly: Common zoonotic diseases transmitted and control. *J. Zoonotic Dis.* **6(1)**: 1-10.

Rahuma, N., Ghenghesh, K.S., Ben Aissa, R. and Elamaari, A. (2005). Carriage by the housefly (*Musca domestica*) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospital and other urban environments in Misurata, Libya. *Ann. Trop. Med. Parasitol.* **99(8)**: 795-802.

VinodhKumar, O.R., Karikalan, M., Ilayaraja, S., Sha, A.A., Singh, B.R., Sinha, D.K., Chandra Mohan, S., Pruthvishree, B.S., Pawde, A.M. and Sharma, A.K. (2021). Multi-drug resistant (MDR), extended spectrum beta-lactamase (ESBL) producing and carbapenem resistant *Escherichia coli* in rescued Sloth bears (*Melursus ursinus*), India. *Vet. Res. Commun.* **45(2-3)**: 163-170.

Wadaskar, B., Kolhe, R., Waskar, V., Pawade, M. and Kundu, K. (2019). Isolation of *Escherichia coli* and *Salmonella* species in flies trapped at animal farm premises. *J. Entomol. Zool. Stud.* **7**: 198-201.

Wang, Y.C., Chang, Y.C., Chuang, H.L., Chiu, C.C., Yeh, K.S., Chang, C.C., Hsuan, S.L., Lin, W.H. and Chen, T.H. (2011). Transmission of *Salmonella* between Swine Farms by the Housefly (*Musca domestica*). *J. Food Prot.* **74(6)**: 1012-1016.

Winpisinger, K.A., Ferketich, A.K., Berry, R.L. and Moeschberger, M.L. (2005). Spread of *Musca domestica* (Diptera: Muscidae), from Two Caged Layer Facilities to Neighboring Residences in Rural Ohio. *J. Med. Entomol.* **42(5)**: 732-738.