Antibiogram and Extended Spectrum Beta-Lactamase (ESBL) Profile of *E. coli* Species Isolated from Houseflies in Abakaliki Metropolis, Nigeria

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Abstract: This study determined the antibiogram and extended spectrum beta-lactamase (ESBL) production potentials of *E. coli* species isolated from houseflies from hospital, restaurant and fruit market environments in Abakaliki metropolis. A total of 150 samples (50 from each sample site) were analyzed using Standard Microbiological Methods. Antibiotic susceptibility profile of the isolates was determined using disc diffusion method. ESBL production was screened using Double Disc Synergy Test (DDST). A total of 12 isolates of *E. coli* were obtained. Of the 12 isolates, 6(50 %) were obtained from hospital while 3(25 %) each were respectively isolated from fruit market and restaurant environments. The antibiotic susceptibility and resistance patterns of *E. coli* isolated from the three environments (hospital, fruit market and restaurant) to different antibiotics showed that meropenem (100 %) had the highest activity while 100 % resistance to ceftriaxone, ampicillin-sulbactam and cefoxitin were recorded. Resistance of the isolates from hospital to gentamicin up to 83.33 % was also recorded. Their susceptibility to other antibiotics used including nalixidic acid, ciprofloxacin, cefoxitin, ceftaxidime, gentamicin, ceftriaxone, sulfamethoxazole-trimetoprim, cefotaxime and ampicillin-sulbactam ranged from 16.67 % to 66.67 %. The extended spectrum beta-lactamase screening showed that none of the isolates tested positive. In conclusion, the study revealed that houseflies from hospital, fruit market and restaurant environments harbor multidrug resistant *E. coli* and that the multidrug resistance were not ESBL mediated.

***Keywords*:** Antibiotic resistance, ESBLs, fruit market, hospital, houseflies, restaurant,

INTRODUCTION

Flies in the order Diptera and suborder Cyclorrhapha, live in unhygienic environment in close association with bacteria and nourish on contaminated materials such as filthy garbage, animal beddings, human excrement and other decaying substances rich in microorganisms (Chaiwong *et al.,* 2012; Graczyk *et al.,* 2001). They move freely and rapidly to food and human environments where transmissions of human pathogens through close association are made easier (Chaiwong *et al.,* 2012; Graczyk *et al.,* 2001).

Filth flies especially muscoid flies (such as housefly (*Musca domestica*) are proficient in mechanically or biologically transmitting different pathogens that can cause dysentery, infant diarrhea, typhoid fever, food poisoning, cholera, helminthiasis, eye infections, poliomyelitis and certain skin infections (Yang *et al.,* 2013; Graczyk, *et al.,* 2001; Khobdel *et al.,*2008). They pick up microorganisms that cause diseases even as they crawl and feed. Thus, houseflies are widely recognized as potential reservoirs and vectors of human and animal pathogens (Khobdel *et al.,* 2008; Pandian and Asumtha, 2001).

In addition to acting as mechanical vector, flies might spread antibiotic resistant genes within the microbial community (Boulesteix *et al.*, 2005; Fotedar *et al.*, 1992). Therefore, the wide spread use of antibiotics in the treatment of infections caused by Gram-negative bacteria has led to the emergence of multi-drug resistant genes which is a very important public health concern all over the world (Yang *et al.,* 2013). This study is therefore designed to determine the antibiogram and presence of extended spectrum beta-lactamases in the *E. coli* isolated from house flies in fruits market, restaurants and hospital environments in Abakaliki metropolis.

MATERIALS AND METHODS

Sample collection

A total of 150 houseflies (50 each from hospital, restaurant and fruit market environments) were collected using sterilized insect scoop nets. The flies were trapped between 10 am - 12 pm in the study environment. Each housefly was aseptically transferred into a sterile culture bottle and properly labeled. All samples collected were transported to the laboratory section of Applied Microbiology Department of Ebonyi State University for bacteriological analysis.

Bacteriological analysis

All houseflies collected were put in the refrigerator for about 30 minutes to inactivate them. After inactivation, 1 ml of normal saline was added into each container with a fly. The content of each container was vigorously shaken to dislodge the external content of a fly. A 0.5 ml each of the mixtures was aseptically transferred into test tubes containing 3 ml of nutrient broth. The tubes were incubated at 37^{o}C for 24 hours. Each tube was properly mixed after incubation and a loopfull was inoculated aseptically onto MacConkey and Eosine methylene blue agar plates and incubated in an inverted position for 24 hours at 37^{o}C. Suspected discrete colonies of *E. coli* were transferred to a freshly prepared MacConkey and Eosine methylene blue agar plates and incubated for 24 hours at 37^{O}C to get the pure colonies. The pure colonies of suspected isolates were transferred onto nutrient agar slants and incubated for 24 hours at 37^{o}C and then stored in the refrigerator between 4^{o}C for further identification (Davian *et al.,* 2010; Nazariet *et al.,* 2017).

Bacteria identification

All isolates obtained were further identified using biochemical tests including Indole, Methyl red, Voges Proskauer and Citrate tests.

Antibiotic susceptibility test

A 24 hours old culture (young culture) of each organisms was standardized using 0.5 Mac Farland equivalents and was inoculated on the surface of aseptically prepared Mueller-Hinton agar plates using sterile swab stick. With sterilized forceps, discs of ciprofloxacin (30 µg), gentamicin (30 µg), nalixidic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ampicillin-sulbactam (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), sulfamethoxazole-trimethoprim (30 µg) and meropenem (30 µg) (oxoid Uk) were placed on the surface of the agar plates about 15 mm from the edge of the plate and not close than 25 mm from one disc to another. The antibiotics were allowed to diffuse for about 10 minutes and the plates were incubated in inverted position at 37^{o}C for 18-24 hours. After 24 hours of incubation, their zones of inhibitions were determined in millimetre (mm) using meter rule. Any isolate resistant to any of the cefotaxime, ceftaxidime and ceftriaxone were further subjected to ESBL phenotypic screening (Afiukwa *et al.,* 2016; NCCLS, 2000; Iroha *et al.,* 2008).

Phenotypic determination of extended spectrum beta-lactamases (ESBL)

Extended spectrum beta-lactamase productions of the test organisms were determined using Double Disc Synergy Test (DDST). Test organism of 0.5 MacFarland equivalent standard of a young culture (18-24 hours old) was inoculated on the surface of aseptically prepared Meuller-Hinton agar plates using sterile swab stick for each test organism (NCCLS, 2000). Discs of ceftazidime (30 µg) and cefotaxime (30 µg) were placed at a distance of 15 mm centre to centre from the centre disc containing Amoxicillin plus Clavulanic acid (20 µg and 10 µg, respectively) (Afiukwa *et al.,* 2016; Iroha *et al.,* 2008; Pitout *et al.,* 2004). The plates were incubated at 37^{O}C for 18-24 hours in an inverted position. Synergistic effect of 5 mm and above towards the central disc indicated ESBL positive and are ESBL negative if it measures lower than 5 mm towards the centre disc (Pitout, 2004).

RESULTS

Results obtained from this study showed that out of the 150 samples analyzed, only 12 isolates of *E. coli* were obtained. Of the 12 isolates, 6(50 %) were isolated from hospital while 3(25 %) each were respectively isolated from fruit market and restaurant environments (Table 1).

Antibiotic Susceptibility and Resistance Pattern of *E. coli* Isolated from the Body wash of Houseflies from Hospital, Restaurant and fruit market

E. coli species obtained from hospital environment when subjected to different antibiotics susceptibility test showed that all the six isolates from hospital environment were 100% susceptible to meropenem; whereas 66.67% each were susceptible to nalixidic acid, cefotaxime, ceftazidime and sulfamethoxazole-trimethoprim; and 50% to ciprofloxacin. The least susceptibility value of 16.67% was observed with gentamicin (Table 2). However, 100% resistance of the *E. coli* isolates was recorded for ceftriaxone, ampicillin-sulbactam and cefoxitin. Their resistance to nalixidic acid, cefotaxime, ceftazidime and sulfamethoxazole-trimethoprim and ciprofloxacin ranged from 33.33% - 83.33% while three *E. coli* isolates screened from restaurant environment were 100% susceptible to meropenem, ciprofloxacin and gentamicin. Their susceptibility to ceftriaxone, ceftazidime, nalixidic acid and sulfamethoxazole-trimethoprim ranged from 33.33-66.67% (Table 2). They were 100% resistant to ampicillin-sulbactam, cefotaxime and cefoxitin whereas 3 isolates of *E. coli* from fruits market screened were 100 % susceptible to meropenem and gentamicin (Table 2). This was followed by 66.67 % each to nalixidic acid, ciprofloxacin, sulfamethoxazole-trimethoprim, cefotaxime and ceftazidime (Table 2). They were 100% resistant to ampicillin-sulbactam, cefoxitin, and ceftriaxone (Table 2). Only 33.33 % each was resistant to nalixidic acid, ciprofloxacin, sulfamethoxazole-trimethoprim, cefotaxime and ceftazidime (Table 2).

Occurrence of ESBL Producing *E. coli* Isolated from the Body wash of Houseflies from Hospital, Restaurant and Fruit market Environments.

In this study, out of the 12 isolates (6 from hospital; 3 from fruit market and 3 from restaurant) screened for the presence of ESBLs, none of the isolates was ESBL positive (Table 3).

Table 1: Percentage prevalence of *e. coli* isolated from the body wash of houseflies from hospital, restaurant and fruit market environments in Abakaliki metropolis

Sample source	No. of samples analyzed	No. and percentage of organisms isolated	Organisms isolated
Hospital	50	6(50)	*E. coli*
Fruit market	50	3(25)	*E. coli*
Restaurant	50	3(25)	*E. coli*
Total	150	12	

Table 2: Percentage Susceptibility and Resistance Pattern of *E. coli* Isolated from Body Wash of Houseflies from Hospital environment

Antibiotics	Hospital		Restaurant		Fruit market	
	E. coli		*E. coli*		*E. coli*	
	% Susceptible	% Resistant	% Susceptible	% Resistant	% Susceptible	% Resistant
Meropenem	6(100)	0(0.0)	3(100)	0(0.00)	3(100)	0(0.00)
Nalixidic acid	4(66.67)	2(33.33)	2(66.67)	1(33.33)	2(66.67)	1(33.33)
Ceftriaxone	0(0.0)	6(100)	0(0.00)	3(100)	0(0.00)	3(100)
Ciprofloxacin	3(50)	3(50)	2(66.67)	1(33.33)	2(66.67)	1(33.33)
Sulfamethozazole-trimethoprim	4(66.67)	2(33.33)	2(66.67)	1(33.33)	2(66.67)	1(33.33)
Cefotaxime	4(66.67)	2(33.33)	2(66.67)	1(33.33)	2(66.67)	1(33.33)
Ampicillin-subactam	0(0.0)	6(100)	0(0.00)	3(100)	0(0.00)	3(100)
Ceftazidime	4(66.67)	2(33.33)	2(66.67)	1(33.33)	2(66.67)	1(33.33)
Cefoxitin	0(0.0)	6(100)	0(0.00)	3(100)	0(0.00)	3(100)
Gentamicin	1(16.67)	5(83.33)	3(100)	0(0.00)	3(100)	0(0.00)

Table 3: Percentage occurrence of ESBL in *E. coli* isolated from the body wash of houseflies from the hospital, restaurant and fruit market environment

Sample source	Isolates obtained	Number of isolates screened for ESBL production	Percentage ESBL positive	Percentage ESBL negative
Hospital	*E. coli*	6	(0.0)	6(100)
Restaurant	*E. coli*	3	(0.0)	3(100)
Fruit market	*E. coli*	3	(0.0)	3(100)
Total		**12**	**0**	**12**

DISCUSSION

In this study, body wash of houseflies from hospital had the highest isolates of *E. coli* 6(50 %) while 3(25 %) each was obtained from fruit market and restaurant environments. This indicates that the body wash of houseflies from hospital environment had the highest number of *E. coli* when compared to those from fruit market and restaurant. The prevalence of *E. coli* from the hospital environment could be because the flies had more contact with human wastes than those from other environments investigated. Davari *et al.,* 2010 reported more pathogenic bacteria from houseflies caught in hospitals than those caught from slaughter house which is in line with our findings. It is also in agreement with the study by Ibrahim *et al.,* 2018 and Yang *et al.,* 2013 who as well isolated *E. coli* from the external body wash of houseflies. Babak *et al*., 2008; Vazirianzadeh *et al.,* 2008; Mawak and Olukose, 2006 also isolated *E. coli* from houseflies which are not out of place from our study.

Antibiotic susceptibility and resistance patterns of *E. coli* isolated from the three environments (hospital, fruit market and restaurant) to different antibiotics showed that meropenem (100 %) had the highest activity but were 100 % resistant to ceftriaxone, ampicillin-sulbactam and cefoxitin. Resistance of the isolates from hospital to gentamicin up to 83.33 % was also recorded. Their susceptibility to other antibiotics used ranged from 16.67 % to 66.67 %. The resistance of the isolates to

various antibiotics could be as a result of presence of some drug resistant genes, additional gain of other genes through horizontal gene transfer or by physiology dependent resistance (Rangrez *et al.* 2006; Mitchell *et al.* 2004). In tandem with our findings, Macovei and Zurek (2006) detected antibiotic-resistant and potentially virulent pathogen in houseflies from food settings. The presence of these pathogens indicates that houseflies (*M. domestica*) are good vectors of pathogens which pose serious health risks to humans.

Of the 12 isolates screened for the presence of ESBLs, none of them from the three environments (hospital, restaurant and fruit market) tested positive. Our study disagrees with the report by Solar-Gines *et al.* (2015) who isolated beta-lactamase-producing *Escherichia coli* from houseflies in Spanish broiler, stating the possible contribution of houseflies to the rise and spread of virulence and resistance genes into different ecological niches.

In conclusion, the study revealed that houseflies from hospital, fruit market and restaurant environments harbor multidrug resistant *E. coli* and that the multidrug resistance were not ESBL mediated. This suggests that houseflies are potential spreaders of multidrug resistant agents, thus constituting a serious threat to human health since they readily interact with them. It is therefore recommended that appropriate steps be taken to control human interactions with houseflies especially in hospital environments. Food and fruit vendors should be alerted and monitored to protect their products from houseflies.

REFERENCES

Boulesteix, G., Le Dantec, P., Chevalier, B., Dieng, M., Niang, B. and Diatta, B. (2005). Role of *Musca domestica* in the transmission of multiresistant bacteria in the centres of intensive care setting in sub-Saharan Africa. *Ann FrAnesth Reanim* **24:**361-365.

Fotedar, R., Banerjee, U., Singh, S., Shriniwas, A. and Verma, K. (1992). The housefly (*Musca domestica*) as a carrier of pathogenic microorganisms in a hospital environment. *J Hosp Infect* **20**:209-15.

Graczyk, T.K., Knight, R., Gilman, R.H. and Cranfield, M.R. (2001). The role of non-biting flies in the epidemiology of human infectious diseases. *Microb Infect* **3**:231-5.

Yang Liu1, Yu Yang, Feng Zhao, Xuejun Fan, Wei Zhong, Dairong Qiao and YiCao (2013). Multi-drug resistant gram-negative enteric bacteria isolated fromflies at chengdu airport, china. *Southeast Asian J Trop Med Public Health* **44**(6):988-996.

Vazirian, Z. B., Shams, S. S., Rahdar, M., Hajhossien, R. and Mehdinejad, M. (2008). Identification of bacteria which possible transmitted by *Musca domestica* (Diptera: Muscidae) in the region of Ahvaz, SW Iran. *Jundishapur Journal of Microbiology*. **1**(1):28-31.

Davari, B., Kalantar, E., Zahirnia, A. and Moosa-Kazemi, S.H. (2010). Frequencyof resistance and susceptible bacteria isolated from houseflies. *Iranian J Arthropod-Borne Dis*. **4**(2):50-55.

Babak, V., Setareh, S., Mahmoud, R., Reza, H. and Manijeh, M. (2008).Identification of bacteria which possible transmitted by *Musca domestica*(Diphtera: Muscidae) in the region of Ahvaz, SW Iran. *Jundishapur Journal of Microbiology* **1**(1):28-31.

Mawak, J.D. and Olukose, O.J. (2006). Vector potential of houseflies (*Muscadomestica*) for pathogenic organisms in Jos, Nigeria. *J. Pest, Dis. Vector Manag*. **7**:418-423.

Solà-Ginés, M., González-López, J.J., Cameron- Veas, K., Piedra-Carrasco, N.,Cerdà-Cuéllar, M. and Migura-Garciaa, L. (2015). Houseflies (*Muscadomestica*) as vectors for extended-pectrum β-lactamase-

producing *Escherichiacoli* on spanish broiler farms. *Appl. Environ. Microbiol.*, **81**(11):3604 – 3611.

Khobdel, M., Jonaidi, N. and Seiedi, M. (2008). Blowfly and flesh (Diptera: Cyclorrhpha) fauna in Tehran, Iran. *J. Entomol*. **5**(3):85-92.

Mitchell, J., Tali De-Medina and Yehuda, C. (2004). Epidemiological interpretationof antibiotic resistance studies: what are we missing? *Nat. Rev. Microbiol.,* **2**:979-983.

Pandian, R. and Asumtha, A. (2001). Vector competence of *Musca domestica* Linn.in slum areas. *Insect Environ.,* **7**:118-119.

Rangrez, A., Dayananda, K., Atanur, S., Joshi, R., Patole, L. and Shouche, Y. (2006). Detection of conjugation related type four secretion machinery in *Aeromonas culicicola. PLoS One,* **1**(1):115.

Nazari, M., Mehrabi, T., Mostafa, S.H. and Alikhani, M.Y. (2017). Bacterial contamination of adult house flies (*Musca domestica*) and sensitivity ofthese bacteria to various antibiotics, captured from Hamadan City, *Iran. J Clin Diagn Res*.**11**(4):04–7.

Afiukwa, F.N. Iroha, I.R., Afiukwa, A.C. and Igwe, O.D. (2016). First report of *bla*CTX-M-15 extended spectrum beta-lactamase (ESBL) producing *E. coli* isolated from cloacal swabs of birds in South Eastern Nigeria**.** *International Archives of BioMedical and Clinical Research.***2**(2):35-39.

Iroha, I.R., Oji, A.E. and Esimone, C.O. (2008). Antimicrobial resistance pattern of plasmid-mediated extended spectrum beta-lactamase producing strains of *Escherichia coli. Sci. Res. Ass.* **3**(**6**):215-218.

National Committee for Clinical Laboratory Standards (NCCLS) (2000). *Performance standard for antimicrobial susceptibility testing*. NCCLS approved standard M 100-S9, National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

Pitout, J.D., Hossain, A. and Hanson, N.D. (2004). Phenotypic and molecular detection of CTX-beta-lactamases produced by *E. coli* and *Klebsiella* spp. *J. Clin. Microbiol.* **42**:5715-5721.

Macovei, L., Miles, B. and Zurek, L., (2008). The potential of house flies to contaminate ready-to-eat food with antibiotic resistant enterococci. *J. Food Prot.* **71**:432–439.

Chaiwong, U., Yimyam, N., Rerkasem, K. and Rerkasem, B. (2012). Green manures for highland paddy in a mountainous area. *J. Nat. Sci,* 11(1):103-168.