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Isolation of potentially pathogenic bacteria from *Musca domestica* captured in hospitals and slaughterhouses, Khartoum state, Sudan

Isam-Eldeen Itaype Ibrahim Bashir[1], AlaaEldin Yousri Muatsim Hamid[1], Mohamed Ahmed Ibrahim Holi[1] and Eltayib H. Ahmed-Abakur[1,2,3*]

[1]Department of Microbiology and Immunology, Faculty of Medical Laboratory Science, Alzaiem Alazhari University, Khartoum north, Sudan, Postal code 11111, Sudan.
[2]Medical laboratory Technology Department, Faculty of Applied Medical Science, University of Tabuk, P. O. Box 741, Tabuk -Postal code 71411, Saudi Arabia.
[3]Prince Fahad Research Chair, Department of Medical Laboratory Technology (FAMS), University of Tabuk, P. O. Box 741, Tabuk -Postal code 71411, Saudi Arabia.

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This study aimed to isolate and identify bacterial and parasitic pathogens from houseflies captured in hospitals and slaughterhouses. The present study involved 300 houseflies, of which 150 houseflies were collected from hospitals and 150 from slaughterhouses. Two samples were obtained from each housefly; one sample was obtained from the surface of the housefly, and the second was extracted from the intestine of the fly. The bacteria were isolated using cystine–lactose–electrolyte-deficient agar (CLED) agar, while the parasites were studied using direct microscopic examination. Two hundred eighty-three bacteria were isolated from hospitals houseflies; 56.2% of them recovered from the surface of houseflies and 43.8% from the intestine of the flies. This result indicated that each housefly carried 1.9 bacteria. Three hundred sixty-six bacteria were isolated from slaughters houseflies; 53.8% of them recovered from the surface of houseflies and 46.2% from the intestine of the flies. This result showed that each housefly carried 2.44 bacteria. *Escherichia coli, Enterococci* spp and *Pseudomonas aeruginosa* were the most common bacteria isolated from the houseflies. This study identified high virulence bacteria such as *E. coli*, *Klebsiella pneumoniae* and *Shigella* spp. This finding reflects the level of hygiene in the studied area and arise alarm of consequent complications for human health.

Key words: Housefly, bacteria, parasite, culture, cystine–lactose–electrolyte-deficient agar (CLED).

INTRODUCTION

Housefly, *Musca domestica Linnaeus*, is an important medical insect and the most common fly in the world (Khamesipour et al., 2018; Zurek and Nayduch, 2016; Solà-Ginés et al., 2015) representing about 90% of flies in human habitation (Balla et al., 2014). Housefly is able to complete its entire lifecycle (larvae, pupae, adults) within human habitations and domestic animals (Khamesipour et al., 2018). During these developmental phases, the houseflies are strictly associate with microorganisms (Park et al., 2019) and due to their

breeding properties, saprophytic foraging behavior, and hematophagous playing an significant role in the transmission and spread of a wide variety of bacterial, fungal and viral pathogens (Stelder et al., 2021). Houseflies have been identified as mechanical vector and reservoirs for more than 100 pathogenic microorganisms (Reuben et al., 2020; Issa, 2019; Neupane et al., 2019; Nazari et al., 2017); they carry a variable and complex prokaryotic microbiota (Park et al., 2019). Some of the bacteria isolated from houseflies were highly virulent species such as *Pseudomonas*, *Escherichia coli, Klebsiella* spp., Vibrio cholera, *Bacillus anthracis*, *Streptococci*, *Enterococci, Staphylococci* and *Clostridium* spp (Khamesipour et al., 2018; Zurek and Nayduch, 2016). Recently houseflies were identified as a potential carrier of the bird flu virus which is a serious threat to human health, and livestock (Zurek and Nayduch, 2016; Davari et al., 2010). Furthermore, severe acute respiratory syndrome coronaviruses (SARS-CoV) have been reported to be mechanically transmitted by insects, particularly CoV-19 can survive on the faeces and surfaces for elongated periods, likely no report has linked the human transmission of CoV-19 with insects (Reuben et al., 2020) .

Houseflies have the capability to act as vector expanded by its ability to move several kilometers in a couple of days (Park et al., 2019). Some microorganisms live in or on the body of houseflies for up to 35 days (Ranjbar et al., 2016), the transfer of pathogenic agents occur by several means like; attaching them to their mouth or body surface or through regurgitation of vomitus and passage through the alimentary tract (Neupane et al., 2019; Ranjbar et al., 2016).

Houseflies lives closely with humans and are often found in abundance in areas of human activities such as food centers, restaurants, food markets, hospitals, livestock and slaughterhouses causing serious health problems (Reuben et al., 2020; Issa, 2019; Khamesipour et al., 2018). One of the major challenges facing the developing countries is control of communicable diseases in which housefly play significant role. Therefore, the present study aimed to determine the frequency and type of bacteria and parasites in the houseflies collected from hospitals and slaughterhouses environments in Khartoum, Sudan.

MATERIALS AND METHODS

This study was cross sectional study, conducted in Khartoum state, Sudan. *Musca domestica* flies represented the population in this study.

Sample collection and identification of houseflies

Samples were obtained via simple random technique. Three hundred houseflies were collected; 150 captured in hospitals (Al-Nao hospital and Al-Boluk pediatric hospital) and 150 captured in slaughterhouses and surrounding area (Omdurman, Kartoum State). From each housefly two samples were obtained, one sample obtained from the body surface of the fly and the second sample was extracted from the intestine of the fly. The houseflies were captured individually using sterile entomological nets and immediately transported to medical parasitology and entomology department, University of Science and Technology, Sudan. Houseflies were identified through observation of the morphological features, macroscopically and microscopically properties as mentioned in fly management handbook (Kirby, 2008).

Preparation of housefly body surface samples

To detect the microorganisms (bacteria and parasites) attached to the surfaces of the a housefly, each fly was immersed in 3 ml sterile peptone water buffer for 2 min and then the fly was kept into sterile Petri-dish. The peptone water buffer was incubated at 37°C for 4 h to encourage the growth of bacteria. A loop full of well mixed peptone water buffer was inoculated into sterile CLED agar and incubated aerobically at 37°C for 48 h, the remaining peptone water buffer was concentrated by centrifugation at 5000 rpm for 5 min. The deposit was examined microscopically using 10X and 40X objective lenses to detect the attached parasites

Preparation of housefly intestinal samples

Each housefly was disinfected by dipping into 70% Ethanol 'two times', sterile distilled water 'three times', 0.05% sodium hypochlorite 'two times' and sterile distilled water 'three times' and finally placed into sterile filter paper. The housefly was dissected under aseptic condition, the intestine was extracted and suspended in sterile 3 ml peptone water buffer, mixed thoroughly to allow bacterial release, and then the medium was incubated at 37°C for 4 h to encourage the growth of bacteria. A loop full of well mixed peptone water buffer was inoculated into sterile CLED agar and incubated aerobically at 37°C for 48 h.

Identification of detected microorganisms

The identification of isolated bacteria was done based on Chessborough's scheme (Cheesborough, 2006). Briefly it relies on colonial morphology, Gram stain reaction and biochemical tests. The biochemical tests for Gram positive cocci were catalase test, coagulase test, esculin hydrolysis, DNase test, whereas the biochemical reaction used for Gram negative rod were oxidase test, motility test, indole test, urease test, citrate utilization test and triple sugar iron. The analytical profile index 20 E identification system for *Enterobacteriacea*e and other non-fastidious Gram negative rods a long with RapiDEC *Staph* identification of frequently isolating *staphylococcus* were used to confirm the identification. The parasites had been identified using Arora scheme for identification of intestinal helminthes and protozoa depending on the morphology

*Corresponding author. E. mail: eltayib1974@yahoo.com, eosman@ut.edu.sa. Tel: +966534609127.

Table 1. Type and frequency of bacteria isolated from hospitals houseflies.

Isolate	Surface		Intestine		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
E.coli	23	14.5	21	17	44	15.5
K.pneumoniae	16	10	25	20.2	41	14.5
P.aeruginosa	16	10	8	6.5	24	8.5
P.stuartii	3	1.9	12	9.7	15	5.3
P.vulgaris	5	3.1	8	6.5	13	4.6
K.oxytoca	6	3.8	5	4	11	3.9
Y.enterocolytica	6	3.8	4	3.2	10	3.5
Shigella spp	8	5	1	0.8	9	3.2
S.paratyphi B	5	3.1	2	1.6	7	2.5
P.mirabilis	3	1.9	4	3.2	7	2.5
S.paratyphi A	2	1.3	2	1.6	4	1.4
S.typhimurium	2	1.3	3	2.4	5	1.8
K.aerogens	2	1.3	--	--	2	0.7
C.freudii	13	8.1	4	3.2	17	6
E.caloacae	6	3.8	--	--	6	2.1
C.davisae	1	0.7	1	0.8	2	0.7
Enterococci spp	20	12.6	20	16.1	40	14.1
S.aureus	15	9.4	3	2.4	18	6.4
Coagulase–ve Staphylococci	7	4.4	1	0.8	8	2.8
Total	159	100	124	100	283	100

of the parasite (Arora and Arora, 2010).

Data analysis

The bacteria per housefly was calculated by dividing the summation of isolated bacteria to total number of houseflies, data was analyzed using Microsoft excel 2007.

RESULTS

Two hundred eighty three bacteria were isolated from hospital's houseflies; 159 (56.2%) of them were isolated from the surface of the flies and 124 (43.8%) from the intestine of the flies. This result indicated that each housefly carried 1.9 bacteria. Sixty six (23.3%) of isolated bacteria were Gram positive cocci and (76.7%) were Gram negative rods. The identification of isolates revealed 19 different species, the most frequent isolate was *E. coli* 44 (15.5%), followed by *Klebsiella pneumoniae* 41 (14.5%) and *Enterococci* spp. 40 (14.1%). The most dominant bacteria isolated from the surface of houseflies was *E. coli* 23 (14.5%) followed by *Enterococci spp* 20 (12.6%), whereas the most dominant bacteria isolated from the intestine was *K. pneumoniae* 25 (20.2%) followed by *E. coli* 21 (17.0%), Table 1.

Three hundred and sixty six bacteria were isolated from slaughterhouses houseflies; 197 (53.8%) of them were isolated from the surface of the flies whereas 169 (46.2%) bacteria were isolated from the intestine of the flies. These results showed that the housefly carried 2.44 bacteria. The majority of isolates 249 (68.0%) were Gram negative rods, 117 (32.0%) bacteria were Gram positive Cocci. The biochemical tests showed 17 different species. The most frequent isolate was *E. coli* 73 (19.9%) followed by *Enterococci spp.* 66 (18.0%) and *P.aeruginosa* 53 14.5%), the most frequent bacteria isolated from the surface was *P. aeruginosa* 38 (19.3%) followed by *E. coli* 33 (16.8%) *and Enterococci* 29 (14.7%), whereas the most dominant bacteria isolated from the intestine was *E. coli* 40 (23.7%) followed by *Enterococci* spp. 37 (21.9%) and *K. pneumoniae* 20 (11.8%) (Table 2). Thirty six parasites were detected in hospital houseflies; the most dominant parasite18 (50%) was *G.lamblia* followed by 9 (25%) *E.histolytica*. The samples collected from slaughters houseflies revealed 21 parasites; the most dominant parasite was *G. lamblia* 9 (42.9%) followed by *E*. *histolytica* 7(33.3%). Only eggs of helimenth (*H.nana*) were detected in hospital houseflies (Table 3).

DISCUSSION

Houseflies significantly increase the risk of exposure to a

Table 2. Type and frequency of bacteria isolated from slaughters houseflies.

Isolate	Surface		Intestine		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
E.coli	33	16.8	40	23.7	73	19.9
K.pneumoniae	9	4.6	20	11.8	29	7.9
P.aeruginosa	38	19.3	15	8.9	53	14.5
P. vulgaris	4	2.0	10	5.9	14	3.8
K. oxytoca	1	0.5	3	1.8	4	1.1
Y. pseudotuberclosis	2	1.0	1	0.6	3	0.82
Shigella spp	5	2.5	1	0.6	6	1.6
S.paratyphi B	3	1.5	13	7.7	16	4.4
P. marabilis			3	1.8	3	0.82
S. typhi A	6	3.0	2	1.2	8	2.2
S. typhimurium	8	4.0	6	3.6	14	3.8
C.freudii	8	4.0	6	3.4	14	3.8
E.cloacae	6	3.0	5	3.0	11	3.0
S. marcescence	1	1.0			1	0.27
Enterococci spp	29	14.7	37	21.9	66	18.0
S.aureus	26	13.2	6	3.6	32	8.70
Coagulase negative *Staphulococc*i	18	9.1	1	0.6	19	5.2
Total	197	100	169	100	366	100

Table 3. Type and frequency of parasites in houseflies collected from hospitals and slaughterhouses.

Parasite	Hospitals		Slaughterhouses	
	Frequency	Percentage	Frequency	Percentage
G.lamblia	18	50.0	9	42.9
E.histolytica	9	25.0	7	33.3
Entamoeba coli	5	13.9	5	23.8
Egg of H.nana	4	11.1	0	00
Total	36	100	21	100

wide range of foodborne pathogens due to their uncontrolled movements, ability to fly long or short distances, and attraction to cooked and uncooked food material (Ghalehnoo, 2015). Moreover, houseflies easily access to hospitals setting in developing countries and play critical role in transmission of nosocomial infections.

In this study, CLED agar was the medium of choice for bacterial isolation to recover all Enterobacteriaceae, most pathogenic bacteria because it inhibits swarming of *proteus* and related genera due to deficiency of electrolytes and enable the differentiation between lactose fermenting from non-lactose fermenting bacteria due to presence of bromothymol blue indicator (Collee et al., 1996).

The result of present study showed that the houseflies collected from hospitals environment were less contaminated, where each housefly carried 1.9 bacteria, compared to that obtained from slaughterhouses in which the housefly carried 2.44 bacteria. This result is relatively higher than that reported by Zurek and Nayduch (2016), who carried out study to investigate the bacteria on houseflies collected from hospital environment and stated 1.4 bacteria per housefly. Zhang et al. (2017) reported 1 bacteria per housefly. Our finding was contradictory to Zurek and Nayduch (2016), report who stated that houseflies collected from hospital were more contaminated. However, the number and type of bacteria is a function of place where these flies are captured (Nazari et al., 2017). The differences in the rate of isolation also related to the techniques used in the isolation of the microorganisms (Acevedo et al., 2009). The high rate of isolation in the present study may be

owing to the low level of hygiene services in studied environments. The majority of isolates in our study belong to the family *Enterobacteriaceae* (Gram negative rod), which could be interpreted by the fact that *Enterobacteriaceae* is the main family that inhabits the gastrointestinal tract of human and animal and it is excreted in their stool, which is an excellent source of nutrition for the housefly.

The present study revealed that the most dominant bacteria isolated from the hospitals houseflies were *E. coli* (15.6%), followed by *K. pneumoniae* (14.5%) and *Enterococci spp* (14.1%). These bacteria "*E. coli*, *K. pneumoniae*, *Enterococci*" are among most important pathogens causing different diseases ranging from urinary tract to pneumonia infections and septicemia, and have the ability to acquire and transfer antibiotic resistance genes (Park et al., 2019; Zurek and Nayduch, 2016; Ahmad et al., 2011). Ranjbar et al. (2016) showed that houseflies are potential vectors of antibiotic resistant *K. pneumoniae* and Ahmad et al. (2011) stated that *Enterococci* are considered a reservoir of antibiotic resistance genes to a wide range of antibiotics.

The presence of houseflies in slaughterhouses reduces the meat hygiene standards and can transfer a variety of pathogenic organisms (Songe et al., 2016). The present study showed that the most common bacteria isolated from slaughterhouses was *E. coli* 73 (19.9%), followed by *Enterococci spp* 66 (18.0%) and *P. aeruginosa* 53 (14.5%). These findings were relatively in alignment with different reports in which *E. coli*, *Klebsiella* spp and *Pseudomonas* spp., were the most dominant bacteria isolated from hospitals and slaughters environments (Songe et al., 2016; Davari et al., 2010; Cheesborough, 2006). Other reports showed *Bacillus* spp, *Staphylococcus* spp. (Zurek and Nayduch, 2016), *Providencia stuartii* (Zhang et al., 2017) and *Proteus mirabilis* (Davari et al., 2010) as common bacteria.

Results of the study indicated that the bacteria were isolated more frequently from the body surfaces than gut of the housefly. These findings agreed with Issa (2019); and Khamesipour et al. (2018) reports and disagreed with other reports that stated the amount of pathogens present in the intestine is generally higher than the quantity present on the body surfaces (Boiocchi et al., 2019; Davari et al., 2010). Houseflies have the ability to transfer the foodborne bacteria to their eggs and newly emerged generation adults (Pava-Ripoll et al., 2015), this process may provide a plausible environment for emerging bacterial strains with new properties involving acquired virulence and antibiotic resistance genes (Akhtar et al., 2009).

Very few studies reported parasites from the housefly (Khamesipour et al., 2018). Our study showed that *G. lamblia* and *E. histolytica* were most the dominant parasites detected in houseflies, similarly Manandhar and Gokhale (2017) mentioned that the *G. lamblia* is the most common human protozoan entero-pathogen worldwide.

Conclusions

Our study showed high load of bacteria per housefly and identified high virulence bacteria such as *E. coli*, *K. pneumoniae* and *Shigella* spp. This findings reflect the level of hygiene in the studied area and arise the alarm of consequent complications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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