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International Journal of Biotechnology and Bioengineering

Volume 7 Issue 2,
Febuary 2021

Citation

Khalid A Enan et.al. (2021), Bacterial and Rotavirus Contaminations of Adult House Flies (Musca domestica), Captured from Khartoum State, Sudan. Int J Biotech & Bioeng. 7:2.

ISSN 2475-3432

Published by
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Research Article

Bacterial and Rotavirus Contaminations of Adult House Flies (Musca domestica), Captured from Khartoum State, Sudan.

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Article History: **Received:** Febuary 23, 2020;
Accepted: Febuary 27, 2020;
Published: Febuary 28, 2021.

Abstract

Background: Identifying disease vectors and pathogens is one of the key steps in controlling vector-borne diseases. The objective of this study was to isolate and identify medically important bacteria and rotavirus from adult Musca domestica flies.

Methods: Musca domestica (House fly) were captured by sticky trap methods during the daytime, from household kitchens, cattle farms, human hospitals, slaughter houses and vegetable markets at random locations in Khartoum State of Sudan, and subsequently transported to the laboratory for detection of bacteria and rotavirus. In the laboratory, flies were identified and killed by refrigeration at 0 °C, then placed in 4 ml brain heart infusion broth and left at room temperature for two hours, sub- cultured onto blood agar plates aerobically at 37°C for 24 hours. Isolates were preliminarily identified to genus level based on colony morphology and Gram staining and further confirmed by biochemical tests. On the other hand, RNA extraction was done from house flies to detect the Rota Viruses by Real-time (RT-PCR) .

Results: Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Enterococcus faecalis, Staphylococcus aureus, and other unidentified bacteria were isolated and two samples were positive for rotavirus.

Conclusions: Houseflies can play an important role in the epidemiology and transmission of bacteria and rotavirus infections in Khartoum State, Sudan.

Keywords

Musca domestica, Real-time PCR, Khartoum, Sudan

Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Introduction

Houseflies (*Musca domestica*) are the most common of domestic flies. Their feeding and reproductive habits make them important mechanical vectors of several human and veterinary pathogens including those causing nosocomial, enteric infections.[1-5] Flies are major epidemiologic factors responsible for the spread of acute gastroenteritis especially among infants and young children in developing countries and transmission of nosocomial infections with multiple antibiotic-resistant bacteria in hospital environment.[6] Regular contact of the fly with wastes and animals provide an opportunity to transmit pathogens to both humans and animals [5-8]. House flies are known to transmit to

transmit some important pathogens such as *Pseudomonas spp., Enterobacteriaceae spp., Staphylococcus aureus, Vibrio cholera, Chlamydia trachoma, Salmonella spp*. and *Klebsiella spp*. [5, 9—16]. These pathogens are carried on the fly's legs and other body parts [16].A study conducted in Malaysia on mechanical transmission of rotavirus by the legs and wings stated that house fly can transmit rotavirus[6]. The objective of this study was to isolate bacterial organisms and rotavirus in *Musca domestica* captured in different settings and locations in Khartoum State

Material and Methods

Study area and sample collection

This was Quasi-Experimental Design conducted in Khartoum State – Sudan. This experiment involved 500 houseflies collected at household kitchens (n=50), cattle farms (n = 80), vegetable market (n = 59), human hospitals (n = 280) and slaughter houses (n = 31). The houseflies were captured by sticky trap methods. The fly samples were then transported to the laboratory using separate sterile tubes to prevent cross-contamination between samples. In the laboratory, the flies were quickly anaesthetized by keeping them at 0 °C for 5 min, then pooled in10 house flies/pool.Each pool was then placed into sterile bottle containing 4mL brain heart infusion broth and grounded with sterile cotton swabs, theeafter the suspensions were divided into two tubes (2ml in each), one was used for rotavirus detection and the other for bacterial isolations.

Isolation of Bacteria from samples

Samples were processed at the faculty of Medical laboratory sciences - Omdurman Ahlia University (OAU). Standard culture and identification methods were used (WHO, 1987). Briefly,the tube with flies presuspended in 2 ml BHI-broth was shaken for 2 min, and incubated at 37 °C for 2 h, then small amount of the suspension was sub cultured onto blood agar plates and keep at 37°C for 24 hours. Then the cultures were examined for growth detection, the bacteria was colony purified by sub culturing in nutrient agar medium and overnight incubation. Obtained growth was identified to the genus level starting from colony morphology, texture, Gram staining and biochemical tests such as growth in kilgler iron agar, motility test, urease production test, peptone water and citrate utilization test, sugar reactions, catalase test, coagulase test, dnase test, litmus milk test and aesculin hydrolysis test.[20]

RNA extraction

Total RNA was extracted by using the QIAamp Viral RNA Mini spin according to the protocol of the manufacturer (Qiagen, Germany). Briefly, 140 µl of flies suspension sample was added to 560 µl buffer AVL containing carrier RNA, and then incubated at room temperature for 10 minutes. Subsequently, 560 µl of ethanol (96- 100%) was added to the sample after which 630 µl of the resulting solution was applied to a column. A volume of 500 µl of AW1 and AW2 was added for washing and the nucleic acids were eluted with 60 µl AVE buffer and stored at -80°C until used.

Real time RT-PCR

Real-time one step RT-PCR was done to detect viral RNA by using a commercial kit following the manufacturer's instructions (One-Step Real-Time RT-PCR Master Mixes Kit, Invitrogen, genesig standard kit).

With the primer/probe set 1, RNaseP was used as an internal control and real-time PCR was carried out following the protocol provided by manufacturer's instructions. The realtime PCR master mix for one reaction was prepared as follows: 4 µl of 5X PCR reaction mix (consisting of a proprietary buffer system, MgSO4, dNTPs, and stabilizers),6 µl of primer/probe, 1 µl of 1U enzyme mix, 5.5 µl of molecular grade water, 0.5 µl of 10X ROX and 3 µl of total RNA (90.3 ng/µl). The final volume was 20 µl for a single reaction. The reaction was performed in an automated 7500 real-time PCR (AB Applied Biosystems, USA). The thermal cycling conditions were 10 minutes at 55°C for reverse transcription, 2 minutes at 95°C for initial denaturation and 50 cycles of 15 seconds at 95°C for denaturation and 60 seconds at 60°C for annealing and extension. A sample whose growth curve crossed the threshold line within 40 cycles (Ct < 40) was considered as positive.

Results

Bacteria isolated from the house flies pools were from the external body surface as will as the internal organs.. Suspension of house flies samples were obtained from household kitchens, cattle farms, vegetable market, human hospitals and slaughterhouses. The bacteria isolated from these sites are as presented in Table1. The bacteria identified to the genus and species level were *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Enterobacter, Enterococcus faecalis, Staphylococcus aureus* in addition to unknown bacteria.

Only two samples were positive for rotavirus which was isolated in pools of flies collected in cattle farms (1 isolate) and vegetable markets(1 isolate).

Collection Sites	Micro-organisms isolated	Total No.of bacterial isolates
Human hospitals	E.coli (15) Klebsiella pneumoniae(23) Proteus vulgaris(10) Staphylococcus aureus(20) Enterococcus faecalis(6) Unknown spp(2)	76
Cattle farms	E.coli (3) Klebsiella pneumoniae (8) Proteus vulgaris (2) Enterococcus faecalis (5) Rotavirus (1)	18
Vegetable markets	E.coli (5) Klebsiella pneumoniae (2) Enterococcus faecalis (4) Rotavirus (1)	11
Household kitchens	Klebsiella pneumoniae (3) Staphylococcus aureus (2) E.coli (5)	10
Slaughter houses	Klebsiella pneumoniae (3) Proteus vulgaris (2) Enterococcus faecalis (3)	8

Table 1 Bacterial and rota virus isolates from pools of house flies collected in different areas in Khartoum state

Discussion

This study is the first of its kind in Sudan. In the recent years much attention has been given to the house fly as a potential mechanical vector of disease agent. Several pathogens were identified on captured flies, including rotavirus.[17]

This study demonstrates that flies carry and presumably disseminate seven bacterial spp as will as rotavirus, however, further studies are necessary to determine the importance of flies as carriers of rotavirus. *Klebsiella pneumoniae* was the most common bacterial pathogen isolated from *M.domestica* and was detected in samples collected in all locations.

These organisms have been implicated as a cause of nosocomial infections with multiple antibiotic-resistant bacteria in hospital environment.[18-19-21]

This study also indicated that a variety of bacteria species were isolated from house flies in the hospital setting. Some of the bacterial species isolated are causative agents for diarrhea and food poisoning and other disease conditions. Gram negative bacteria are known to be pathogenic and are usually associated with immunosuppression or malignancy but occasionally are seen in apparently normal hosts.[6] *Klebsiella spp* is present in the respiratory tract and faeces and at a small proportion causes bacterial pneumonias. It occasionally produces urinary tract infection and bacteremia with focal lesions in debilitated patients. *Klebsiella spp* also cause hospital acquired infections and is associated with inflammatory conditions of the upper respiratory tract. *Enterobacter spp* and *Proteus vulgaris* are also known to cause urinary tract infection. *Enterococcus faecalis* often are normal flora but occasionally cause nosocomial infection[6].

Efforts to decrease the fly population or reduce their numbers in areas in which people live and eat would be a reasonable approach to decreasing diarrheal disease and nosocomial infection. Prospective Studies performed before and after active interventions to reduce the fly populations may be required to determine the importance of flies in disseminating enteric pathogens. Our work suggests that such a study would be worthwhile.

Conclusion

Houseflies can play important roles in the epidemiology and transmission of bacteria and rotavirus infections in Khartoum State,Sudan.

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