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POTENTIALS OF FLIES IN THE TRANSMISSION OF *Escherichia coli* 0157:H7 AND OTHER ENTERIC BACTERIA ASSOCIATED WITH WASTE WATER

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Abstract: The design was to determine the prevalence of enteroheamorrhagic *Escherichia coli* 0157:H7 (EHEC) carried by Flies (*musca domestica*) around waste water irrigation sites of Jakara and Sharada waste water channels in Kano city. Thirty one (31) samples of flies were collected for the analysis, 15 from along Jakara waste water river and 16 from along Sharada waste water river and screened for *E. coli* 0157:H7 on Sorbitol MacConkey agar supplemented with Cefexim and Potassium tellurite (CT-SMAC) agar and using Latex agglutination test. Other enterobacteriaceae were isolated on McConkey agar and identified by biochemical tests, using Microbact 24E Identification Kit. There was a mean (*M*) mesophilic bacteria count of 126.07×10^{-4} and standard deviation (SD) of ±122.38 and $M = 112.00 \times 10^{-4}$, SD = ±75.72 from Jakara and Sharada river sample sites respectively. *Citrobacter spp*. had the highest percentage occurrence of 83.87% among the thirteen enterobactericeae species isolated, while *Yersinia* spp and *Providentia* spp have the least occurrences of 6.45% and 3.23% respectively. Serologically *E. coli* 0157:H7 was 70.97% amongst which only Six (27.6%) were biochemically confirmed to be *E. coli* 0157:H7, Ten (45.5%) were positive for Cellobiose fermentation and Potassium cyanide. There were no significant differences in the prevalence of both enterobacteriaceae and *E. coli* 0157:H7 between the sites. The study demonstrated the presence of *E. coli* 0157:H7 in flies found at irrigation site not directly connected to animal farm- yard, and in strong relationship with other enterobacteriaceae.

Keywords: Occurrence, *Ctrobacter* spp, *Klebsiella* spp, Jakara, Sharada.

Introduction

Most of the water sources used are waste waters from domestic sewers, which consist of waste Jakara and Sharada river collect and channel this waste water from Laundries, Kitchens, Bathroom (Private and Commercial), Abattoirs and storm water from various out let in Kano municipality. The river is mostly utilized as source of irrigation water. Waste water of this nature contains and can contribute to the spread of potentially pathogenic bacteria within the environment (Chapman *et al*. 1993; Cizek *et al*. 1994; Hancock *et al*. 1994

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and Davies and Wray 1997). Gram-negative bacteria within the family Enterobacteriaceae, including *Salmonella* spp., *Shigella* spp., *Escherichia* spp., *Yersinia* spp., *Klesiella* spp., *Citrobater* spp and even *E. coli 0157*:H7are of special concern, because of their opportunistic pathogenic nature, in causing disease to humans, domestic animals, and wildlife, Janda and Abbott, (1998).

Therefore bacteria from waste water columns and sediments can be release back into stream when it's disturbed (Sherer *et al*. 19992), thereby giving flies opportunity of carrying and transmitting pathogenic organism to fresh vegetables write from the farm. Pathogens present in animal carcasses or shed in animal wastes may include Rotaviruses, Hepatitis Ebola virus, *Salmonella* spp., *E. coli* O157: H7, *Yersinia enterocolitica*, *Campylobacter* spp., and *Vibrionaceae* (Sobsey *et al*. 2002). These are mostly normal intestinal flora of Cattles, Sheep, Goats and Birds, and are associated with potentially contaminated environments, such as refuse dumps, sewage treatment facilities, compost manure, dead animal carcasses, agricultural sites, and bird feeders, (Felon, 1985; Casanovas *et al*. 1995; Cezek *et al*. 1994) which is normal habitat of fly.

A number of species of flies have been reported to transmit *E. coli* 0157:H7. Kobayashi *et al*. (1999) studied contamination of flies in an investigation of a nursery-associated *E. coli* 0157:H7 outbreak and reported detection of the agent in fly intestines, excretion by contaminated flies for a 3-day period. Several studies have detected *E. coli* O157:H7 in flies collected from both dairy and beef cattle production environments Alam and Zurek, 2004; Hancock *et al*. 1998 and Iwasa *et al*. 1999).

Heuvelink *et al*. (1998) also isolated *E. coli* 0157:H7E from stable flies (*Stomoxys calcitrans*) on Dutch dairy farms. Szalansky *et al*. (2004) determined that 0.4–1.3% of pools of flies of two different species (*Musca domestica* and *Hydrotaea aenescens*) on a turkey farm were PCR positive for *E. coli* O157:H7 markers, and Keen and colleagues (2006) demonstrated a 5.2% *E. coli* O157:H7 carriage rate in flies sampled at agricultural fairs. It was also reported flies can disseminate *E. coli* O157:H7 contamination from one spinach plant to another (Tally *et al*. 2009). Janisiewicz and colleagues (1999) similarly noted that fruit flies (*Drosophila melanogaster*) could spread *E. coli* O157:H7 contamination to fresh-cut apple tissue.

Ahmad and colleagues (2007) showed that eight cattle exposed to contaminated flies became colonized with and shed *E. coli* O157:H7, whereas eight other cattle not exposed to the flies remained culture negative. Similarly, most pulsed-field gel electrophoresis patterns of *E. coli* O157:H7 were sometimes indistinguishable in fly and livestock isolates, indicating transfer of

the pathogen (Keen *et al*. 2006), also a Chinese study isolated the bacterium from the intestine of 4 of 113 dung beetles (*Catharsius molossus*) and found that its PFGE pattern and virulence genes were identical to those in ten strains isolated from humans with diarrhea in the same geographic region (Xu *et al*. 2003). The persistence and proliferation of *E. coli* O157:H7 in and on houseflies suggested to Kobayashi *et al*. (1999) that houseflies are more than just mechanical vectors for this pathogen, retention of viable pathogens in the flies' crops for 4 days, adhering to the mouthparts of culture-positive flies, suggesting a biological association.

However, the relationship on the role of house fly in transmission of bacterial pathogens to vegetables on farms is relatively unknown. To date, there has been no systematic assessment of pathogenic bacteria carried by house fly that associate with waste water used for agricultural practices in Kano state. However, isolation of fecal coliform bacteria was reported in United State (US), on routine basis from waters of local creeks and urban streams Cole, (2003). This study was undertaken to determine if house flies pose a threat to on farm vegetables, and therefore playing possible transmission role in the epidemiology of *E. coli* 0157:H7. As such, the primary objective of this study was to assess the prevalence and diversity of enteric bacteria carried by house fly, associating with waste water used for irrigation purposes in Kano state.

Material and Methods

Thirty one samples of flies (*Musca domestica*), 15 were collected along Jakara and 16 along Sharada waste water river by sweep net method and immediately brought to laboratory for analysis. A whole fly is immersed in 10 ml sterile water and serially diluted to 10^{-4} for bacterial mesophlic counts.

McConkey agar was used for isolation of other enterobacteriaceae, by taking 1ml homogenate of whole fly in 10ml sterile water and incubated at 37^0C for 24hrs. Lactose and non lactose fermented colonies with different colonial morphology were sub-cultured on nutrient agar (NA) and a suspension of pure colony from NA plates was emulsified in sterile distilled water, and identified using Microbact 24E test, for Oxidase negative *Enterobacteriacea*e, (Koneman *et al*. 1994; Jay *et al*. 2007; Islam *et al*. 2004)

Escherichia coli 0157:H7: Isolation and identification of *Escherichia coli* 0157:H7 was by enrichement on Tripticase soy broth supplemented with 0.5% Sodium thioglycolate for 4hrs at 37^0C (Dahiru e*t al*. 2008; Shin Sata *et al*. 2003), and sub -cultured on Sobitol McConkey

agar containing Cefixime and potassium Tellurite (CT-SMA), incubated for 24hr hours at 37^0C. CT-SMA non sorbitol fermented colonies, were biochemically screened for growth in Potassium cyanide, Cellobiose fermetation, Motility, Oxidase and with 0157:H7 Latex agglutination Kit (Oxoid) Dahiru e*t al*. (2008).

Result

A mean mesophilic count of 126.07×10^{-4} and standard deviation (SD) of ±122.38 was recorded from Jakara river samples and 112.00×10^{-4} with SD of ±75.72 from Sharada river samples. Thirteen species of enterobactericeae and one strain (*E. coli* 0157:H7) were isolated from the two site. Of the species *Citrobacter fruendii* recorded the highest prevalence 77.42% followed by *E. coli* 0157:H7 with 68.75% and *Serratia liquifaciens*, *Salmonella cholera-sius*, *Klebsiella oxytoca*, *K. rhinoscleromatis* and *Providentia rettgerii*, each having 3.23% (Table 1).

Similarly among the enterobacteriaceae genera isolated from flies was *Citrobacter* spp still recods the highest with (83.87%), consisting of *C. freundi* and *C. diversus*, among the eight genera and thirteen species isolated from *Musca domestica*. *E. coli* 0157:H7 was 68.75%, other *E. coli* species were (32.26%), *Klesiella* spp (9.68%), *Salmonella* spp (12.90%) *S. cholera-sius* and *S. typhi*, *Seratia* spp (9.68%) *S. mescenscens*, *Enterobacter* spp (9.68%) *E. agglomerans*, *Yesinia* spp (6.45%) *Y. enteocoletica*, and lastly *Providentia rettgeri* (3.23%). Only *E. coli*, *Salmonella* and *Citrobacter* species were isolated from both sites and *Citrobacte*r was having the highest occurrence of n = 21 from Jakara river (Table 2). There was however no significant difference in the prevalence for Jakara (*M*=4.78, *SD*=7.48) and Sharada [*M* =3.44, *SD*=2.69; *t* (10.047) =.503, *p*=.626]. The magnitude of the differences in the means was very small (*eta squared* = 0.015).

Table: 1: Frequency distribution of abundance and relative abundance of Bacterial species isolated from Flies samples.

Bacterial Species	**Abundance n= 31**	**Relative Abundance**
Escherichia coli 0157:H7	22	70.97
Serratia marcenscens	3	9.68
S. rubidae	2	6.45
S. liquifaciens	1	3.23
Citrobacter freundi	24	77.42

C. diverus	3	9.68
Enterobacter agglomerancs	3	9.68
Escherichia. Coli	10	32.26
Salmonella spp	5	16.13
S. cholera-sius	1	3.23
Klebsialla oxytoca	1	3.23
K ozaenae	2	6.45
K. rhinoscleromatis	1	3.23
Providentia rettgerii	1	3.23
Yersinia enterocoltica	2	6.45

Key: n = number of individual.

a. Predictors: (Constant), Other Ecoli

b. Predictors: (Constant), Other Ecoli, Citrobacter

c. Predictors: (Constant), Other Ecoli, Citrobacter, Klebsiella

d. Predictors: (Constant), Other Ecoli, Citrobacter, Klebsiella, Salmonella

e. Predictors: (Constant), Other Ecoli, Citrobacter, Klebsiella, Salmonella, Enterobacter

f. Predictors: (Constant), Other Ecoli, Citrobacter, Klebsiella, Salmonella, Enterobacter, Seratia

g. Predictors: (Constant), Other Ecoli, Citrobacter, Klebsiella, Salmonella, Enterobacter, Seratia, Yersinia

h. Predictors: (Constant), Other Ecoli, Citrobacter, Klebsiella, Salmonella, Enterobacter, Seratia, Yersinia, Providentia

i. Dependent Variable: E. coli 0157: H7

Figure 1: Multiple regression *E. coli* 0157:H7 and other enterobacterieaceae from flies along waste water river bank.

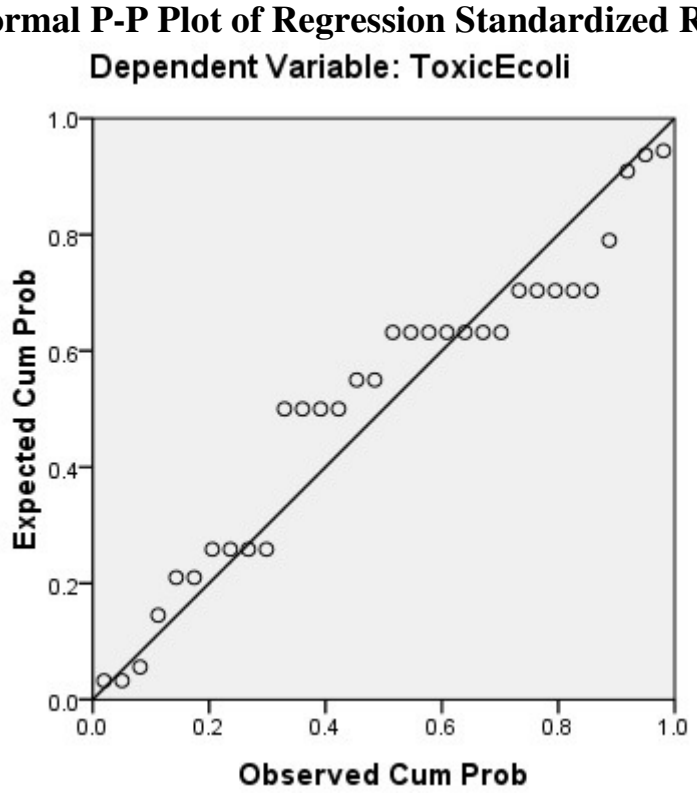

Table: 2 Prevalence of Enterobacteriaceae in Flies (Musca domestica) collected along Jakara and Sharada rivers in Kano.

Bacterial Isolates	No (%) Isolates from Jakara River n=15	No (%) Isolates from Sharada River n=16	Total No. (%) Isolated n = 31
E. coli 0157:H7	12(80)	10(62.5)	22(70.97)
Other E. coli spp	8 (53.33)	2 (12.50)	10 (32.26)
Klebsialla spp	0 (00)	3 (18.75)	3 (9.68)
Salmonella spp	2 (13.33)	2 (12.50)	4 (12.90)
Yersinia spp	0 (00)	2 (12.50)	2 (6.45)
Citrobacter spp	21 (140)	5 (31.25)	26 (83.87)
Providentia spp	0 (00)	1 (6.25)	1 (3.23)
Serratia spp	0 (00)	3 (18.75)	3 (9.68)
Enterobacter spp	0 (00)	3 (18.75)	3 (9.68)

Key: n = number sampled, spp = species

Table: 3 Distribution of Latex agglutination positive Escherichia coli 0157:H7 Isolated from *Musca domestica* and their profile to some basic biochemical reactions.

Sample Number	Oxidase Test	Motility Test	Cellobiose fermentation.	Growth Potassium Cyanide
FS1	+	+	_	_
FS2	+	+	+	_
FS3	+	+	+	+
FS4	+	+	+	+
FS7	+	+	+	−
FS8	+	+	+	−
FS9	+	+	+	−
FS10	+	+	+	+
FS11	+	+	−	−
FS12	+	+	+	+
FS13	+	+	+	+
FS15	+	+	+	−
FS16	+	+	+	+
FS17	+	+	+	+
FS18	+	+	+	+
FS19	+	+	−	−
FS20	+	+	+	+
FS21	+	+	−	−
FS22	+	+	−	+
FS25	+	+	+	+
FS26	+	+	−	−
FS30	+	+	−	−

Kay: FS = sample number, + = positive, − = Negative.

All the isolates were motile and oxidase positive and mostly grow well in Cellobiose fermentation test. Only 31.82 % fail to grow in cellobiose fermentation and 50% also fail to grow in the presence of potassium cyanide. A large proportion of the isolate 45.5% were positive for both Cellobios and potassium cyanide test, 27.6% were negative for both and only 4.5% was negative for Cellobiose and positive Potassium Cyanide test.

Discussion

The risk of infection can be better predicted by monitoring microbial contamination at points of potential contamination in the field during harvesting, during processing and distribution, or in retail markets (Beuchat and Ryu, 1997).

The common house fly, *Musca domestica* L., is medically-important insect worldwide. In addition to causing annoyance and myiasis it is forensically-important fly specie, being reported as mechanical carrier and/or reservoir of several pathogens, *ie*, bacteria, viruses, protozoan cysts and helminth eggs (Sukontason *et al*. 2007). This has remained to be true to the present day, even in flies not directly connected to human excreta, animal carcasses, garbage, dumping sites, food ruminants and sewages or any other unsanitary, filthy looking environment. It was also reported as one of the potential modes of dissemination of *E. coli* 0157:H7 in the environment, by associating with human and animal feces and manure Alam and Zurek, (2004). In a research on Association of *Escherichia coli* O157:H7 with Houseflies on a Cattle Farm, have detected a fecal coliform of (95.4%) from 350 house fly screened, and counts ranging from 3.0×10^1 to 3×10^6 CFU/fly with a mean count of 2.1×10^5 CFU/fly and a median count of 2.4×10^4 CFU/fly and prevalence of *E. coli* O157:H7 was 2.9 and 1.4% in feed bunks and a cattle feed storage shed respectively. This is per below the count in this work and is not directly connected to cattle farm, but a mean of 126.07×10^{-4} and *SD* of ±122.38 and 112.00×10^{-4}, SD of ±75.72 of flies counts from Jakara and Sharada river sampling sites. This could be related to the degree of contamination of the water source, which contains both domestic and waste water from abbortours from the municipality, not only waste water but deposition of fresh septic tank content (sludge). Nazni *et al*. (2005) have isolated *Bacillus* sp., *Coccobacillus* sp., *Staphylococcus* sp., *Microccus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Enterobacter* sp., *Proteus* sp., *Escherichia* sp., *Klebsiella* sp. and yeast cells from feaces, vomitus, external surfaces and internal organs of house fly. This in harmony with our finding of isolating of large number of enterobactriaceae from flies (Table 1) with significant number of pathogenic species (*Salmonella* spp, *Klebsialla spp, Yersinia* spp, *Citrobacter* spp, and *Enterobacter* spp), and *E. coli* O157:H7 living in the same ecological niche, which remain a public health risk to vegetables, whose leaves are the resting places for the scavenging flies and the farmers. Sulaiman *et al.* (2000) isolated eighteen species of bacteria from *M. domestica*, twelve species of bacteria from *M. sorbens*, twelve species from *Chrysomya megacephala* and five species from *Chrysomya rufifacies*. Kuzina and colleagues, (2001) also identified a total of 18 bacterial species belonging to the family

Enterobacteriaceae, Pseudomonadaceae, Vibrionaceae, Micrococcaceae, Deinococcacea, Bacillaceae, and the genus *Listeria*. They found *Enterobacter*, *Providencia*, *Serratia*, and *Staphylococcus* spp. as the most frequently isolated genera. *Bacillus cereus*, *Enterobacter sakazakii*, *Providencia stuartii*, and *Pseudomonas aeruginosa, Aeromonas hydrophila* and *Klebsiella pneumoniae* spp. *pneumoniae* were also identified from *Anastrepha ludens* (Diptera: Tephritidae). This is a large community of microorganisms isolated from same ecological niche with a significant bacterial abundance.

The bacterial abundance (Table 1) also demonstrated a high level of relationship between species of bacteria isolated, and therefore can play important role in the occurrence of *E. coli* O157:H7. Polleya *et al*. (2007) note that Net Biodiversity Effect of mixtures of grasses between perennial grasses and forbs (grass/forbs and grass/grass and forb/forb) is sensitive to effects of species ratios on complementarity. Similarly Kinkel *et al*. (1996) also report that indigenous bacteria enhance the survival of introduced strains, using *Pseudomonas syringae as introduced strain,* this show a positive predictable phenomenon, where the occurrence of particular species of bacteria can influence the growth and survival of another. Lopez-Velasco *et al*. (2011) in a research on the characterization of interaction between with epiphytic bacteria reported a reduction in vitro, between *E. coli* O157:H7 and epihpytic bacterial on spinach leaf surface as opposed to symbiotic relationship.

Hence, a model was calculated to test the occurrence of serologically positive isolates in relation to other enterobacteriaceae isolated from flies. We found good model (r = 516 (51.6%), {F (3, 28) = 3.393}, P= .032) it suggest frequent isolation of *Citrobacter* spp and *Klebsiella* spp as indicator of indicator of the presence of Latex agglutination (for *E. coli* 0157:H7) positive isolates. *Citrobacter* show the highest percentage significance contribution of B .492, at P = .016 and *Klebsiella* species B. 402, P = .029 (Figure I and II). These indicate the possibility for the occurrence of *E. coli* O157:H7 in particular ecological environment may be seriously influenced by the presence and number of certain enterobacterial species in the community. Since, some strains of *Citrobacter freundii* and *Enterobacter* spp. were reported to produce *Stx*2 toxin and contain *stx2* gene with high homology to those found in *E. coli* (Nataro and Kepper, 1998). And interactions with native microbial flora could influence the survival and establishment of immigrant bacteria and their persistence after post-harvest operations (Nataro and Kepper, 1998).

Kobayashi *et al*. (1999) report a number of flie species capable of transmit EHEC O157. Hancock and colleagues (1998) isolated the bacterium from dairy farms and from *Stomoxys*

calcitrans (stable fly) by Heuvelink *et al*. (1998) on Dutch dairy farms. Iwasa and colleagues (1999) reported five flies positive for cultures of 310 collected from four farms. In this work we report the highest prevalence of 70.97% ever of *E. coli* 0157:H7 in flies as compared with some previous works (Alam and Zurek 2004; Hancock *et al*. 1998 and Kobayashi *et al*. 1999). This result may include latex positive *E. coli* which are not 0157:H7 like *E. hermanii, E. vuneris* and *E. fergusonii*, that are biochemically and serologically similar to *E. coli* O157 but can be distinguished by cellobiose fermentation and growth in the presence of potassium cyanide (*E. coli* is negative for both, and *E. hermanii* is positive for both) Nataro and Kepper (1998). Talley *et al.* (2009) noted the important role of flies in dissemination of EHEC O157 by their ability to transmit contamination from one spinach plant to another.

Conclusion

This study have demonstrated a high level of prevalence of Enteroheamohrragic *E. coli* and other entero-pathogenic enterobacteriaceae including the virulent *E. coli* O157:H7 strain, in house fly, scavenging around waste water river used for irrigation purposes. This development is further strengthening various previous findings on the potentiality of fly in the transmission of pathogenic bacteria to un-infected surfaces and bodies. And always biochemical screening should be attached to the isolation and identification of *E. coli* O157:H7 to avoid false positive interpretations, since the occurrences of other enterobactriaceae could influence the presence of latex positive isolates that are not *coli* O157:H7 in a particular ecosystem and isolation on CT-SMAC increased the selectivity and decreased (not inhibit) growth of non-O157 organisms.

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