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Flies and water as reservoirs for bacterial enteropathogens in urban and rural areas in and around Lahore, Pakistan

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SUMMARY

The study was conducted to isolate and characterize campylobacter, enterotoxigenic *Escherichia coli*-labile toxin (ETEC-LT), shigella and salmonella in flies and water.

The material for the study, flies ($n = 300$) and water samples ($n = 148$), was collected from different localities in and around Lahore, Pakistan. Cultivation of the samples was performed on conventional standard media. Membrane filtration technique was used for water prior to culture. Determination of ETEC-LT was done by GM1 ELISA.

Results of our study showed that flies and water were reservoirs for all the four pathogens, campylobacter, ETEC-LT, shigella and salmonella. Flies from the village were carrying fewer enteropathogens, while water from the village was found to be more contaminated as compared to the city. Campylobacter and ETEC-LT were the most frequently isolated pathogens in both flies and water.

Thus the incidence of diarrhoeal disease in children of developing countries may be decreased by providing plenty of safe drinking water, improving excreta disposal, toilet facilities and giving education in personal hygiene.

INTRODUCTION

Diarrhoeal disease constitutes a major health problem and is recognized to be the cause of high morbidity and mortality rates among children in developing countries [1–4]. Environment plays an important role in the transmission of enteric pathogens. Human beings, water, food, insects, domestic and wild animals are known to be reservoirs for the most common and classical enteropathogens [1, 5–8].

In many developing countries flies are in abundance, water is scarcely available and is contaminated by faecal matter due to a defective and ill-planned water supply system. There is indiscreet disposal of excreta, insufficient toilet facilities and lack of knowledge in personal hygiene [9–13].

Children of the four selected areas in this study were included in a longitudinal study for growth and development, followed from birth up to the age of 5 years [14], where diarrhoea was considered as one of the determinant factors of health,

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growth and development [15]. Our study was aimed at the isolation and characterization of campylobacter, enterotoxigenic *Escherichia coli* (ETEC-LT), shigella and salmonella in flies and water from different localities, from main water supplies, hospitals, market places, river and canal in and around Lahore, Pakistan.

MATERIALS AND METHODS

Area

Two areas were chosen for the study, an urban, Lahore, and a rural, Halloki village. Lahore is the second largest city of Pakistan, having a population of more than four million. It is located at the left bank of the river Ravi, which passes through a major part of the city. A prospectively followed population representing four levels of urbanization [14] was selected; a village, situated in the suburbs of Lahore, about 40 km from the centre of the city, a periurban slum, a mud hut area situated at the periphery of the main city, an urban slum area which is located in the centre of the city, and an upper middle class (UM class) area, scattered around the centre of Lahore. The socio-economic characteristics, level of education and standard of living of the four areas are shown in Table 1 and have been described in detail elsewhere [14, 16, 17].

Five major hospitals located in the areas selected were also included in the study. The density of flies was high in the wards of the hospitals at the time of the study. The water supply system of some of the hospitals investigated was more than 70–80 years old.

Samples were obtained from restaurants, road-side barbecue shops, sweet candy shops, butchers, hawkers and vendors selling fruit and various kinds of food. In the market places domestic animals are present and horse-drawn carriages are used for transport. There are uncovered refuse dumps; human and animal excreta are scattered on the roads. The shops and the food sold on open trollies are not protected against flies and pollution.

The main river and canal which run through most parts of Lahore are used by humans and animals for swimming, bathing and washing purposes. Garbage and excreta are frequently thrown in the river.

Flies ($n = 300$) and water ($n = 148$) samples were simultaneously collected during two phases of the year, phase 1, hot and dry (April to June), and phase 2, hot and humid (July and September), the rainy monsoon season. Water was obtained from different sources such as wells, water taps, hand pumps, storage tanks, utensils and from various points in the river and the canal.

Flies

Flies were *Musca domestica* according to standard criteria by the use of taxonomic keys. House flies were captured either by using a fly killer, which was cleaned between each killing with a disinfectant, or by hanging a special fly tape (Tal fly paper, Walco-Link Corp., Clifton N.J., USA) inside the houses, shops and hospital wards for 3–4 h. Flies were removed with a sterilized forceps and placed in brain–heart-infusion (BHI) broth. They were processed the same day as of the sampling. The tube with flies (two in each tube) having 1 ml BHI-broth, was shaken for 2 min and incubated at 37 °C for 2 h. Later on, inoculation was done

Table. 1 *Distribution of socio-economic status, housing and sanitary conditions in households of the four areas of living included in the study in Lahore, Pakistan* [*16, 17*]

Socio-economic, housing and sanitary measures		Area of living: Village (n = 1000) %	Periurban slum (n = 1005) %	Urban slum (n = 993) %	UM class (n = 240) %
Monthly income	< Rs 1000	74·6	87·4	41·5	0·0
	RS 1000–4000	25·4	12·3	57·7	0·1
	> Rs 4000	0·0	0·3	0·8	99·9
Education					
Mother	Illiterate	93·2	94·6	50·7	0·0
Father	Illiterate	76·7	82·5	35·9	0·0
Rooms/house	Mean	1·5	1·1	2·0	4·7
Persons/room	Mean	5·3	5·7	4·3	1·2
Water supply	Hand pump	87·0	100·0	0·0	0·0
	Well	13·0	0·0	0·0	0·0
Tap	Own	0·0	0·0	10·0	100·0
	Shared	0·0	0·0	52·0	0·0
	Community	0·0	0·0	38·0	0·0
Lavatories	Open areas	100·0	100·0	0·0	0·0
	Share	0·0	0·0	30·0	0·0
	Private	0·0	0·0	70·0	100·0
Closed sewage system	—	0·3	0·5	7·1	100·0
Garbage disposal	Inside house	6·0	94·0	0·0	0·0
	Outside	94·0	6·0	30·0	20·0
	Refuse dump	0·0	0·0	70·0	80·0
Domestic animals	Transport	20·0	5·0	1·0	1·0
	Big dairy	44·0	3·0	1·0	2·0
	Small dairy	12·0	4·0	1·0	0·0
	Poultry	24·0	13·0	3·0	4·0
Flies	—	59·0	84·0	9·0	1·0
Surroundings hygiene	Dirty	54·0	81·0	19·0	1·0

with 0·1 ml/plate on Endo-agar for *E. coli* (Mast), deoxycholate citrate (DC) agar for shigella (Mast) brilliant green agar for salmonella (Oxoid), and on blood-free selective (BFS) agar for campylobacter (Oxoid). The remaining BHI-fly-fluid was equally divided into two tubes, one filled up with 4 ml of Rappaport broth (Merck) for enrichment of salmonella and one with 4 ml for enrichment of campylobacter [18].

The direct cultivations of Endo and DC agar, were analysed after 24 h at 37 °C and on BFS agar after 48–96 h in microaerobic atmosphere in a candle jar at 42 °C. After enrichment (37 °C for 24 h), cultivation of salmonella was done on brilliant green agar and incubated for 24 h at 37 °C. The enriched samples for campylobacter were incubated microaerobically for 24 h at 42 °C and thereby subcultivated on BFS agar for 48–96 h in the same conditions as above.

Water

Water was collected in 100 ml sterilized screw-capped glass bottles. A water sample (50 ml × 2) was withdrawn from the bottle by a sterilized syringe and

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filtered through 0·22 micrometre Millipore filters (×2) [19]. One filter was divided into two pieces with a sterilized scissors and scalpel. One piece was put on a BFS agar plate for cultivation of campylobacter, while the second was put on DC agar for growth of salmonella and shigella. The other filter was divided into three pieces: one piece was put on Endo agar for *E. coli*, one piece into 4 ml Rappaport broth for enrichment of salmonella and the third piece into 4 ml enrichment broth for campylobacter. Filters on agar plates were removed after 60 min and cultivation of broths and agar plates was done as for flies.

Identification

Shigella and salmonella were identified biochemically by triple sugar iron slants (Mast), Simmon's citrate slants (Mast), Phenylalanine slopes and fermentation of sugars.

Confirmation and sero-grouping of shigella was done by using specific antisera and a slide agglutination technique. Polyvalent pool antiserum was used for agglutinating salmonella.

Campylobacter was confirmed by its growth on BFS agar, its oxidase reaction and Gram-staining characteristic.

Six separate lactose-fermenting colonies for each individual sample identified as *E. coli* were selected at random from Endo agar, inoculated on nutrient agar and incubated for 24 h at 37 °C, which was utilized later for the determination of ETEC-LT by GM1 ELISA [20].

Statistical methods

Difference of probabilities was carried out using the two-tailed chi-square (χ^2) and Fisher's exact test.

RESULTS

Results of our study indicated that flies and water were reservoirs for campylobacter, ETEC-LT, shigella and salmonella (Figs 1 and 2).

In water samples from the urban slum we were unable to isolate any of the four enteropathogens (Fig. 2).

Campylobacter. Flies from the periurban slum and the urban slum carried campylobacter more frequently than flies obtained from the village (Fig. 1). Additionally, campylobacter was the most frequently identified enteropathogen in water samples from the city and the village (Fig. 2).

ETEC-LT. Flies collected from all the sampling areas were found to be carrying ETEC-LT (Fig. 1). In water samples ETEC-LT was isolated only from the river and canal and to some extent from the UM class houses (Fig. 2).

Shigella. Flies from the village were carriers of shigella more often as compared to flies from the city, except for flies from the hospitals which carried shigella more frequently (Fig. 1). Water samples from the UM class houses, hospitals, river and canal were contaminated by shigella (Fig. 2).

Salmonella was isolated in flies obtained from most areas of the study, except the urban slum and the hospitals (Fig. 1). Water samples from the periurban slum

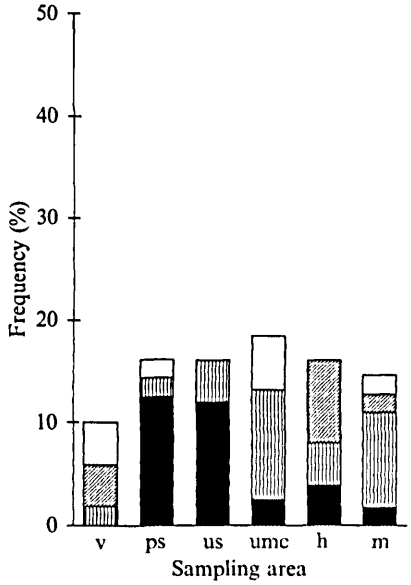

Fig. 1. Frequency (%) of four bacterial enteropathogens in flies ($n = 300$) from different areas in and around Lahore, Pakistan (v = village, $n = 50$; ps = periurban slum, $n = 56$; us = urban slum, $n = 50$; umc = upper middle class, $n = 38$; h = hospitals, $n = 50$; m = markets, $n = 56$). ■, Campylobacter, ▥, ETEC (LT); ▨, shigella; □, salmonella.

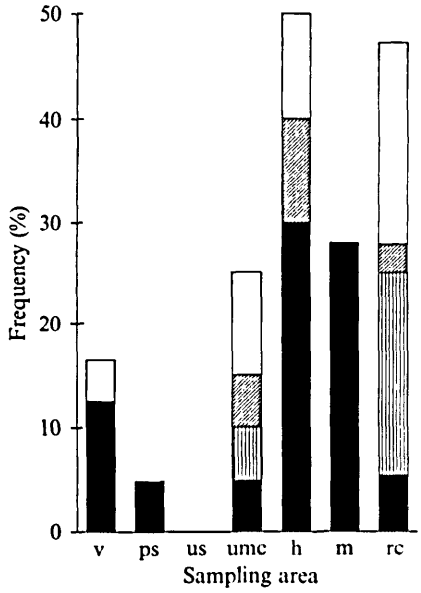

Fig. 2. Frequency (%) of four bacterial enteropathogens in water ($n = 148$) from different areas in and around Lahore, Pakistan (v = village, $n = 24$; ps = periurban slum, $n = 20$; us = urban slum, $n = 20$; umc = upper middle class, $n = 20$; h = hospitals, $n = 10$; m = markets, $n = 18$; rc = river and canal, $n = 36$). ■, Campylobacter, ▥, ETEC (LT); ▨, shigella; □, salmonella.

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and the urban slum were free of salmonella while water from the UM class houses, hospitals, river and canal was often contaminated with salmonella (Fig. 2).

DISCUSSION

This study is the first of its kind in Pakistan, in which flies and water are investigated for common enteropathogens associated with diarrhoea. The results of the present study indicate that flies and water could be reservoirs and vehicles for spreading bacterial enteric agents. In most developing countries the conditions are quite different in rural and urban areas. To elucidate this, two population groups were selected, which fairly reflected environmental conditions that mostly prevailed in the country.

The results showed that campylobacter, shigella and salmonella were found to be equally distributed among flies but the relative frequency of ETEC-LT was comparatively higher. The small inoculum required to cause shigellosis [21] and campylobacteriosis [22] readily enables transmission of infections where hygiene is compromised.

Indiscriminate disposal of human and animal excreta in open spaces was observed in all the three areas of our study [17]. Toilets were non-existent in the village and periurban slum. Open defaecation in the field and inside the houses was common, particularly in very young children. These children seldom use toilets as a habit, which promotes the spread of enteric organisms shed in the faeces of infected persons as for domestic animals.

Flies can become contaminated by dwelling on excreta of human and animal origin, then transmitting enteric agents to food, water, human beings and animals [23]. In Thailand, it was shown that flies collected from refuse dumps in a village and a city carried enteropathogens [8]. Additionally, during the hot and wet season ETEC-LT was isolated from flies when ETEC-LT infections were most frequent among the inhabitants with diarrhoea [12].

Flies collected from garbage dumps have been known to carry salmonella and shigella for up to 20–24 days [24]. The effect of control of houseflies on the incidence of diarrhoea, particularly shigellosis, was evaluated in a prospective intervention study [25].

It is possible that house-flies may function as an epidemiological link between human beings and domestic animals, particularly for campylobacter [26] which remains viable for several days on the body surfaces and in the discharges of the flies after they have been desiccated [27].

Although the environment and living conditions are satisfactory in the houses of families of UM class, ETEC-LT (10·5%) isolated from flies obtained from these houses was frequent. Salmonella (5·3%) and campylobacter (2·6%) were also present. Incidence of diarrhoea in the UM class was reported to be less in Lahore [15], which could probably be associated with specific hygiene measures and child care practices of this class [17]. It appeared to be in agreement with the studies on specific behaviours such as water handling, food handling, hand washing and the importance of their role in promoting the transmission of enteric pathogens [1, 28].

In the hospitals flies were carriers of shigella (8%), campylobacter (4%) and ETEC-LT (4%). No salmonella was isolated.

There could be a relation between fly density and the peak of diarrhoeal incidence in the developing countries [8]. It was observed that large numbers of flies are present throughout the year in all areas of our study, though more in the village and the periurban slum. In cooler months like January and February, the density of flies is comparatively less [17]. In the few studies available, it has been elucidated that the housefly acts as a mechanical vector, transmitting enteropathogens, thus causing diarrhoeal disease [8, 12, 24].

Water samples collected from different sources from the village were found to be harbouring campylobacter (12·5%) and salmonella (4%). In water samples from the periurban slum, only campylobacter (5%) was isolated. The presence of large numbers of domestic animals, type of water supply and excreta disposal in the village and periurban slum may be interlinked. Domestic animals are reported to be reservoirs for campylobacter [7, 29]. Water and food are probably contaminated by campylobacter directly or indirectly through infected animal excreta.

Potable water, especially during heavy rains, has been directly implicated in several outbreaks of campylobacter and traveller's diarrhoea [5, 11, 30]. It has also been found that *Campylobacter jejuni* inoculated into unchlorinated water kept at +4 °C can remain viable for weeks [31]. However, the virulence of campylobacter present in water for causing diarrhoea has still to be established. It has been suggested by Newell and co-workers that environmental campylobacter are non-pathogenic. In their study the water isolates were not as virulent as the clinical isolates [32]. Further investigations are needed to identify the virulence markers for the detection of pathogenic campylobacter in the environment.

For the evaluation of bacteriological quality of water, *E. coli* is generally accepted as an indicator. A water sample is considered contaminated if any *E. coli* or faecal coliform is isolated from it [33]. A high inoculum dose required for *Vibrio cholerae*, salmonella and ETEC (10^9 organisms) infections indicates that a highly contaminated source or sufficient time is needed to achieve such large doses in vehicles of transmission. Consequently, water and food rather than person to person spread are the cause for ETEC and related coliform pathogen infections [34]. ETEC infections require a grossly unsanitary environment for the transmission and diarrhoeal outbreak, which explains that enteritis by enterotoxigenic *E. coli* is rare in countries with generally high water hygiene [1, 10].

In the present study, water supplied in the village and the periurban slum administered by hand pumps was found to be highly contaminated with bacterial enteropathogens probably due to seepage of faecal matter from ground level. In a study from South Africa, it was shown that the incidence of diarrhoea decreased when the quality of water was improved by building deep and enclosed wells and the use of potable water from surface wells was discontinued [35]. A morbidity survey revealed a significant relationship between the sanitary quality of the water supply system of a community and the incidence of gastrointestinal illness [36].

Many bacteriological studies have suggested that level of toilet hygiene might

influence the incidence of enteric illnesses [37]. Heavy surface contamination of water by enteropathogens has been found in areas close to the disposal of excreta, especially in outbreaks of shigellosis [23].

Water from the houses of the urban slum was free of any bacterial enteropathogen as compared to even the water obtained from the houses of UM class which was found to be contaminated with all the four enteropathogens. One possible explanation could be that the source of water supply in the urban slum was through pipes which were not concealed and the drains were open, while in the UM class houses all the plumbing, drainage and septic tanks were laid down under the ground, where any leakage from rusting and damaged pipes could possibly contaminate the water with faecal matter from the sewerage. Tap water was available in the UM class but the water was often collected and stored in overhead/underground tanks which were generally uncovered and easily accessible to birds, dust and insects.

The water from the hospitals was found to be unsafe for human consumption. It was heavily contaminated with campylobacter (30%), shigella (10%) and salmonella (10%). The water supply of most of the hospitals investigated was almost a century old and was probably damaged. It will be worthwhile to study the incidence of diarrhoea in attendants and staff working in the hospitals.

The water samples from river and canal contained all the four types of enteropathogens with a relatively higher frequency of ETEC-LT (19·4%) and salmonella (19·4%) than campylobacter (5·5%) and shigella (2·8%).

In less developed countries the environment is unhygienic, there is overcrowding and the standard of living is poor. Water is scarce, not easily accessible and generally piped water is not available to most people. In many developing countries water is frequently contaminated by human and animal excreta due to a defective and ill-planned water supply system, substandards of sanitation and insufficient facilities for excreta disposal and toilets [36]. Faecally contaminated water can take part in the transmission of enteric agents directly by ingestion, or indirectly due to scarcity of water and insufficient knowledge in personal hygiene [1, 10, 13], which may contribute to an increase in the incidence of diarrhoeal disease.

Availability of clean and safe water should be a basic necessity for every individual in this world but it has become a luxury for people in the third world. From our results we could conclude that flies were carrying most of the enteric agents and water was an important reservoir for enteropathogens in the areas of the study The original sources of water may not be that unsafe but it becomes contaminated after distribution and storage by faecal matter in unhygienic and inadequate sanitary conditions [36]. If a child in a developing country is provided a sanitary environment and plenty of safe water, her/his chances of survival and leading a healthy life could be increased.

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