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Frequency of Bacterial and Viral Infections in the Environment of Different Areas of Karachi

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Abstract
Karachi, the capital of Sindh is the largest city with the health issues caused by air and water pollution, improper sanitation and unhygienic measures. This study focuses on survey the human health issues of bacterial and viral pathogens in different age groups of the male and female patients. A total of 305 and 259 (total 564) different samples of stool and blood of the male and female patients of 1-15; 16-30, 31-45 and 46-60 age groups from Liyari (LY), Baldia Town (BT), Orangi Town (OT), Sachal Goth (SCG), Sohrab Goth (SBG) and Korangi Industrial Area (KIA) were collected to determine the occurrence (percentage) of major diseases prevailing in the environment of Karachi. In this study, the bacterial isolates were identified by culturing on Blood agar, MacConkey's agar and Pylori agar for *E.coli, Shigella dysentery* and *Helicobacter pylori* respectively. HBVsAg method was used to detect the presence of antibodies in patients' serum. Our results revealed the greater prevalence of *E. coli / Shigella dysentery* infection in females and males at SCG; *Helicobacter pylori* infection in at BT in males and no infection was observed in females and HBV infection resulted at greater ration in both genders at OT in 1-15 years of age respectfully. The observation of 16-30 years of age groups revealed greater occurrence of *E. coli / Shigella dysentery* SCG in males and in females at LY; *Helicobacter pylori* infection at LY in males and in females at KIA, whereas HBV infection at LY and KIA in males and females, respectively. The age groups 31-45 years showed greater incidence of *E. coli / Shigella dysentery* infection at LY and KIA; *Helicobacter pylori* infection at SCH and OT in females and males, whereas HBV resulted greatly at LY in males and in females at LY and OT, respectively. The findings of *E. coli / Shigella dysentery; Helicobacter pylori* infections revealed the greater incidence at LY, BT, LY, BT in males and females, respectively, whereas the HBV was observed at KIA in both genders of 46-60 years of age groups. These observations are the alarming increase in the risk factors and the dissemination of infections with greater morbidity and mortality rates in Karachi.

Keywords: GIT infections, Hepatitis B, Incidence rate, Environmental microbiology

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Introduction

The environment is a vital source of transmission of the microbial agent, which are the vehicles of disseminating mild to severe diseases in humans [1]. The microorganisms including bacteria, fungi, protozoa and viruses are the natural habitants of the different environments particularly air, soil, water and food. The environment consists of biological and non-biological factor interacting with each other with climate, weather, and natural resources that affect human survival [2]. Humans are sensitive to the climate change that may affect globally; this may endanger humans in terms of

rising in poor air quality, sea level, drought, flooding and temperature [3]. Some of the chemicals are by-products of industrial activity. Apart from the microbial diseases, several chemicals found in the environment affect the human health such as pesticides; the source of in-fertility, cancer, damage DNA, diabetes, and endometriosis, disorders of the respiratory tract, endocrine system, natural immune system, hormone imbalance and the complications of the nervous system. Heavy metals like cadmium, lead and mercury causes intestinal damage, kidney and bone disease and also the digestive problems [4].

In human, the environmental microorganisms show some sequestered activities that explore the threatening risk factor. The poor population and the substance abusers, mentally ill people are exposed to the increased risk due to the changes in climatic factors and also the vector transmission of pathogenic microorganisms. The children of different ages, women at pregnant stages and the elderly age are more sensitive to the environmental pathogens that may be transferred by water, food, air, and vector or by the direct contact [5-6].

WHO reported twenty nine thousand deaths per year in Pakistan due to environment problems such as vehicle emissions, industrialization, emission of greenhouse gases, improper waste management [7-9] sanitation and unhygienic conditions, unhygienic food is also the common sources. Water in Karachi may lead to amoebic dysentery, gastroenteritis, diarrhea [10] and to some extent bacterial dysentery. The aim of this study is to survey the prevalence of bacterial gastrointestinal diseases and hepatitis B infection in male and female population of different age groups at different areas of Karachi.

Materials and Method
Ethical Protocol

This research topic was approved by supervisors and the research work was performed at the Institute of Microbiology and Center for Environmental studies, University of Sindh, Jamshoro. The clinical samples were collected from the patients with the consent of attendants on the regular basis and processed with in the limited time period.

Study Population

This study was based on the detection of gastrointestinal diseases and hepatitis B from various areas of Karachi in the male and female patients of different age groups.

Sampling Technique

A total of 564 (n=305 males and n=259 females) different samples of stool (n=202 males, 161 females) and blood (103 males, 98 females) were collected in sterilized bottles and sterilized syringes of 1-15, 16-30, 31-45 and 46-60 years of age groups, respectively. Sample processing was carried out in three different phases, culturing on laboratory media, microscopy and HBVsAg kit method.

Culture

The laboratory media were prepared according to the Oxoid manual and kept for sterility for 24 at 37°C. Pylori agar plates were kept in the plastic bags to moisten them. The blood samples were inoculated in Bac-Tee bottles and kept in rolling incubator for 7 days. The specimens were also inoculated separately on to the surface of agar plates and incubate at 37°C for 24 h [11]. Modified *H. Pylori* selective media plates composed of Columbia blood agar base, vancomycin, amphotericin b, trimethoprim and polymyxin B were cultured and kept for 5-7 days along with CampyGen CN025 gas generating kit in 10% CO_2 incubator [12-13]. In addition, the medium was supplemented with blood, meat extract, agar, and corn starch [14]. Catalase, oxidase and urease tests for confirmation of *H. pylori* were performed according to the [15-17].

Microscopic Observation

Blood containing stool samples were stained and examined by Gram staining technique. The smears were flooded with crystal violet and a

drop of Gram iodine (primary stain) was mixed to form CVI complex and allowed for 1 min. The slides were then washed with water, air dried and flooded safranin (counter stain) and observed under an oil immersion lens [18]**.**

Detection of HBVsAg

The serum of all blood samples were separated, kept at room temperature and HCV, HBVsAg kits were opened in the room temperature. Added 60μL of serum in the well separately of the test kit and the reading was recorded using timer and allowed for 15 min [19].

Results and Discussion

A total of 564 different samples of stool and blood were collected in sterilized bottles of the male and female patients of different age groups to determine the frequency of major GIT diseases and viral hepatitis B and C in the environment of Karachi in 2017. The number of samples of patients from LY, BT, OT, SCG, SBG and KIA were 113 (37%), 64 (21%), 57 (19%), 72 (24%) and 87 (34%), 67 (26%), 58 (22%), 43 (17%) of 1-15, 16-30, 31-45, 45-60 years (Y) age of male and female patients respectively (Table 1-2).

The population of LY, OT, SCG, SBG and KIA revealed *E. coli / Shigella dysentery; Helicobacter;* HBV infection percentage 46, 33; 0, 0; 9, 0% (LY), 39, 36; 5.5, 0; 17, 0% (BT), 29, 54,; 0, 0; 18, 13% (OT), 73, 67; 0, 0; 0,8% (SBG) and 33, 18; 5.5, 11; 22, 18% (KIA) of 1-15 years. (Table 3). The results of 16-30 years of both genders showed 12, 40; 37.5, 0; 25, 0 % (LY), 44, 17; 0, 8; 22, 17% (BT), 20, 31.5; 30, 5, 20, 10.5 % (OT), 41, 31, 12, 8; 18, 8% (SCG), 55.5, 25; 0,1; 22, 12.5% (SCG), 18, 0; 0, 20, 18, 20% (KIA) in male and female population, respectively (Table 4). The patients of 31-45 years showed 30, 33; 20, 13; 30, 27% (LY), 8, 0; 17, 0; 25, 17% (BT), 0, 27; 20, 18; 20, 27% (OT), 23, 0; 8, 25; 15, 25% (SCG), 9, 19; 9, 12.5; 27, 25% (SBG); 67, 25; 17, 12.5; 17, 25% (KIA), respectively (Table 5). The age groups of 46-60 years of male and females revealed 45, 33; 27, 0; 18, 17% (LY), 27, 44; 13, 22; 13, 11 % (BT), 18, 29; 9, 0; 18, 14% (OT), 36, 25; 0, 12.5; 9, 25% (SCG), 11, 33; 11, 0; 22, 17%

(SBG) and 33, 28.5; 20, 0; 27, 28.5% (KIA), respectively (Table 6).

Area wise analysis showed the high incidence rate of bacterial and viral infections 93% (LY), 92% (BT) in females, 78% (OT), 91% (SCG) in males, 89% (SBG) in females and 93% (KIA) in males of 1-15 years of age, whereas 87.5% (LY), 80% (BT) in males, 76.4% (OT), 69.2% (SCG) in females, 80% (SBG) and 79% (KIA) in males of 16-30 years. The population of 31-45 years showed 80% (LY), 87% (BT), 67% (OT) in males, 75% (SCG), 83% (SBG) in females and 90% (KIA) in males and the age groups of 46-60 years. The higher rate of infections resulted 91% (LY) in males, 87.5% (BT) in females, 73% (OT), 78 (SCH) in males, 87% (SBG) in females and 71% (KIAS) in both genders (Table 7).

Cultural characters revealed colorless, non-lactose-fermenting, convex colonies on SS agar and smooth, colorless colonies on MacConkey's agar of *Shigella dysentery*, Medium to large, raised, pink, smooth, halo, colonies of *E.coli* on MacConkey's agar whereas small, shiny and to some extent pale grayish confluent growth of *H. pylori* on modified *H. pylori* selective medium and they were confirmed by catalase, oxidase and urease positive with bubble formation, purple discoloration on filter paper and appearance of pink color in urea agar respectively. Morphologically the *Shigella dysentery* appeared as short rods, G-ve cells and the *E. coli* showed short to medium sized oval shape, G-ve cells under oil immersion object. *H. pylori* cells appear straight rods after 24 hours but also appear pleomorphic, irregular curved rods when incubated for 72 h in 10% CO_2 incubator.

The findings revealed the prevalence of gastrointestinal tract bacterial infection in different areas of Karachi that may be due to the contaminated water supply and food such as fruits and vegetables, which are usually contaminated by the soil borne microorganisms and fertilization of crop fields with human feces. Many people eat meals in groups with unwashed hands [20] and eating in contaminated papers provided by street food sellers, living standard in polluted areas, lack

of sanitation, food shops near overcrowdings and over traffic areas.

The potent pathogens of bacterial gastrointestinal infections are *E. coli* [21] and *Shigella dysenterie due* to the adhesion molecules and toxins; a heat labile cholera like toxin and heat-stable toxin that act through guanylate cyclase [22-23]. *Shigella* is commonly found in fecal contaminated water [21] and produce shiga toxin that have cytotoxic effect on human intestine to cause dysentery [22-23]. In our findings the higher incidence occurred in all age groups with some exceptions of *Helicobacter pylori* infection. This may be due to the fact that mostly gastroenteritis occurs by eating at school canteens, low quality restaurants, picnic spots, and social gatherings through contaminated meat, poultry and dairy products, raw fish, contaminated potable water and also due to unhygienic food preparation, handling, storage, overcrowded areas, sharing house with infected persons and oral-fecal route [19, 24-26] and also the increased pH *in the stomach when the pathogen converts* urea to ammonia by urease enzyme because *H. pylori* have 10-20% lifetime risks of developing peptic ulcers [27].

The hepatitis B virus (HBV) infection is an alarming threat worldwide. The WHO reported this infection in two billion people with 600,000 deaths per annum. In Pakistan, 2.5% HBV infection is reported in general, whereas 6.3, 5.9, 4.3 and 4 percent in Khairpur, Ghotki Larkana and Noshero Feroze, prospectively. Our work in various areas of Karachi revealed the greater percentage of HBV infection varies among the male and female populations of 1-15, 16-30, 31-45, and 46-60 all areas. This incidence rate of HBV may be due to the poverty and over population, unawareness, refuse of health care, gender discrimination and vulnerability of the environmental pollution. This observation is in accordance to [28]. Colonial characters, morphological and biochemical characters, HBVsAg test and microscopic observations of parasites, according to the author's instructions confirmed the presence of clinical isolates in male and female patients of different age groups [15-17].

Table 1. **Total number of samples collected from male patients of different age groups various sites of Karachi.**

Age	Liyari	Baldia Town	Orangi Town	Sachal goth	Sohrab Goth	Korangi Industrial Area	Total samples collected	Percentage of samples (%)
1-15	23	18	17	15	22	18	113	**37**
16-30	08	09	10	09	17	11	64	**21**
31-45	10	12	05	13	11	06	57	**19**
46-60	11	15	11	11	09	15	72	**24**
						Total	**305**	******

Table 2. **Total number of samples collected from female patients of different age groups various sites of Karachi.**

Age	Liyari	Baldia Town	Orangi Town	Sachal goth	Sohrab Goth	Korangi Industrial Area	Total samples collected	Percentage of samples
1-15	13	11	15	12	08	28	87	**34**
16-30	05	12	19	08	13	10	67	**26**
31-45	15	06	11	04	16	08	58	**22**
46-60	06	09	07	08	06	07	43	**17**
						Total	**259**	******

Table 3. **Laboratory investigation and percentage infections from the positive (+ve) cases of male (M) / female (F) patients of 1-15 years of age from various areas of Karachi.**

Pathogens	Liyari (23/13 samples)				Baldia Town (18/11 samples)				Orangi Town (17/15 samples)			
	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%
E.coli / Sh.dysenty	06	33	06	46	07	39	04	36	05	29	08	53
H.pylori	00	00	00	00	01	5.5	00	00	00	00	00	00
HBV	02	9	00	00	03	17	00	00	03	18	02	13
Pathogens	Sachal Goth (15/12 samples)				Sohrab Goth (22/8 samples)				Korangi Industrial area (18/28 samples)			
	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%
E.coli / Sh.dysenty	11	73	08	67	08	36	03	37.5	06	33	05	18
H.pylori	00	00	00	00	01	4.5	00	00	01	5.5	03	11
HBV	00	00	01	8	00	00	00	00	04	22	05	18

Table 4. **Laboratory investigation and percentage infections from the positive (+ve) cases of male (M) / female (F) patients of 16-30 years of age from various areas of Karachi.**

Pathogens	Liyari (8/5 samples)				Baldia Town (9/12 samples)				Orangi Town (10/19 samples)			
	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%
E.coli / Sh.dysenty	01	12.5	02	40	04	44	02	17	02	20	06	31.5
H.pylori	03	37.5	00	00	00	00	01	8	03	30	01	5
HBV	02	25	00	00	02	22	02	17	02	20	02	10.5
Pathogens	Sachal Goth (9/8 samples)				Sohrab goth (17/13 samples)				Korangi Industrial area (11/10 samples)			
	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%
E.coli / Sh.dysenty	05	55.5	02	25	07	41	04	31	02	18	00	00
H.pylori	00	00	01	12.5	02	12	01	08	00	00	02	20
HBV	02	22	01	12.5	03	18	01	08	02	18	02	20

Table 5. **Laboratory investigation and percentage infections from the positive (+ve) cases of male (M) / female (F) patients of 31-45 years of age from various areas of Karachi.**

Pathogens	Liyari (10/15 samples)				Baldia Town (12/06 samples)				Orangi Town (5/11 samples)			
	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%
E.coli / Sh.dysenty	03	30	05	33	01	08	00	00	00	00	03	27
H.pylori	02	20	02	13	02	17	00	00	01	20	02	18
HBV	03	30	04	27	03	25	01	17	01	20	03	27
Pathogens	Sachal Goth (13/4 samples)				Sohrab goth (11/16 samples)				Korangi Industrial area (6/8 samples)			
	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%
E.coli / Sh.dysenty	03	23	00	00	01	09	03	19	02	67	02	25
H.pylori	01	8	01	25	01	9	02	12.5	01	17	01	12.5
HBV	02	15	01	25	03	27	04	25	01	17	02	25

Table 6. **Laboratory investigation and percentage infections from the positive (+ve) cases of male (M) / female (F) patients of 46-60 years of age from various areas of Karachi.**

Pathogens	Liyari (11/6 samples)				Baldia Town (15/9 samples)				Orangi Town (11/7 samples)			
	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%
E.coli / Sh.dysenty	05	45	02	33	04	27	04	44	02	18	02	29
H.pylori	03	27	00	00	02	13	02	22	01	9	00	00
HBV	02	18	02	17	02	13	01	11	02	18	01	14

Pathogens	Sachal Goth (11/8 samples)				Sohrab goth (9/6 samples)				Korangi Industrial area (15/7 samples)			
	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%	+ve cases (M)	%	+ve cases (F)	%
E.coli / Sh.dysenty	04	36	02	25	01	11	02	33	05	33	02	28.5
H.pylori	00	00	01	12.5	01	11	00	00	03	20	00	00
HBV	01	9	02	25	02	22	01	17	04	27	02	28.5

Table 7. Determination of total percentage of all *E.coli / Sh. dysentery, H. pylori,* HBV, infections in male and female patients of different age groups of various areas of Karachi.

Age groups	Gender	LY	BT	OT	SCG	SBG	KIA
1-15	Male	65	77	78	91	76	93
	Female	93	92	77	87.5	89	78.5
16-30	Male	87.5	80	67	67	80	79
	Female	78	62.5	76.4	69.2	54.5	40
31-45	Male	80	87	67	67	60	90
	Female	69.2	50	64	75	83	75
46-60	Male	91	67	73	78	64	71
	Female	73	87.5	55	50	87	71

Conclusion

During the survey of the environmental diseases in 2017, it is concluded that the poverty is the main cause of the transmission of diseases, the population of Liyari, Baldia Town, Orangi Town, Sachal Goth, Sohrab Goth, and Korangi Industrial Area are surviving in the low standard environment. The air, water, and food supply is substandard and also the pollution caused by industrialization, local transport could be the significant reasons of prevailing diseases caused by environmental pathogens. It is concluded that the greater incidence of the diseases occurred in various areas of Karachi in males as compared to the females (M > F) of 1-15, 16-30, 31-45 and 46-60 years of age ranging from 93% to 78% (as per samples collected), respectively. It is also concluded that the test percentage is a threat of dissemination of other environmental diseases such as tuberculosis, whooping cough, cancer and other diseases due to the weak immune system of the residents.

It is suggested that the public sector and the private sector should take initiatives for better water supply, hygienic foods, and sanitary measures. The poor population may be little educated for their own preventive measures and personal hygiene. Street hawkers and low standard hotels, shops may be monitored for quality standard.

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