REPORT

Prevalence of shigellosis in three different areas of Karachi

Tasneem Qureshi, Aftab Saeed and Khan Usmanghani*
Department of Basic Clinical Sciences, Faculty of Eastern Medicine, Hamdard University, Karachi, Pakistan

Abstract: Shigellosis is communicated when the pathogen is swallowed down through the factors like stool or polluted hands with dust. Inappropriate hygienic conditions may reach such clinical manifestations. Shigellosis is frequently originated in crowded places and where hygienic conditions are insufficient and sanitation is poor. Infections easily transmissible from person to person like family members or infected playmates. The disease can easily transmit through infected food handlers with inappropriate hygienic conditions, crops nurturing in contaminated water with sewage line system, swimming in contaminated pool water. Similarly houseflies can also form an important vector for its transmission. In this study, Ecological factors were also the causative indicators for the disease. The issue was the water contamination of the different areas of Karachi mainly the Pehlwan Goth, Safora Goth. Then another area we focused was Patel Para in which *Salmonella* species were present. Other indicator was the individual-to-individual transfer that was common in Safora Goth.

Keywords: Shigellosis, contaminated water, prevalence, Karachi.

INTRODUCTION

Shigella gram negative organism related to the family Entero-bacteriacae embrace subdivisions as: *Shigella flexneri, Shigella sonnei, Shigella dysenteriae and Shigella boydii*. The main focus is the gastrointestinal tract causing bacillary dysentery, severity depends on the multiple serotypes consisting of carbohydrate antigen mainly gram-negative lipopolysaccharides responsible for infection (Linderberg *et al*., 1991).

Nevertheless, remarkably lethal effects are produced by *Shigella dysenteriae* releasing shiga toxin that can result in epidemics while other species namely *Shigella flexneri* and *Shigella sonnei* are supposed to be labeled as causing endemic infections (Dupont *et al*., 1989).

Clinical manifestations range from mild to severe dysentery moreover, the disease accompanies wide variety of symptoms including abdominal pain and cramps, fever, tenesmus, nausea and anorexia. Diagnostic stool examination give a clear picture of presence of pus cells and red cells indicating bloody mucoid stool. This reflects the invasion of pathogens towards colon and rectal regions eliciting the inflammatory process, leading to immense mucosal devastation (Kotloff *et al*., 1999).

Pathophysiology

Shigellosis is referred to as the foremost communal health dilemma especially in developing countries related to prevailing hygienic conditions. Humans are the innate reservoir, even though other primate may be infected. *Shigella* spp. infection mainly occurs as a result of contamination, infection can occur by ingesting as few 10 *Shigella dysenteriae* bacilli whereas 100-200 bacilli are needed for *Shigella sonnei* or *Shigella flexneri*. The means of spread incorporate intake of contaminated food or water contact with a contaminated inanimate object, and certain modes of sexual contact. Houseflies play an important role in the transmission of infected faeces. Shigellosis, the disease is sometimes self-limiting comprising twelve hours to minimum four days or seven days for recovery. The individual after this period continuously sheds the pathogen in the stool and the infection is considered to be transmissible. *Shigella* species assume to survive the acidic pH of the stomach, that's the reason the infection is enormous even with considerable low pathogen count. After accessing the gastric epithelial lining the organism initiates the inflammatory processes including infiltration of defensive cells, apoptosis with advance obliteration turning to further invasion that present the picture of mucosal edema, erythematic, friability, ulceration, and mucosal hemorrhage.

Other microscopic findings include accumulation of infiltrative cells in lamina propria and crypt abscess formation, further invasion through M cells overlying the lymphoid nodules causing cell death leading to dysenteric symptoms (Phalipon *et al*., 2007).

Geographical distribution

Shigella is extremely acclimatized to human, being the only known natural host. The least infective measured quantity is fewer than about 200 cells; assist spread in

**Corresponding author:* e-mail: ugk_2005@yahoo.com

regions where there are multitude packed in population, inadequate sanitation in addition to pitiable hygiene (Levine *et al*., 1991; DuPont *et al*., 1989).

In developing countries shigellosis is referred to as primarily a pediatric disease, with the urban areas to be more decisively effected (Keusch *et al*., 1989; Pickering *et al*., 1986).

Besides the high risk factors, shigellosis as a result of food contamination appears in low risk individuals. Other than sudden climatic change such as monsoon season that result in changes like faecal contamination of drinking water (Hossain *et al*., 1990).

Musca domestica, a housefly with low inoculum, is a vector for the spread of Shigellosis (Levine *et al*., 1991).

The ecological allocation and the pathogenesis of the four species of *Shigella* is diverse, the rationale for this are still uncertain (Keusch *et al*., 1989).

In emerging states *Shigella flexneri* causes high rates of death. Whereas, less infections occurs by *Shigella dysenteriae* as epidemics. In developed states, the predominant species is *Shigella sonnei* which is less severe *(*Acheson *et al*., 1995). Ailments with *Shigella* protect against subsequent infection with the same serotype however, because there are multiple serotypes, individuals may become infected several times.

One of the reports regarding Centre for Disease Control (CDC) (full name?) evaluated that occurrence of *Shigella* infections was 1,780; 3.8 per 100,000 population in 2010 (CDC 2011).

Shigellosis mostly occurs during the summer season. *Shigella sonnei* is prevalent 78% of the incidences while remaining due to *Shigella flexneri* and *Shigella boydii*, 18% of *Shigella flexneri* occurs in the United States.

Shigella dysenteriae is considered to be uncommon in the United States. The peak occurrence per 100,000 populations for shigellosis (27.77 cases) was between children less than 5 years.

In general, the pace of hemolytic uremic syndrome (HUS) in children less than 15 years is nearly 0.49 cases per 100,000 population while frequency of *Shigella* infection rate is found to be 4.67 cases per 100,000 population. It is predictable that yearly occurrence of *Shigella* infection did not altered widely since three years in contrast to 2004-2006 (Bishop *et al*., 2008). Furthermore 95% of *Shigella* infections may possibly be asymptomatic; therefore, the definite incident may be 20 times advanced is documented. Each year, Center for Disease Control (CDC) estimation shows that 450,000 total cases of shigellosis take place in the United States. In February 2010, a *Shigella sonnei* foremost epidemic is documented from Illinois. In general, statistics reveal that the occurrence of shigellosis is predictable to be 164.7 million cases per year, of which 163.2 million were in developing countries, where 1.1 million deaths occurred. Statistics illustrates that occurrence of *Shigella* infections are twenty times higher in developing countries. It is also recorded that nearly 60% of incidence and 61% deaths accounts for shigellosis in children less than five years of age. Even though the relative significance of a range of strains causing the disease is imprecise, a probable prediction is 30% of these infections result due to *Shigella dysenteriae.* In one study it is illustrated that out of 56,958 individuals 2,927 were suffering from diarrhoeal disease in Bangladesh, China, Pakistan, Indonesia, Vietnam, and Thailand between 2000-2004. It was also highlighted that *Shigella* incidence increased after 40 years of age. *Shigella flexneri* was the most commonly isolated *Shigella* species in all above countries except in Thailand, where *Shigella sonnei* was most recurrently detected. It was assumed that *Shigella flexneri* serotypes were exceedingly diverse in nature varying from site to site as well as annually. The vast numbers of *Shigella flexneri* isolates were found resistant to amoxicillin and co-trimoxazole. Ciprofloxacin-resistant *Shigella flexneri* isolates were identified in China 6%, Pakistan 3% and Vietnam 2%. On the whole, it is illustrated that Shigellosis rates of the population in adults as well as in children less than five years in Bangladesh were statistically significantly higher than the shigellosis rates in China, Pakistan and Indonesia which in turn were significantly higher than those in the two countries with the lowest shigellosis rates, Vietnam and Thailand (Von Seidlein *et al*., 2006).

Shigellosis can be treated with alternate therapies as the multidrug resistance is increasing further research studies are required to investigate the efficacy of other formulations.

The aim of this investigation was to determine the efficacy of herbal formulation ShigelDysent (test group) to be as effective as Ciprofloxacin (control group).

MATERIALS AND METHODS

Experimental design

It is a Comparative study to determine the efficacy of herbal formulation ShigelDysent (test group) to be as effective as ciprofloxacin (control group).

Study population

The study was carried out among patients of age group between 25-45 years from 2010-2012. The study population consists of 250 patients fulfilling the criteria of the project, 125 each for a control group and test group residing in areas comprising of all socioeconomic classes, especially the lower class. Area of Karachi/patients Our

study was restricted for the cases suffering from shigellosis, for that purpose we emphasized on the areas having inadequate sanitary conditions in addition there was water contamination resulted in increased chance of revealing *Shigella* species. Two areas Pehlwan Goth and Safoora Goth were targeted for our study, as individual-to-individual spread is also one of the important causative factor. When we later on investigated other areas of Karachi like Patel Para to further investigate *Shigella* prevalence we in turn, found *Salmonella species* prevalent in stool specimens, which we also included in the data.

Patients age profile

Age group has been divided into 4 intervals 25-29 years, 30-35 years, 36-40 years, 41-45 years, cumulative percent of both test and control groups are mentioned below:
Patients of the age group 36-40 years were mostly effected in this study. In both test and control group the female/male ratio was high as it is shown below.

Drugs

The selected drugs for bacillary dysentery were purchased from the local market at Jodia Bazar. All the drugs were examined for their impurities and adulterations in Pharmacy of Hamdard Al-Majeed College of Eastern Medicine, Hamdard University and were identified and authenticated by Dr. Iqbal Azhar, Chairman, Department of Pharmacognosy, University of Karachi.

Dosage

Two ShigelDysent capsules three times per day for test group while one 500mg Ciprofloxacin capsules three times per day for control group.

At the premises of the prevalent area according to the feasibility of our clinic faecal specimens were collected for the investigation, as a basic tool for diagnosis. Maximum number of samples were collected in the temporary made sample test room, some were advised to give sample at the Mother Care Hospital lab depending on the feasibility, as all the tests conducted without any charges from the participant that were barred by the investigator. For the investigation portion of the specimen especially that contains mucus, blood was collected in a clean, dry leak proof container. The colour of the specimen, consistency of the specimen whether it is formed, unformed, semisoft was noted for documentation. Delayed specimens were carried in Cary-Blair medium.

Stool culture report has been categorized as *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii* and *salmonella spp*., number of species been calculated with cumulative percent in either group.

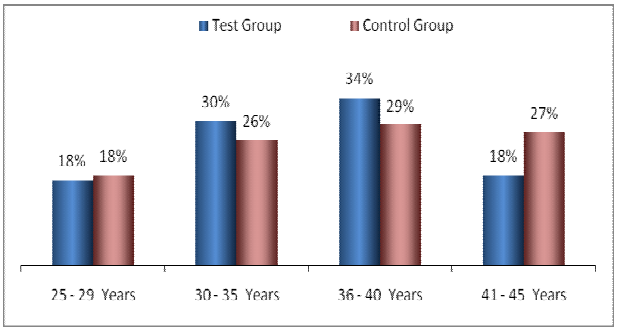
STATISTICAL ANALYSIS

This data has been analyzed by using ANOVA test, applied to conclude the prevalence of *Shigella* species in some areas of Karachi.

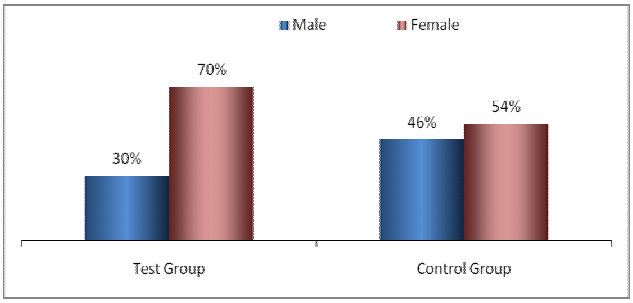
RESULTS

Observations

During the study we come across the main factor responsible for the spread of bacillary dysentery (shigellosis) as under:



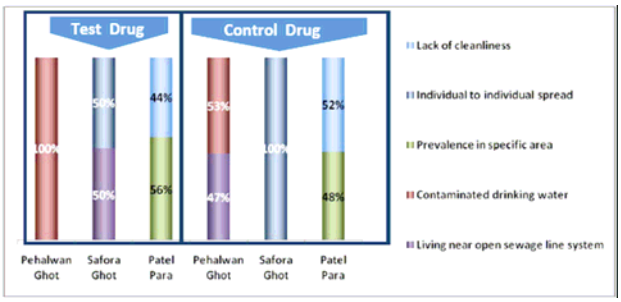
Graph 1: Percentage of frequency in different age intervals between test and control groups



Graph 2: Patient Gender Profile indicating percentage of prevalence

Ecological factors

In this study, Ecological factors were also the causative indicators for the disease. The issue was the water contamination of the different areas of Karachi mainly the Pehlwan Goth and Safora Goth. Then another area we focused on was Patel Para in which salmonella species were present. Other indicator was the individual-to-individual transfer that was common in Safora Goth due to adjacent infected area that is Pehlwan Goth. According to the research protocol specifically designed for shigellosis, patients were randomly selected through the OPD and camps in that premises.



Graph 3: Representing percentage ecological factors involved in the spread of disease

Table 1: Frequency in different age intervals between test and control groups

Age group	Test Group Frequency			Control Group Frequency		
	Frequency	Percent	Cumulative Percent	Frequency	Percent	Cumulative Percent
25-29 years	22	17.6	17.6	23	18.4	18.4
30-35 years	37	29.6	47.2	32	25.6	44.0
36-40 years	43	34.4	81.6	36	28.8	72.8
41-45 years	23	18.4	100	34	27.2	100
Total	125	100		125	100	

Table 2: Patients gender profile

Gender	Test Group	Control Group
Male	30%	46%
Female	70%	54%

Table 3: Percentage of different ecological factors involved in the spread of disease

Ecological Factors	Test Group			Control Group		
	Pehlwan Goth	Safora Goth	Patel Para	Pehlwan Goth	Safora Goth	Patel Para
Living near open sewage line system	--	50%	--	47%	--	--
Contaminated drinking water	100%	--	--	53%	--	--
Prevalence in specific area	--	--	56%	--	--	48%
Individual to individual spread	--	50%	--	--	100%	--
Lack of cleanliness	--	--	44%	--	--	52%
Number of patient	34	66	25	34	66	25

Table 4a: Frequency of *Shigella* and *Salmonella species* in test and control groups

Pre Treatment	Test Group Frequency			Control Group Frequency		
	Frequency	Percent	Cumulative Percent	Frequency	Percent	Cumulative Percent
Shigella sonnei	47	37.6	37.6	49	39.2	39.2
Shigella flexneri	41	32.8	70.4	33	26.4	65.6
Shigella boydii	12	9.6	80.0	18	14.4	80.0
Salmonella spp.	25	20.0	100	25	20.0	100
Total	125	100		125	100	

Table 4b: Age group related prevalence of infection by *Shigella* and *Salmonella*

Organisms	Prevalence of Infection			
	25-29yrs.	30-35yrs.	36-40yrs.	41-45yrs.
Shigella sonnei	0	40	37	19
Shigella flexneri	0	27	14	33
Shigella boydii	0	2	28	0
Salmonella spp.	45	0	0	5
Total	45	69	79	57

Table 5: Prevalence of *Shigella species* related to age intervals

Descriptive								
Stool Culture								
AGE	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
25	9	4.00	.000	.000	4.00	4.00	4	4
26	10	4.00	.000	.000	4.00	4.00	4	4
27	6	4.00	.000	.000	4.00	4.00	4	4
28	12	4.00	.000	.000	4.00	4.00	4	4
29	8	4.00	.000	.000	4.00	4.00	4	4
30	12	1.50	.674	.195	1.07	1.93	1	3
31	9	1.56	.527	.176	1.15	1.96	1	2
32	11	1.55	.688	.207	1.08	2.01	1	3
33	13	1.31	.480	.133	1.02	1.60	1	2
34	13	1.46	.519	.144	1.15	1.78	1	2
35	11	1.36	.505	.152	1.02	1.70	1	2
36	20	1.90	.968	.216	1.45	2.35	1	3
37	18	1.72	.895	.211	1.28	2.17	1	3
38	16	1.88	.885	.221	1.40	2.35	1	3
39	12	2.00	.953	.275	1.39	2.61	1	3
40	13	2.00	.913	.253	1.45	2.55	1	3
41	15	1.87	.743	.192	1.46	2.28	1	4
42	13	1.69	.480	.133	1.40	1.98	1	2
43	12	2.00	1.044	.302	1.34	2.66	1	4
44	10	2.00	1.155	.365	1.17	2.83	1	4
45	7	1.57	.535	.202	1.08	2.07	1	2
Total	250	2.14	1.136	.072	1.99	2.28	1	4

ANOVA

Stool Culture					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	201.503	20	10.075	19.247	.000
Within Groups	119.873	229	.523		
Total	321.376	249			

Stool culture: Prevalence of *Shigella* species, mean, standard deviation, Standard error

Table 5a: Gender Stool Culture Cross tabulation

Count						
		Stool Culture				Total
		Shigella sonnei	*Shigella flexneri*	*Shigella boydii*	*Salmonella* spp.	
Sex	Male	29	30	11	25	95
	Female	67	44	19	25	155
Total		96	74	30	50	250

Table 5b: Test of significance showing gender difference

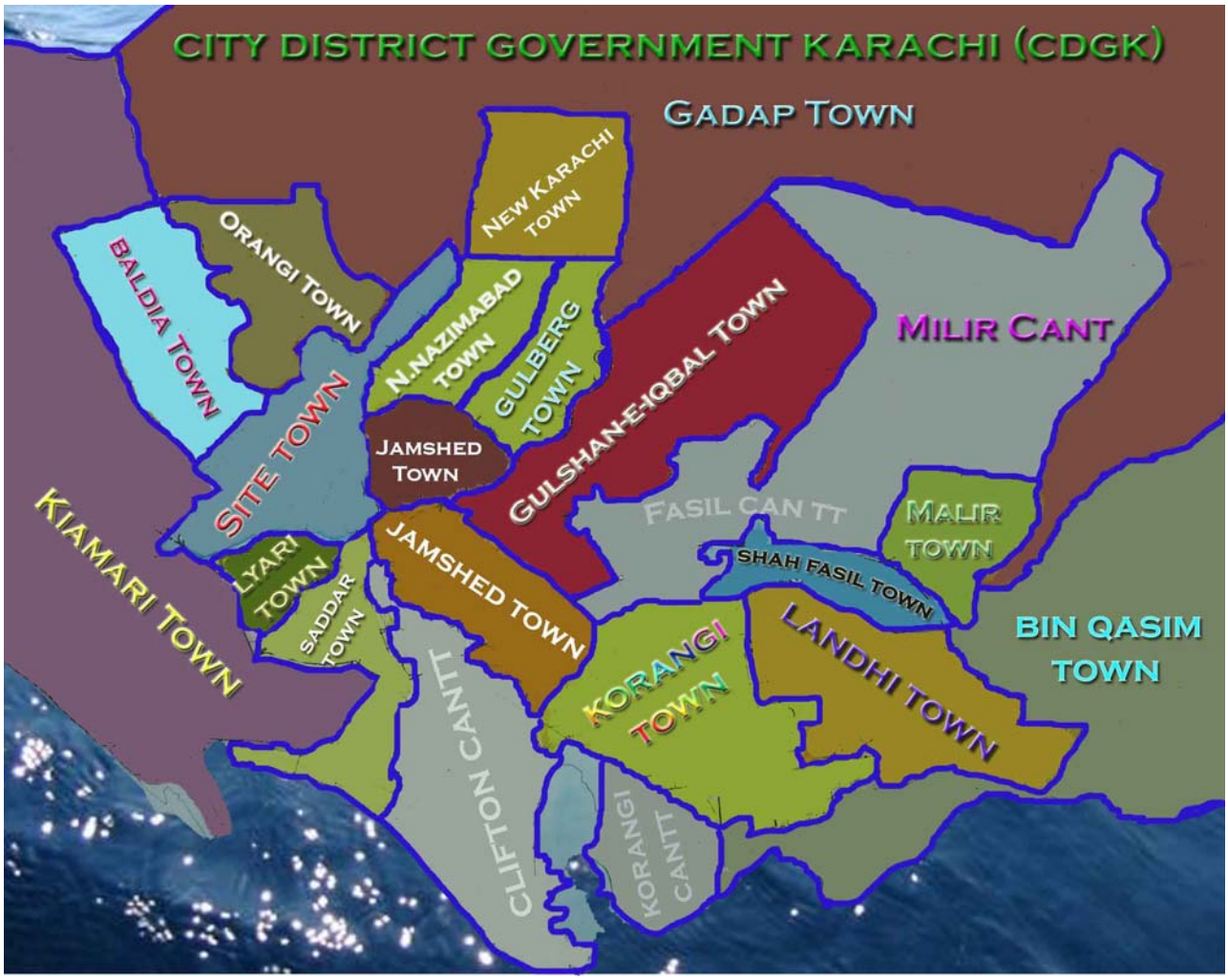
Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.755[a]	3	.124
Likelihood Ratio	5.744	3	.125
Linear-by-Linear Association	4.789	1	.029
N of Valid Cases	250		
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.40.			

Table 6a: Mean of the prevalence of infection depending on gender

Group Statistics					
	Sex	N	Mean	Std. Deviation	Std. Error Mean
Stool Culture	Male	95	2.34	1.172	.120
	Female	155	2.01	1.099	.088

Table 6b: Frequency of infection by test of significance

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% confidence Interval of the Difference	
									Lower	Upper
Stool Culture	Equal variances assumed	3.317	.070	2.205	248	.028	.324	.147	.035	.613
	Equal variances not assumed			2.171	189.02	.031	.324	.149	.030	.618



Stool culture

Stool culture report has been categorized as *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii* and *Salmonella spp*., number of species been calculated with cumulative percent in either group.

Stool culture was the basic diagnostic tool that was applied for the confirmation of the *Shigella* species, in our study the most prevalent species was *Shigella sonnei* that was found in around (96) number of cases, *Shigella flexneri* in about (74) cases, *Shigella boydii* in about (30) cases whereas in one of the areas we found (50) cases of *Salmonella species* effecting gastro-intestinal tract.

The highest number (40) of *Shigella sonnei* was found in age interval 30-35 years, highest number (33) of *Shigella flexneri* was found in 41-45 years of age group whereas for *Shigella boydii* the highest number (28) was in age interval 36-40 years. For *Salmonella species* the only and highest number (45) was the age group 25-29 years.

Difference between male and female in prevalence of infection

According to different age groups *Shigella* infection was found to be prevalent in areas of Karachi due to drinking water contamination. Prevalence of Shigella species found to be significant with (p-value 0.000 or p<0.0001?) in Pehlwan and Safoora Goth of Karachi with exclusion of Patel Para that was free of shigellosis. Statistics become also relevant when other city parts of Karachi are free of Shigellosis.

DISCUSSION

The epidemiological and antibiotic resistance regarding *Shigella* species is altered as the passage of time. The study was undertaken to evaluate the shigellosis cases and to establish an appropriate effectual treatment for shigellosis. The present study highlights that shigellosis can occur at any stage of life. In the present study the clinical findings reveal that patients were suffering from dysentery comprising of blood and mucus. In addition with abdominal cramps/pain, tenesmus, nausea and anorexia. Few cases of dehydration is also seen, although no major complications were seen.

In the present study the predominate species was found to be *Shigella sonnei* due to contaminated drinking water, then *Shigella flexneri* and lastly *Shigella boydii*.

Stool culture was the basic diagnostic tool that was applied for the confirmation of the *Shigella* species, in our study the most prevalent species was *Shigella sonnei* that was found in 96 number of cases, *Shigella flexneri* in 74 cases, *Shigella boydii* in 30 cases whereas in one of the areas patel para (which one?) we found 50 cases of *Salmonella species* effecting the gastro-intestinal tract.

The highest range (40) of *Shigella sonnei* was found in age interval 30-35 years, highest number (33) of *Shigella flexneri* was found in 41-45 years of age group whereas for *Shigella boydii* the highest range (28) was in age interval 36-40 years. For *Salmonella species* the highest range (45) was the age group 25-29 years. In patel para *Salmonella* species mostly effected the age group from 25-29 years of age.

Shigella sonnei was the most frequently isolated species (96 cases) at Pehlwan Goth and Safora Goth whereas *Shigella flexneri* (74 cases) except in Patel Para where *Salmonella* was most common (50 cases). *Shigella boydii* was infrequently isolated (30 cases), of shigellosis episodes. In all of the three study location *Shigella sonnei* was considerably more commonly isolated from age group 30-35 years old. In contrast, *Shigella flexneri* was more recurrently isolated from age group 41-45 years old.

This multicentre shigellosis research work evaluated that shigellosis is further omnipresent than earlier consideration. At three researches oriented locations on the whole, culture results established shigellosis in age groups other than children.

Vigorous observation would have make available a more complete recognition of all diarrhea incidents at the risk of capturing insignificant episodes that do not require medical care. Data collected in the during 2010-2011 in a mixed community in Karachi indicating that among adults, episodes of bacillary dysentery (shigellosis) can occur due to contaminated drinking water lasting 7-10 days. It is the comparative study between two groups control (Allopathic Drug ciprofloxacin) and test group (Herbal Formulation ShigelDysent), as increasing emergence of multidrug resistant among Shigella species, studies using alternative treatments plus dynamic case recognition could offer an additional comprehensive awareness of the shigellosis load in the research work areas. Ciprofloxacin is no allopathic drug, has nothing to do with extremely small doses,

Ciprofloxacin was given in dosage of 500 mg capsule three times a day.

In conclusion, shigellosis is a frequent cause of diarrhea in the more impecunious areas of Asia. Although there were few medical complications associated with shigellosis, control of this disease could reduce overall diarrheal burden globally. The progress in the formulation of an alternative treatment may be defensive against shigellosis is a highly desirable public health goal.

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