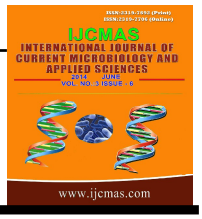
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 2 (2014) pp. 950-957**
http://www.ijcmas.com



Original Research Article

Clinico-epidemiology of shigellosis in children suffering from Diarrhea in District Lahore, Pakistan

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A B S T R A C T

For the present study a total of 126 stool samples were examined during February to April 2012. Out of these diarrheic samples *Shigella* accounted for 5.5% (7/126), *Enterobacter* 10.3 % (13/126), *Klebsiella* 11.9% (15/126), *Salmonella* 16.6 (21/126) and *E.coli* 18.2 % (23/126).The prominent age groups for shigellosis were 1-6 months (28.6%) and 19-24 months (28.6%) and having complaints of bloody diarrhea and fever. Cases were prominent (57.2%) in warmer month of April than (28.6%) in March and (14.3%) in April. Factors which were significantly associated with shigellosis were breastfeeding practices to children (p=0.036), child feeding on fresh/raw/ unboiled milk (p=0.029), sterilization of feeder with boiling water and disinfectant (p=0.008), family members using common toilets (p=0.044), other family members suffering from diarrhea (p=0.030), blood and mucous present in the stool samples(p=0.000) and knowledge of mothers about "transmission of disease through contaminated food/water and through direct contact" (p=0.015) and (p=0.017) respectively. The rest of the factors were not significantly associated with shigellosis were gender of the child (p=0930.), children playing in muddy places (p=0.752), crawling children (p=0.319), children admitted in Day care centers (p=0.223), boiled water used by family members (p=0.223), flies access to water tank (p=0.384), community water supply to houses (p=0. 367), hand washing practices of mothers before feeding their child (p=0.146), cloth washing of children with detergents (p=0.420), pets in home (p=0.136), free movement of pets in home (p=0.223), free access of pets to kitchen utensils (p=0.883) and fresh or uncooked vegetables and salad used by family members (p=0.717).

K e y w o r d s

Shigella; diarrheic samples; bloody diarrhea and fever.

Introduction

Shigellosis is reported to be one of the major microbial infections manifested by diarrhea or dysentery, vomiting, headache, fever, stiff neck, convulsions and joint pain (Benenson et al. 1995). The causative organism belongs to the genus

Shigella and was first described by Kiyoshi Shiga in 1897 (Pelczar et al. 1981). The pathogenic species of Shigella to humans includes *S. dysenteriae*, *S. boydii*, *S. flexneri* and *S. sonnei* (Subekti et al., 2001).

According to an estimate 165 million people suffers from shigellosis worldwide and 99% cases occur in developing countries, 69% victims are children in the age group of 1-5 years (Kotloff et al. 1999). The overall death toll is about 0.4 million. Children and immune-compromised person are prone to the infection. The mortality rate is greater in children younger than 5 years of age in Asia (Niyogi 2005).

The natural host of Shigella is only humans and mode of transmission is fecal-oral or by contact with contaminated food and water (Du Pont et al. 1989). The site of infection is colonal mucosa; but sometimes it causes extra-intestinal complications including bacteraemia, urogenital and neurologic manifestations (Barrett et al. 1970). Prevention of shigellosis is difficult due to its ability of not being effected by gastric juice in the stomach very low infectious dose (10-100 organisms) can causes infection, hence Shigella is a highly contagious pathogen (Abu-Elyazeed et al. 2004). The pathogenic organisms attach to epithelial cells of the intestinal mucosa, multiply in the cells and spread to neighboring epithelial cells, causing destruction, the characteristic pathology of dysentery (Todar 2009). Sometimes the patient may develop severe hemolytic haematuria when infected by Shiga toxin strain, which may cause renal failure and ultimately death (Benenson et al. 1995).

Shigella has been documented to survive in salt water for 12-30 hours, in kitchen wastes for 1-4 days, in fresh water for 5-11 days, in sour milk for four weeks, in dust at room temperature for six weeks and in soiled linen for up to seven weeks. Shigella species have been found in most of sewage, water surface, crops and food contaminated by human feces (Taylor et al. 1964).

The purpose of this study is to evaluate the most prevalent pathogen responsible for diarrhoea in children and to study the descriptive epidemiology of shigellosis in children suffering from diarrhea/dysentery in Lahore, Pakistan.

Materials and Methods

Rectal swabs (sterile) along with stool samples in a sterile container were collected from 126 children with complaints of diarrhea/dysentery at Emergency Departments of three randomly selected hospitals i.e. Mayo, Children and Shalimar Hospitals Lahore. Verbal consent was taken from the mothers and guardians and questionnaire was filled on the spot containing information regarding socioeconomic status, demographic characteristics and routinely practiced activities. The specimens were place in buffered glycerol saline (transport medium) and transported to laboratory in ice box at 4°C. Each specimen was cultured on MacConkey and XLD (Xylose Lysine Deoxycholate) agar media as soon as possible after arrival to the laboratory, and incubated at 37°C for 18 to 24 hours (Harwana et al. 2010). The suspected colonies were grouped as Gram Positive and Gram Negative by Gram's staining reaction. Non-lactose fermenting colonies on MacConkey and XLD agar were selected and tested for their biochemical reactions (Farmer 2003) by

conventional method using Oxidase test, Catalase test, Sugar Fermentation test, Indole Production test, Methyl Red test, Vogues Prausker's test, Citrate Utilization test, Urease test, Motility test, and TSI (Triple Sugar Iron) and by using API 20E (Biomerieux, France). The data were coded, entered and analyzed by using software SPSS 16.0.

Results and Discussion

A total of 126 stool samples were examined during February to April 2012. Out of these diarrheic samples *Shigella* accounted for 5.5% (7/126), *Enterobacter* 10.3 % (13/126), *Klebsiella* 11.9% (15/126), *Salmonella* 16.6 (21/126) and *E.coli* 18.2 % (23/126) Table-1.

The prominent age groups for shigellosis were 1-6 months (28.6%) and 19-24 months (28.6%) and having complaints of bloody diarrhea and fever. Cases were prominent (57.2%) in warmer month of April than (28.6%) in March and (14.3%) in April. Factors which were significantly associated with shigellosis were breastfeeding practices to children (p=0.036), child feeding on fresh/raw/ unboiled milk (p=0.029), sterilization of feeder with boiling water and disinfectant (p=0.008), family members using common toilets (p=0.044), other family members suffering from diarrhea (p=0.030), blood and mucous present in the stool samples(p=0.000) and knowledge of mothers about "transmission of disease through contaminated food/water and through direct contact" (p=0.015) and (p=0.017) respectively , Table-2.

The rest of the factors were not significantly associated with shigellosis were gender of the child (p=0930.), children playing in muddy places (p=0.752), crawling children (p=0.319), children admitted in Day care centers (p=0.223), boiled water used by family members (p=0.223), flies access to water tank (p=0.384), community water supply to houses (p=0. 367), hand washing practices of mothers before feeding their child (p=0.146), cloth washing of children with detergents (p=0.420), pets in home (p=0.136), free movement of pets in home (p=0.223), free access of pets to kitchen utensils (p=0.883) and fresh or uncooked vegetables and salad used by family members (p=0.717).

Our study showed that out of 126 stool specimens Shigella was recovered from 5.5%. This is comparable with studies conducted i.e 3% in China (Wang et al. 2005), 3.8% Indonesia (Subekti et al. 2001), 4% in Nepal (Bhattacharya et al. 2005), 4.8% in Pakistan (Zafar et al. 2008), 5% in Ghana (Opintan et al. 2007), 5.3% in Thailand (Hiranrattana et al. 2005), 8% in southern Trinidad during 1997-2006 (Orrett 2008), 8.5% in Malaysia 1992-2003 (Alici et al. 2006). 8.8% in Iran (Ghaemi et al. 2011), 9.8% in Iran (Mashouf et al. 2006), 10.1% in India (Patil et al. 2012) and 14.01% in North Iran (Savadkoohi et al. 2007). And different from other studies like 19.8% in Pakistan (Khalil et al. 1998), 20 % in Ethiopia (Mache et al. 2001) and 58% in Bangladesh (Shahid et al. 1985).

Gender distirubtion of children was no significantly assocated with shigellosis. These findings are coreleated with the findings of Abu-Elyazeed, which stated that gender distribution had no association with shigellosis in Iranian children and diverse from Egyptian and Bangladeshian children where male suffered more from shigellosis than females (Hossain et al. 1990, Abu-Elyazeed et al. 2004).

Table.1 Frequency of microorganisms isolated from samples

Bacteria	**Organisms (isolated)**	**Percentage**
Shigella	7	5.5
Enterobacter	13	10.3
Klebsiella	15	11.9
Salmonella	21	16.6
E. coli	23	18.2
No Organism Detected	47	37.3
Total	126	100.0

Table.2 Association of different factors with shigellosis

Parameters		***Shigella***		**Chi-Square**	**p-value**
		Detected	**Not Detected**		
N		7	119		
Child breast feeding	Yes	0	47	4.410	0.036
	No	7	72		
Child feeding on	Fresh Raw	1	51	7.104	0.029
	Local Vender	4	62		
	Un boiled	2	6		
Washing feeder with hot water	Yes	0	61	6.956	0.008
	No	7	58		
Use of common Toilets	Yes	4	102	4.04	0.044
	No	3	17		
Diarrhea to other family members	Yes	7	70	4.717	0.030
	No	0	49		
Disease spread through contaminated water and food	Yes	0	56	5.92	0.015
	No	7	63		
Disease spread from diseased children to healthy children	Yes	0	55	5.742	0.017
	No	7	64		
Blood or mucous in feces	Yes	6	29	12.40	0.000
	No	1	90		

p-value: Significant* ≤ 0.05

The results of our study showed that 28.6 % of Shigella was isolated from children with age group of 1-6 months and 19-24 months, 14.3% from 7-12, 13-18 and 43-48 month and no any case was found in the rest age of groups. These results are supported by other studies conducted Savadkoohi Iran and Wang in China where shigellosis was common in children under 5 years of age and unsupported by prevalence was least in children less than 1 year of age. This may be due to breastfeeding practices no adapted by mothers in Pakistan and feeding their child with milk from other sources (Savadkoohi et al. 2007, Wang et al. 2005).

These results of our study about season distribution showed that there was 1 (14.3%) case of shigellosis in February, 2 (28.6%) cases in March and 4 (57.2%) cases in April. Various studies claimed that the seasonal tendency of shigellosis was summer, (Abu-Elyazeed et al. 2004, Alici et al. 2006, Savadkoohi et al. 2007) and peak prevalence was found in April and May in Bangladesh (Hossain et al. 1990). Our results showed the same results as there were more cases occurred in April followed by March and February. In April the temperature rises and summer season started so the cases of shigellosis occurred more than the other two months.

Some of the factors showed significant associtions with shigellosis like "Breastfeeding practices of mother to their children were significantly associated with shigellosis i.e (*P*= 0.036). Out of 7 children suffering from shigellosis no single child was on breastfeeding. The findings of the present study about breastfed children were similar to the findings of other studies in Bangladesh, Zaire, Tanzania and Pakistan where breastfed children were generally spared from shigellosis (Khalil et al. 1998). There was significant association of shigellosis with children feeding on fresh raw/local vender/ unboiled milk. Out of these 7 children, one child was feeding on fresh raw milk, 4 were on milk from local vender and 2 were unbioled milk (*P*=0.029). According to the study conducted by Robinson, (1981) who found that various species of shigella intially multiply in milk. However, survival rate of these species are different for different food or other liquid products. Water was found to be the most reliable medium for its survival, about upto six months. Hence, in our study the occurrence of shigella species in milk from local venders also showed higher cases. This might be due the adulteration of water in milk from local venders.

Sterilization of feeders with hot water and disinfactant was signifiacntly assocaited with shigellosis. Since we found in our study (Questionare) obtained from mothers of 7 children suffering from shigellosis indicated that they did not wash feeders with hot water and disinfactants (*P*=0.008). Our results are therefore in collaboration with the findings of Ghaemi and Wilson in Iran and Nepal, where shigellosis was significantly associated with lack of hygienic practices by the mothers (Ghaemi et al. 2008, Wilson et al. 2006).

Similarly, use of common toilets was also significantly associated with shigellosis, because 4 out of the 7 cases of shigellosis (*P*=0.044) were using common toilets. This results is coreleated with the results of (Khalil et al. 1998) about the transmission of shigellosis, where the disease occurred commonly by direct person to person contact and through contaminated food, water and fomites.

A significant association was found between risk factor, like "other family members suffering from diarrhea" with shigellosis. Family members of all the 7 cases of shigellosis told that there were other family members already suffering from diarrhea in their houses (*P*= 0.030).

Knowledge of the mothers about spreading of "diseases from contaminated food and water" and "spreading of diseases from diseased children to healthy children" were significantly associated with shigellosis. Out of 7 cases of isolated *Shigella* all the 7 mother didn't have the knowledge that contminated food, water and diseased children can be a source of

disease transmission with (p-value= 0.015) and (p-value= 0.017) repectively. These results are parrallel to a study conducted by Ghaemi in Iran where the education of day care attendants was significantly associated with shigellosis (Ghaemi et al. 2011) and in contrast with study conducted by Abu-Elyazeed in Eygpt concludeed that shigellosis was not associated with meternal education (Abu-Elyazeed et al. 2004).

Blood and mucous present in the feces were significantly associated with shigellosis. Out of 7 children suffering shigellosis, blood and mucos was present in the feces of 6 of the children. i.e. (*P*=0.000). These findings agree well with the findings of Ghaemi in north Iran where fever, abdominal tenusmus and blood in stool were commonly found during shigellosis (Ghaemi et al. 2011) and (Kavaliotis et al. 2002), however, there is a conflicting results which indicated that majority of children didn't have fever and blood in their feces suffering from shigellosis (Abu-Elyazeed et al. 2004). All the 7 children in which *Shigella* was detected, presented 6-10 diarrheal episodes per day which was insignficantly associated with detection of *Shigella*. These outcomes were in aggrement with the findings of Herwana et al. 2010. Children admitted in day-care centers were not significantly associated with shigellosis. These finding are in contrast with findings of (Ghaemi et al. 2007).

Other risk factor like Children playing in muddy places, children who are crawling, flies acess to the water tank, cummunity water supply to the houses, washing hands of mother before feeding the child, washing the body and clothes of children with detergents after defecation, family members using fresh or uncooked vegetables and salad, washing of toilets with disinfactants, pets in home, free movement of pets in home, access of pets to kitchen and utensils were found insignificantly associated with shigellosis. These results don't show revalancy with other studies carried out in Iran, India, Nepal, Bangladesh, China, Egypt, Indonesia and Kenya due to small sample size and short duration of our study.

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