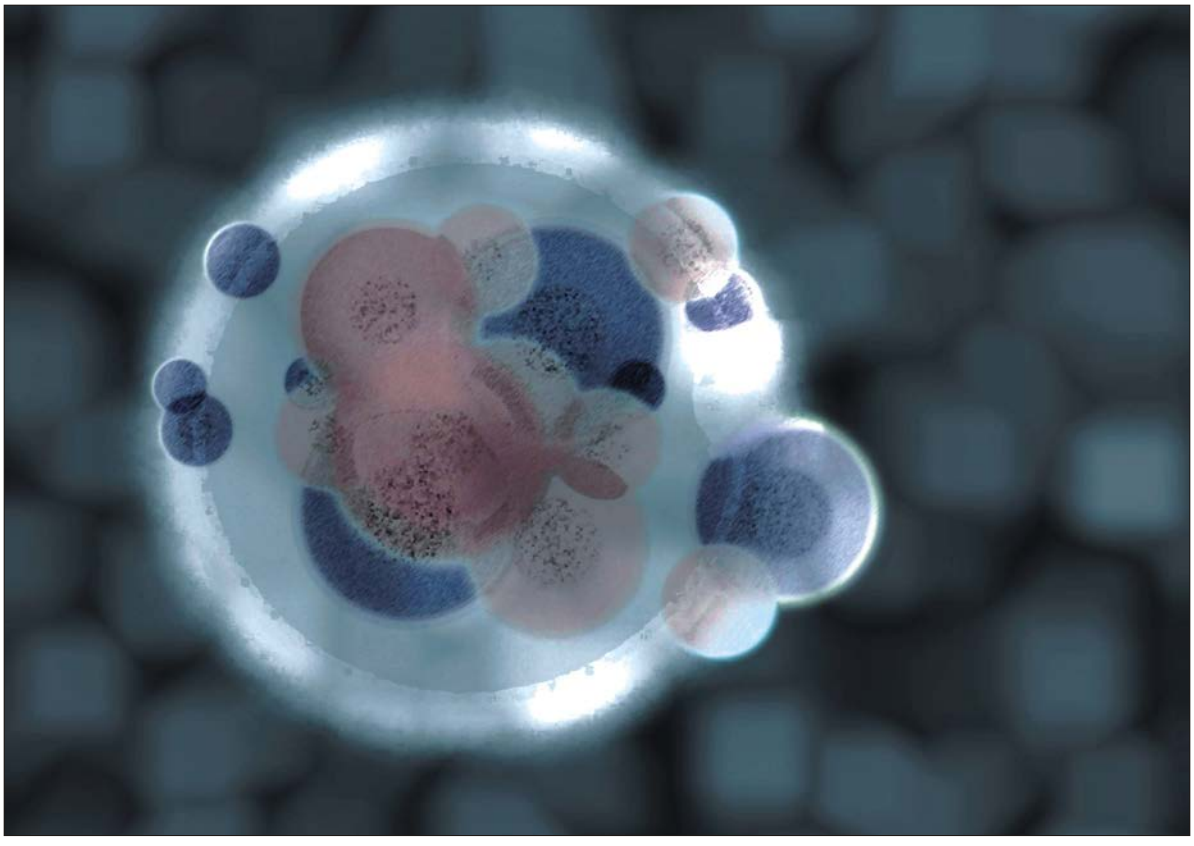

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Distribution Patterns of *Salmonella* Infection in Rawalpindi/Islamabad Area And the Risk Factors Associated with the Disease Prevalence

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Abstract: This study was designed to determine the prevalence of *Salmonella* sp. in 300 clinical samples (stool and blood) isolated from salmonellosis suspected patients. Samples were cultured on Salmonella-Shigella, MacConkey, agars enriched with Selenite F broth. Biochemical and serological confirmatory tests were carried. *Salmonella* sp. were found to be the principal etiological agent in 22 (7.3%) cases. Only 2 serotypes *Salmonella typhi* (63.6%) and *Salmonella paratyphi* A (36.4%) were reported. Prevalence rate was greater in blood (81.8%) than stool samples (18.2%), with higher *Salmonella typhi* (85.7%) than *Salmonella paratyphi* A (75%) isolated from positive blood samples. Incidence rates of salmonellosis were slightly higher among females as compared to males (54.5 and 45.5%, respectively), with equal gender distribution of *Salmonella typhi* cases, but a higher incidence of *Salmonella paratyphi* A among females (62.5%) than males. Of the *Salmonella* positive cases, 63.6% were among the 5-14 years age group, followed by 13.6% in the less than 5 years age group. Of the *Salmonella typhi* cases 71.4% were reported in children aged 5-14 years and 7.1% in infants under 5 years of age. For *Salmonella paratyphi* 50% of cases were reported among children in the 5-14 years age category and 25% in children under the age of 5 years. Fever was the most common symptom (81.8%), followed by chills (75%), abdominal pain (68%), vomiting (59%) and diarrhea (54.5%). Highest number of *Salmonella typhi* cases (42.9%) were reported among those living on the poverty line and 28.5% living in below the poverty line, whereas 50% of the cases of *Salmonella paratyphi*, reported in this study, were among those living in extreme poverty. Of the positive cases 58.8% of the children had illiterate parents. Of the children with *Salmonella typhi* 54.4% had illiterate parents. Of the children with *Salmonella paratyphi*, 65.7% had parents who lacked any sort of formal education. Among the adult population positive for *Salmonella* infection, 40% of them were uneducated. Of those with *Salmonella typhi* 66.6% were uneducated, while 50% of *Salmonella paratyphi* adult cases were among those who had received no formal education. In Rawalpindi, the highest incidence of salmonellosis was reported among patients residing near the Nullah Leh (35.3%). Of those patients residing in the Nullah Leh, 36.4% and 33.3% had *Salmonella typhi* and *Salmonella paratyphi* as their etiological agent, respectively. While 18.2% and 33.3% of the *Salmonella typhi* and *Salmonella paratyphi* cases, respectively, were reported from rural areas surrounding Rawalpindi. For Islamabad only a total of 5 cases of *Salmonella* infections were reported.

Key words: *Salmonella typhi*, *Salmonella paratyphi* A, Rawalpindi, Islamabad

INTRODUCTION

Salmonellosis, a collective term for all human and animal infections caused by members of the genus *Salmonella*, remains a substantive cause of morbidity and mortality globally, even in developed nations where 50,000 cases are reported annually (Baron *et al*., 1994). *Salmonella* sp. gram-negative facultative anaerobes are primarily intestinal parasites of vertebrates that inflict several diseases including enteritis and typhoid like diseases (Boyd, 1995). *Salmonella* species are hardy microorganisms that survive in moist environments and as well as in a frozen state for several months and are able to tolerate hostile environmental conditions such as low gastric pH levels and antimicrobials actions of peptides secreted by the enterocytes (Solano *et al*., 1998).

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Typhoid fever represents a major public health challenge with an estimated 16.6 million cases and 600,000 deaths annually (Ahmed *et al*., 1994) and is predominantly associated with developing world (Pang *et al*., 1995). There are four species i.e., *Salmonella typhi*, *Salmonella paratyphi* A, B and C, *Salmonella choleraesuis* and *Salmonella enteritidis* (Baron *et al*., 1994), that have been associated with typhoid-like diseases, but each of these species have various sub-types based on their serology. The serotyping is based on the nature of antigens include: O antigen (heat-stable polysaccharides present in cell wall as lipopolysaccharide (LPS), H antigens (heat-labile proteins) of the flagella and Vi antigens (heat labile capsular polysaccharide) (Old and Threlfall, 1997).

Salmonella typhi the causative organism of typhoid fever, may manifest itself among all ages in endemic areas where poor sanitary conditions and poor/contaminated water supply persist, such as in countries like Pakistan (Karamat *et al*., 1991). The signs and symptoms associated with typhoid include anorexia, headache, myalgias, sore throat, abdominal pain, chills, vomiting and diarrhea. Conditions like epistaxis, lethargy and delirium may occur after completing the incubation period. *Salmonella paratyphi* A and *Salmonella paratyphi* B are associated with paratyphoid (enteric) fever of a milder nature, whereas *Salmonella paratyphi* C is known to cause septicemia but is an uncommon etiological agent of infections in humans (Cheesbrough, 1991).

The incidence of salmonellosis, as most gastrointestinal diseases, is greater during the summer/warmer months, probably due to ambient temperatures being conducive for *Salmonella* sp. proliferation, particularly in un-refrigerated, or ready-to-eat foods. Humans acquire the infection by ingesting the organisms through contaminated food and or waters (Mahon and Manuselis, 1995). Salmonellosis occurs in all age groups, but it is more severe among infants, children and the elderly (Preston and Borezyk, 1994).

Salmonellosis has an incubation time of about 12 to 36 h, during which the pathogen first invades the intestinal mucosa, where it multiplies and then proliferates to the lymphatic and cardiovascular systems, from where they may spread to many organs. The fever associated with *Salmonella* infections is associated with the endotoxins released during the lysis of the bacterial cells (Brooks *et al*., 2001).

In Pakistan, typhoid fever and others *Salmonella* infections are endemic, particularly among the poor and those residing in slum areas with poor sanitation and inadequate clean water supply (Karamat *et al*., 1991; Qureshi *et al*., 2001).

This study was designed to determine the prevalence of *Salmonella* sp. in various clinical samples (stool and blood) isolated from salmonellosis suspected patients and correlate the prevalence of salmonellosis with socio-economic condition, age, sex, education, eating habits and geographical distribution of the patients. Findings of this study may be of immense importance to physicians as well as Government Development Authorities, to create awareness amongst the masses with the aim of preventing and controlling the spread of such infections by employing good management and good hygiene practices.

MATERIALS AND METHODS

This retrospective study of 300 patients of gastroenteritis, typhoid fever and bacteremia has been carried-out from August 2001 to July 2002, at different hospitals of Islamabad-Rawalpindi (Pakistan Institute of Medical Sciences, Islamabad and Holy Family Hospital, Rawalpindi). Blood and stools samples were taken from those patients, who presented symptoms consistent with salmonellosis.

Following the collection, these samples were immediately transported to the Bacteriology Laboratory at National Institute of Health (NIH), for bacteriological isolation and characterization. Furthermore, detailed history of each patient was also recorded on a prescribed questionnaire. The information included age, sex, locality, level of education (parental education levels were noted for children from 1 to 15 years), eating habits, socio-economic status, signs and symptoms exhibited by these patients were noted.

These samples (blood and stool) were processed for the isolation of *Salmonella* sp. on various selective media: Salmonella-Shigella, MacConkey, enriched with Selenite F broth. Composition of one liter of Selenite F broth is peptone from meat 5.0 g; lactose 4.0 g; sodium selenite 4.0 g; di-potassium hydrogen phosphate 3.5 g; potassium dihydrogen phosphate 6.5 g. Blood cultures were incubated at 37°C for 7 days, with subcultures streaked on MacConkey agar after every 24 h. These plates were incubated aerobically at 37°C for 24 h and isolates were identified through microscopy. If *Salmonella* were not obtained from first subculture then sub-culturing was repeated every 24 h for the next 7 days. The blood cultures were initially examined for any turbidity-indicative of positive growth-subsequent to which microscopy was carried out.

Stool cultures were inoculated on MacConkey, Salmonella-Shigella agar (Typical Composition/liter-peptone 10.0 g; lactose 10.0 g; ox bile 8.5 g; sodium citrate 10.0 g; sodium thiosulfate 8.5 g; ammonium iron (III) citrate 1.0 g; brilliant green 0.0003 g; neutral red 0.025 g; agar-agar 12.0 g) and Selenite F broth and

incubated at 37°C for 72 h, with subcultures from Selenite F Broth re-cultured on Salmonella-Shigella agar (Cheesbrough, 1991).

Salmonella sp. were initially identified by colony morphology as they form pale yellow or colorless, 1-3 mm diameter, non-lactose fermenting colonies on Salmonella-Shigella and MacConkey agar. Confirmatory tests were carried out through gram staining and biochemical tests (Oxidase test, Motility test, Indole test, Citrate test, Urease test, Triple sugar iron test) (Baron *et al*., 1994).

All the pathogenic forms of *Salmonella* sp. were confirmed up to species level by serotyping. *Salmonella* serotypes were done using O, H and Vi antigens (Cheesbrough, 1991).

RESULTS AND DISCUSSION

The results of this study revealed *Salmonella* sp. as the principal etiological agent in 22 (7.3%) of the 300 cases analyzed (Table 1). Of these cases, only two serotypes of *Salmonella* were isolated, i.e., *Salmonella typhi* (63.6%) and *Salmonella paratyphi* A (36.4%). As for the other serotypes, namely *Salmonella paratyphi* B or C, these strains were not identified amongst the isolates in this study. These findings are in accordance with the studies documented by other researchers (Saqib and Ahmed, 2000). Researchers have reported that in Pakistan, the majority of cases of typhoid fever are caused by either *Salmonella typhi*, or *Salmonella paratyphi* A (Rehman and Ahmad, 1994). This is contrast to the findings in the population of western countries, where a majority of the cases of typhoid have been attributed to *Salmonella paratyphi* A, B and C (Bahr and Bell, 1987). However, a few studies have been documented on *Salmonella paratyphi* B being the etiological agent in salmonellosis cases in Karachi and Rawalpindi (Pakistan) (Asghar *et al.,* 2002).

Overall trend of *Salmonella* sp. isolated in this study revealed that the prevalence rate was greater in blood samples (81.8%) as compared to stool samples (18.2%). These findings are consistent with those reported by other researchers, who reported slightly higher incidence (Usman *et al*., 1996), as well as slightly lower incidence (Wain *et al*., 1998) of *Salmonella* sp. as the etiological agent in patients of gastroenteritis, diarrhea and other related ailments. The reason for the contrasting results may be attributed to the different techniques involved in these studies for the isolation of bacteria, sampling area, difference in sample size, the literacy and awareness levels and hygienic practices among the population under study, as well as the availability of adequate infrastructure facilities and provision of basic enmities (Asghar *et al*., 2002).

Table 1: Prevalence of *Salmonella* sp. in blood and stool samples

Clinical samples	No. of samples	Positive for *Salmonella* sp.	Positive for *Salmonella typhi*	Positive for *Salmonella paratyphi* A
Blood	152	18 (81.2%)	12 (85.7%)	6 (75%)
Stool	148	4 (18.2%)	2 (14.8%)	2 (25%)
Total	300	22 (7.3%)	14 (63.6%)	8 (36.4%)

The prevalence of *Salmonella typhi* as the etiological agent was lower (63.9%) than the findings reported in previous studies (Saqib and Ahmed, 2000; Usman *et al*., 1996; Wain *et al*., 1998) and may be attributed to the improvement of living standards among the study population and their increased awareness to use boiled and or filtered water and to visit their doctor for regular routine examinations. The areas covered in this study were quite diverse and included those locales where availability of clean water supply, improved sanitary conditions and level of affluence was high, as well as the slum and poverty stricken areas of the twin cities. Reports have been published that shown an even lower incidence rate of *Salmonella typhi* among patients of gastroenteritis in the Rawalpindi area (Munir *et al*., 2001), while other researchers have reported a similar prevalence rate (Shanahan *et al*., 2000) as that revealed in this study.

On the other hand, the study under discussion has revealed a greater incidence of *Salmonella paratyphi* A (36.4%) as the etiological agent of salmonellosis in the Rawalpindi/Islamabad area. This prevalence of *Salmonella paratyphi* A is relatively high as compared to previous studies carried out in other areas of Pakistan, namely Baluchistan (Mishra *et al*., 1991) and elsewhere (Usman *et al.,* 1996). However, study carried out in Karachi did report a rise in *Salmonella paratyphi* A cases, which may be attributed to massive urban migration, inadequate infrastructure support and facilities to accommodate the population inflow, leading to the establishment of slum areas where hygiene and water qualities are compromised (Saqib and Ahmed, 2000).

Further differentiation revealed that among the *Salmonella typhi* cases, 85.7% of the isolates were derived from blood samples, while 14.8% from stool samples. A similar trend was found for *Salmonella paratyphi* cases as well, in that 75% of the *paratyphi* serotypes were isolated from blood samples (Table 1). Other researchers have also reported similar findings, having documented a *Salmonella* isolation rate of 80-90% yield in blood samples during first week of illness (Shanahan *et al*., 2000). The most likely reason of high yield in blood may be due to fact that in the *Salmonella* causes bacteremia in blood and afterwards it goes to gastrointestinal tract (Baron *et al*., 1994). The low isolation rate of *Salmonella* from stool samples in this study is also consistent with the reports of other researchers (Wain *et al*., 1998). This may be attributed to

Table 2: Prevalence pattern and gender-wise distribution of *Salmonella* sp. among positive patients (n = 22)

Positive for *Salmonella*			Positive for *Salmonella typhi*			Positive for *Salmonella paratyphi* A		
Total	Male	Female	Total	Male	Female	Total	Male	Female
22	10 (45.5%)	12 (54.5%)	14 (63.6%)	7 (50%)	7 (50%)	8 (36.4%)	3 (37.5%)	5 (62.5%)

Table 3: Age-wise distribution pattern of *Salmonella* positive cases (n = 22)

Age groups (years)	Positive for *Salmonella* sp.	Positive for *Salmonella typhi*	Positive for *Salmonella paratyphi* A
<5	3 (13.6%)	1 (7.1%)	2 (25%)
5-14	14 (63.6%)	10 (71.4%)	4 (50%)
15-24	2 (9.1%)	1 (7.1%)	1 (12.5%)
25-34	2 (9.1%)	2 (14.3%)	-
34-44	-	-	-
45-54	1 (4.5%)	-	1 (12.5%)
>55	-	-	-
Total	22	14	8

the difficulties associated with isolating specific pathogens from stool samples, where the normal flora is inundated with numerous bacteria-particularly gram-negative bacilli (Cheesbrogh, 1991; Mahon and Manuselis, 1995; Saqib and Ahmed, 2000), as well as the rampant practice of self-prescription among patients; a come practice in Pakistan (Asghar *et al*., 2002; Mahmood *et al*., 2001; Siddiqi *et al*., 2002). Antimicrobial therapy at the time of sample collection had a considerable influence on the culture result. The presence of antimicrobial agents in specimen may cause false-negative culture results since the growth of susceptible bacteria may be inhibited (Usman *et al*., 1996; Wain *et al*., 1998).

Sex-wise distribution pattern of the *Salmonella* positive cases showed no marked differentiation, as the results of this study recorded a slightly higher incidence of salmonellosis among females as compared to males (54.5 and 45.5%, respectively), with equal gender distribution of *Salmonella typhi* cases. However, the *Salmonella paratyphi* cases showed a marked difference in gender distribution, with a higher (62.5%) incidence among females (Table 2). Most of the women, positive for typhoid, were pregnant, which may help to explain the high incidence as during pregnancy a women's immune response weakens and they become more susceptible to infections. These finding are very much consistent with the previous studies carried out in Bangladesh and Karachi (Pakistan), where a higher incidence of salmonellosis was reported among females (Ahmed *et al*., 1994). Another reason for the higher incidence of *Salmonella* infection among females, particularly in this region, may be the socio-cultural practices of the people of this region, where preference the best possible health care and food stuff are preferentially provided to boys and men over girls and women (Anonymous, 2003; Shehzad, 2003; Anonymous, 2002).

Age-wise distribution pattern of *Salmonella* sp. showed a greater prevalence amongst children and infants as compared to adults. Of the *Salmonella* positive cases, 63.6% were among the 5-14 years age group, followed by 13.6% in the less than 5 years age group (Table 3). A similar pattern was reported among the specific isolates as well, in that 71.4% of the *Salmonella typhi* cases were reported in children aged 5-14 years and 7.1% in infants under 5 years of age. For *Salmonella paratyphi* 50% of cases were reported among children in the 5-14 years age category and 25% in children under the age of 5 years. These results are consistent with the findings of numerous researchers who documented the greatest incidence of *Salmonella* infection among children aged 1-14 years (Wain *et al*., 1998; Shimoni *et al*., 1999). The highest risk of infection in lower age may be because of poor immune response to infection, bad quality of drinking water especially among school going children, being exposed to contaminated ready-to-eat food items available in open-air cafeteria, especially during out-door activities associated with extra-curricular school activities (Asghar *et al*., 2002). Salmonellosis, in its most acute and most often fatal form, is particularly a disease of young, whether humans, animals or birds (Wain *et al*., 1998).

The high incidence among young children may indicate marked susceptibility of young children to salmonellosis, coupled with the lack of good hygiene practices and level of awareness both among the children and their parents. Another potential factor may be the intimate association of children with pets, which are carriers of salmonellosis (Cheesbrough, 1991; Mahon and Manuselis, 1995).

Interestingly enough, no case of salmonellosis among adults over the age of 54 years was reported in the present study. This may be attributed to the possible sub-clinical infection controlled by the development of cell-mediated immunity (Cheesbrough, 1991).

The reason of higher infection in children among uneducated parents, as reported in this study, lays credence to fact that there is a lack of knowledge on the practices of good personal hygiene, correlating dirty hands with spread of infections like *Salmonella* through

Table 4: Prevailing symptoms of positive patients for salmonellosis (n = 22)

Symptoms						
Fever	Chills	Abdominal pain	Vomiting	Diarrhea	Headache	Chest pain
81.8%	75%	68%	59%	54.5%	50%	45.4%

Table 5: Correlation of salmonellosis with household income

Income Categories (Rs./month)	Positive for *Salmonella* sp.	Positive for *Salmonella typhi*	Positive for *Salmonella paratyphi* A
<1000	8 (36.4%)	4 (28.5%)	4 (50%)
1000-3000	6 (27.3%)	6 (42.9%)	-
3000-6000	4 (18.2%)	2 (14.2%)	2 (2.5%)
6000-15000	3 (13.6%)	2 (14.2%)	1 (12.5%)
>15000	1 (4.5%)	-	1 (12.5%)
TOTAL	22	14	8

Table 6: Parental education level among *Salmonella* positive children below 15 years of age (n = 17)

Level of Education	Positive for *Salmonella* sp.	Positive for *Salmonella typhi*	Positive for *Salmonella paratyphi* A
Uneducated	10 (58.8%)	6 (54.4%)	4 (66.7%)
Matriculate	2 (11.76%)	1 (9%)	1 (16.7%)
Intermediate	3 (17.6%)	3 (27.2%)	-
Graduate	2 (11.76%)	1 (9%)	1 (16.7%)
Post Graduate	-	-	-
Total	17	11	6

the preferred fecal-oral route. This was evident from the additional fact that the study also reported a high incidence rate of *Salmonella* infection among uneducated adults as well. These results are in agreement with those reports by other researchers (Khan and Khan, 2000). Furthermore, the common practice of consuming unwashed and partially cooked vegetables by these people enhanced the possibility of spread of infection.

This study revealed that fever was the most common symptom (81.8%), followed by chills (75%), abdominal pain (68%), vomiting (59%), diarrhea (54.5%), headache (50%). Chest pain (45.4%) was the least common symptom reported among these patients (Table 4). This is in conformity with other studies reported worldwide (Mishra *et al*., 1991). However, some researchers, while documenting the very same symptoms have reported varying incidences among patients diagnosed as *Salmonella* positive (Rehman and Ahmad, 1994). It must be noted here that clinical symptoms of typhoid fever are not very precise, as these symptoms may present a variety of ailments. Therefore, diagnosis is often delayed until the results of blood culture and other serological tests are made available.

The correlation pattern between the incidence of salmonellosis and total household income, as defined in this study, revealed that most of the *Salmonella* positive cases (63.7%) occurred in patients who were either living in extreme poverty or just below the poverty line as defined by the National Human Development Report (Anonymous, 2003) (Table 5).

According to the results of this study, 36.4% cases of salmonellosis were reported among those living in extreme poverty (< Rs. 1000/month), while 27.3% of the cases were reported among those living below the poverty line (Rs.1000-3000/month) and another 18.2% among those living on the poverty line. As for the specific etiological agents, *Salmonella typhi* was reported in 42.9% of those living on the poverty line and 28.5% living in abject poverty, whereas 50% of the cases of *Salmonella paratyphi*, reported in this study, were among those living in extreme poverty (Table 5). These findings are also reflected in reports published by experts highlighting the nexus between poverty, environmental degradation and human health (Swati, 2003; Rana, 2003).

The results of this study also revealed an interesting correlation between levels of education and prevalence of salmonellosis. Among the children aged 15 years and below, who were found positive for this infection, parental literacy levels were ascertained. The resulted indicated that the incidence of salmonellosis in children is related to the level of education of the parents. Of the positive cases 58.8% of the children had illiterate parents, 17.6% had parents with 12 years of schooling and 11.76% had parents who had over 10 years of schooling (Table 6).

Among these, those who had *Salmonella typhi* as their etiological agent, 54.4% had illiterate parents and 27.2% had parents with high school diploma. As similar pattern was revealed in the case of *Salmonella paratyphi*, as 65.7% of the positive children had parents who lacked any sort of formal education (Table 6).

Among the adult population positive for *Salmonella* infection, this study revealed that 40% of them were uneducated. Of those with *Salmonella typhi* as their etiological agent, 66.6% were uneducated, while 50% of *Salmonella paratyphi* adult cases were among those who had received no formal education (Table 7).

Table 7: Education level among *Salmonella* positive patients above 15 years of age (n = 5)

Level of education	Positive samples for *Salmonella* sp.	Positive for *Salmonella typhi*	Positive for *Salmonella paratyphi* A
Uneducated	2 (40%)	2 (66.6%)	1 (50%)
Matriculate	1 (20%)	-	-
Intermediate	-	-	-
Graduate	1 (20%)	1 (33.3%)	-
Post Graduate	1 (20%)	-	1 (50%)
Total	5	3	2

Table 8: Locality-wise distribution of *Salmonella* sp. in Rawalpindi (n = 17)

Locality	Positive for *Salmonella* sp.	Positive for *Salmonella typhi*	Prevalence of *Salmonella paratyphi* A
Downtown and its surrounding areas	3 (18.75%)	2 (18.2%)	1 (16.7%)
Nala Leh	6 (35.3%)	4 (36.4%)	2 (33.3%)
Sattelitetown/Posh Area	1 (5.9%)	1 (9.1%)	-
Faizabad	2 (11.8%)	2 (18.2%)	-
Choorchowk	1 (5.9%)	-	1 (16.7%)
Surrounding rural areas	4 (23.5%)	2 (18.2%)	2 (33.3%)
TOTAL	17	11	6

Table 9: Locality-wise distribution of *Salmonella* sp. in Islamabad (n = 5)

Locality	Positive for *Salmonella* sp.	Positive for *Salmonella typhi*	Positive for *Salmonella paratyphi* A
G-sector	3 (60%)	2 (66.7%)	1 (50%)
F-sector	1 (20%)	1 (33.3%)	-
I-sector	-	-	-
Shehzad Town	1 (20%)	-	1 (50%)
otal	5	3	2

Table 10: Hygiene practices displayed by *Salmonella* positive patients (n = 22)

Eating and drinking habits	Total number of positive patients (%)
Homemade food	8 (36%)
Ready-to-eat food (outdoors)	14 (64%)
Use of boiled/filtered water	9 (40%)
Use of un-boiled/in-filtered water	13 (60%)

These findings are in agreement with those reported by other researchers who effectively argue that quality education is essential to holistically address the poverty and environmental issues (Shehzad, 2003; Anonymous, 2002; Haider, 2003)

The overall prevalence pattern of salmonellosis, as reported in this study, based on the locality of the incidence, also revealed interesting results. In Rawalpindi, the highest incidence of salmonellosis was reported among patients residing near the Nullah Leh (35.3%) -a slum area surrounding a large wastewater drain running through the city and into a local river. The second highest incidence was reported among patients living in the surrounding rural areas (23.5%), followed by those residing in downtown Rawalpindi (18.75%) (Table 8).

The species prevalence pattern followed a similar trend, as 36.4% and 33.3% of those patients residing in the Nullah Leh region had *Salmonella typhi* and *Salmonella paratyphi* as their etiological agent, respectively. While 18.2 and 33.3% of the *Salmonella typhi* and *Salmonella paratyphi* cases, respectively, were reported from rural areas surrounding Rawalpindi (Table 8).

For Islamabad, the overall incidence level was much lower, as only a total of 5 cases of *Salmonella* infections were reported over the period in this study (Table 9). However, the distribution pattern did highlight the fact that most cases of *Salmonella typhi* (66.7%) and *Salmonella paratyphi* (50%) were reported in patients residing in the poorer and more congested sectors of Islamabad, particularly the G-sectors where poor sanitation and choked sewerage pipelines have been reported (Mubashir, 2003).

As for the hygiene practices employed by the *Salmonella* positive patients in this study, it is evident from the results revealed that most of patients do not practice basic hygiene methods. Among the patients of salmonellosis, 64% habitually ate outdoors from ready-to-eat vendors, while 60% drank un-boiled and un-filtered water on a regular basis (Table 10).

This study, under discussion also showed that salmonellosis was more prevalent in patients who consumed ready-to-eat food items from outside vendors, as compared to homemade food. Such practice increased their chance of infection by 20%, particularly among those populations that in addition to this drank un-boiled and or un-filtered water. Similar results have also been reported by other researchers (Chaudhary *et al*., 1994).

There is no doubt that typhoid will remain endemic as long as there is inadequate socio-economic development, poor infrastructure maintenance and a rapid population growth. Poverty, lack of quality education and awareness and a lack of the general sense of responsibility for

personal and environmental cleanliness, will allow such pathogens to flourish and persist (Pugh, 1989).

This study also revealed a strong correlation between prevalence of salmonellosis and poverty, as the higher incidence of salmonellosis reported amongst the low-income class may be attributed to contaminated water supply, poor sanitation and unhygienic food practices: aspects prevalent in poorer slum areas of the twin cities of Rawalpindi and Islamabad (Brooks *et al*., 2001).

Prevalence of salmonellosis was greater in Rawalpindi than Islamabad and was more frequent in patients residing in densely populated, less developed areas, such as the G-sector of Islamabad, which is a very congested sector, with poor sanitation and water supply system. Similarly in Rawalpindi, patients living along the Nullah Leh and its adjoining surroundings areas were more prone to *Salmonella* infection than those living in more affluent and developed areas. The main reason for the incidence of salmonellosis in the Nullah Leh region is due to the fact that the Nullah, which originally was a natural causeway rain waters, has now become nothing short of a large drain of sewerage, municipal and industrial wastewaters (Shehzad, 2002), contaminating the area's drinking water supply.

Infection reported in developed areas in these twin cities, as reported in this study, may be attributed to the overall poor quality of water supplied by the municipal government. Constant commuting of household workers and care-givers, from the under-developed areas of the cities, to the affluent regions of the cities, may be another source of infection transmission, particularly among the children and elderly, whom they look after (Mishra *et al*., 1991).

Causes of typhoid in Rawalpindi and Islamabad may be associated with the densely populated urban centers with adjoining suburbs, coupled with poverty and poor infrastructure development, like sanitation and waste disposal, lack of quality education, inadequate primary health care facilities, cumulatively resulting in the contamination of food produce and water. The lack of adequate health care facilities, affordable diagnostic laboratories and proper counseling of parents on methods of good hygiene practices, in particular, has lead to an increase of *Salmonella* related illnesses in urban centers throughout Pakistan (Rehman and Ahmad, 1994; Asghar *et al*., 2002).

Without doubt, like many infectious diseases in developing countries, salmonellosis may also be labeled as a disease of the poor and under privileged. Countries that have inadequate infrastructure to support a growing population, coupled with poor wastewater treatment facilities and improper sanitation mechanisms or systems, will always be prone to gastrointestinal infections, particularly those caused by enteric gram-negative bacilli. To combat such endemic diseases it is essential to prioritize political commitments for sustained socio-economic development, through adequate allocation of resources, quality education, properly managed implementation of urban and rural planning and use of affordable, preferable indigenous, cost-effective technologies to combat the plight of water contamination and wastewater treatment.

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