



RESEARCH PAPER

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Prevalence of typhoid fever in five Southern districts of Khyber Pakhtunkhwa, Pakistan: A preliminary study

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Abstract

Typhoid fever, caused by *Salmonella* Typhi (*S. Typhi*), is one of the deadly diseases and is a serious public health problem around the world. The prevalence of typhoid fever in five flood affected Districts (Bannu, Hangu, Karak, Kohat and Peshawar) of khyber Pakhtunkhwa province of Pakistan during 2010/11 was investigated in this study. The blood samples of 110 hospitalized patients were used for clinical diagnosis applying widal test and Dot immunosorbent Assay (DIA), and their results were compared for analysis. A total of 60 patients were found positive for widal test, however only 67 % patients of them were positive for DIA. More males (52%) than females (48%) were infected by *Salmonella*. The ratio of typhoid patients was greater in young people having ages between 24-38 years. These preliminary informations could be helpful for future mass screening in the country. It is concluded that DIA could be used as an authentic confirmative diagnostic technique for the detection of *S. Typhi*. This study provides important information about the net incidence of typhoid in the region that would help in proposed future health policies.

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Introduction

Salmonella Typhi (*S. Typhi*), responsible for typhoid fever is a non-spore forming, non-capsulated, rod-shaped bacterium, which also causes human diseases such as gastroenteritis, bacteremia and enteric fever (Archer and Young, 1988; Hargrett *et al.*, 1988; Echeita and Usera, 1989; Rodrigue *et al.*, 1990). Typhoid is still one of the most hazardous problems in the Indian sub-continent and parts of sub-Saharan Africa where the hygienic conditions are not satisfactory (Bhan *et al.*, 2005; Crump *et al.*, 2004; Karkey *et al.*, 2008; Parry *et al.*, 2002). *S. Typhi* has been isolated frequently from Asian countries. Approximately 200,000 deaths occur from typhoid fever in the world annually (Karkey *et al.*, 2008). It is also reported that water borne infections cause 250,000 deaths each year in Pakistan among which typhoid fever is a major cause (Shah *et al.*, 2003). More than 2500 serovars of *Salmonella* has been recognized on the basis of "O" and "H" antigens (Blood *et al.*, 2003).

S. Typhi is transmitted by fecal-oral route, either from person-to-person or by ingestion of contaminated food or water. In developing countries large epidemics are related with fecal contamination of water and food (Department of Health, 2008). The incubation period of *S. Typhi* could vary from 1-2 weeks. After incubation period the *S. Typhi* enters into the small intestine, attaches itself to epithelium and then penetrates in sub-mucosa where they are engulfed by the monocytes. *S. Typhi* resists intracellular killing and multiply in the monocytes. They reach mesenteric lymph nodes, multiply there and reach blood stream resulting in primary bacteremia, seeded in the liver, gall bladder, spleen, lymph node, bone marrow, where they continue to multiply. After primary bacteremia bacteria gain access to bloodstream resulting in secondary bacteremia. When bacteria are shed from the gall bladder along with the bile juice, they reach the small intestine again, leading to inflammation (Rao, 2009).

The members of the genus *Salmonella* can be isolated, identified and characterized by various cultural, serological, biochemical and molecular analysis (Deighan *et al.*, 2000; Veling *et al.*, 2000; Buerfeind *et al.*, 2001). Serological techniques are considered the simplest and quick methods for the diagnosis of *Salmonella*. These techniques include widal test, Dot Immunosorbent Assay (DIA), dip stick assays, and semi-quantitative tube agglutination test (Aziah *et al.*, 2007). Widal test is widely used as a recommended screening procedure in developing countries, regarded as a useful test in endemic areas (Pang and Puthuchery 1983), and is used to establish the presence or absence of homologous antibody. This test is based on the immunological reaction between the antibodies produced to viable bacteria (agglutinins) and their other various counterpart febrile antigens. The DIA detects the presence of specific IgG and IgM antibodies to a specific outer membrane protein (OMP) antigen, and is recommended as a confirmative test (Ismail, 1991).

One of the most hazardous problems in Pakistan is typhoid fever. The objective of this study was to investigate the incidence of typhoid fever in five flood affected Southern Districts (Bannu, Hangu, Karak, Kohat and Peshawar) of KP. There are 25 districts in KP. However selected districts were based on high prevalence of typhoid in these districts. Clinical diagnosis of patients was done using widal test and DIA and their results were compared for analysis. Since this was a preliminary study, it will provide a base for the incidence of typhoid in Pakistan. It could also play a significant role in treatment, prevention, and future planning regarding typhoid in Pakistan.

Materials and methods

The districts selected for this study were based on the high prevalence of typhoid, poor health and lower socioeconomic status of the people in the region. These districts are situated in the north-west of Pakistan and are part of KP. Total of 110 blood samples were collected from typhoid patients of five Southern Districts (Bannu, Hangu, Karak, Kohat and

Peshawar) of KP, Pakistan during 2010/11. Samples were collected from both male and female patients of different ages. The blood samples were collected in EDTA (Ethylenediamine tetraacetic acid) tubes and preserved at 4°C till used. The clinical analysis was performed using widal test and DIA. Widal test and DIA were performed according to the manufacturer's protocols (Murex Biotech, Ltd.), and (CTK Biotech, Inc.), respectively. These techniques detect the presence of antibodies against the particular pathogens. Variables recorded were the clinical method of diagnosis, age and sex of the patients. The data collected were analyzed and represented in tabulated and graphical forms.

Results and discussions

Clinical analysis

All positive samples (60 samples) for widal test collected from different hospitals of KP were further

diagnosed with DIA and comparison was made between these two diagnostic procedures. Results obtained show that all widal positive samples did not show positive results for the presence of *S. Typhi* when analyzed by DIA. Only 41 samples were positive and nineteen were negative for the presence of antibodies against *S. Typhi* (Table 2). These results show that widal test alone may not be a reliable index for the diagnosis of *S. Typhi* and it needs to be coupled with DIA for the confirmation of *S. Typhi*. Thus, DIA seems to be a practical alternative to widal test for early diagnosis of typhoid fever. Similar comparative study for the early diagnosis of typhoid fever has already been done by Begum *et al.*, (2009), which also recommend that DIA could be used as an authentic diagnostic procedure in comparison to the simple widal test.

Table 1. Gender-wise distribution of typhoid patients in flood affected districts of Khyber Pakhtunkhwa, Pakistan during 2010/11.

Group	Age	No. of Samples	No. of Affected Females	% of Affected Females	No. of Affected Males	% of Affected Males
A	2 – 9	9	5	55.55%	4	44.45%
B	9 – 17	6	2	33.33%	4	66.67%
C	17- 24	8	4	50%	4	50%
D	24 –31	8	6	75%	2	25%
E	31 –38	8	1	12.5%	7	87.5%
F	38 –45	7	3	43%	4	57%
G	45 –52	9	5	55%	4	45%
H	52 –59	5	3	60%	2	40%
Total	2-59	60	29	48%	31	52%

Gender-wise distribution of patients

More males than females were infected by *Salmonella*, out of 60 samples analyzed thirty eight were males (52%) and twenty two were females (48%) (Table 1). The increase prevalence of typhoid observed in males might be due to the male predominance in Pakistani society, making it easier for males to visit hospitals for the handling of typhoid patients. High burden of workload and the habit of taking no rest or little rest in males could also be the possible cause of high prevalence of typhoid in men. The incidence of other diseases including cancer has

also been reported to be more common in males than in females in Pakistani society (Zeb *et al* 2008).

Age-wise distribution of patients

The highest percentage of blood specimens infected by *Salmonella* was found in the age group of 24-38 years old but gradually declined in old people and children (Figure 2). The higher ratio of typhoid in young people might be due to greater exposure of young people to the outside environment in comparison to the old people and children. Higher workload and little rest in young people (particularly

young males) could also be the cause of high prevalence of typhoid in young people.

The current study was a preliminary study in the five selected districts of KP, Pakistan. The data show information regarding the most affected persons, especially the gender-wise and age-wise, and also highlights the competence of clinical methods used for diagnosis. However, there is still a need to determine the occupation of typhoid patients in the area and to analyze their socioeconomic and

demographic data. It is also desirable to device a more reliable and simple polymerase chain reaction (PCR) based diagnosis procedure for the detection of *S. Typhi* instead of using classical serological methods, which have some limitations. The amplification and sequencing of the PCR amplified products will provide an insight into the phylogenetic relationship of these bacteria with other counterparts, which will allow us to develop novel strategies for the control of this disease.

Table 2. Comparison between widal test and Dot immunosorbent Assay (DIA).

DIA RESULTS					DIA RESULTS				
No of samples	Widal test	igG	igM	Result	No of samples	Widal test	igG	igM	Result
S1	+	-	-	-	S31	+	+	+	+
S2	+	-	-	-	S32	+	-	-	-
S3	+	-	-	-	S33	+	+	+	+
S4	+	-	-	-	S34	+	-	-	-
S5	+	-	-	-	S35	+	+	+	+
S6	+	-	-	-	S36	+	+	-	+
S7	+	-	-	-	S37	+	+	+	+
S8	+	-	-	-	S38	+	-	+	+
S9	+	-	+	+	S39	+	-	-	-
S10	+	+	+	+	S40	+	+	-	+
S11	+	+	+	+	S41	+	-	+	+
S12	+	+	+	+	S42	+	-	-	-
S13	+	+	+	+	S43	+	+	-	+
S14	+	+	+	+	S44	+	+	+	+
S15	+	+	-	+	S45	+	-	+	+
S16	+	-	+	+	S46	+	-	+	+
S17	+	+	-	+	S47	+	+	+	+
S18	+	+	+	+	S48	+	+	+	+
S19	+	+	+	+	S49	+	+	-	+
S20	+	-	+	+	S50	+	-	-	+
S21	+	+	+	+	S51	+	-	+	+
S22	+	+	+	+	S52	+	-	+	+
S23	+	-	+	+	S53	+	-	-	+
S24	+	+	+	+	S54	+	+	-	+
S25	+	-	-	-	S55	+	-	-	-
S26	+	+	+	+	S56	+	+	+	+
S27	+	-	-	-	S57	+	-	+	+
S28	+	-	-	-	S58	+	+	-	+
S29	+	-	-	-	S59	+	-	-	-
S30	+	-	-	-	S60	+	-	+	+

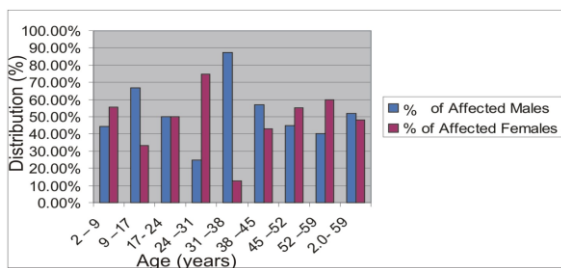


Fig. 1. Age-wise distribution of typhoid patients in flood affected districts of Khyber Pakhtunkhwa, Pakistan.

Conclusions

The precise prevalence and death rates of typhoid in Pakistan (particularly in KP) are still questionable. To our knowledge this is the first study of its nature in

KP. Since this was a preliminary investigation, it could add the information on the prevalence of typhoid in Pakistan. The molecular characterization of *S. Typhi* and construction of phylogeny on the basis of sequence information could find the evolutionary relationship of *S. Typhi* with other members of the family. Public awareness by education is very important which may play a role in preventing the epidemic of the disease.

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