Original Article

Epidemiology, clinical presentation, and patterns of drug resistance of *Salmonella* Typhi in Karachi, Pakistan

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Abstract

Introduction: Enteric fever remains a major public health problem in Asia. Planning appropriate preventive measures such as immunization requires a clear understanding of disease burden. We conducted a community-based surveillance for *Salmonella* Typhi infection in children in Karachi, Pakistan.

Methodology: A *de jure* household census was conducted at baseline in the study setting to enumerate all individuals. A health-care facility-based passive surveillance system was used to capture episodes of fever lasting three or more 3 days in children 2 to 16 years old.

Results: A total of 7,401 blood samples were collected for microbiological confirmation, out of which 189 *S.* Typhi and 32 *S*. Paratyphi A isolates were identified with estimated annual incidences of 451/100,000 (95% CI: 446 – 457) and 76/100,000 (95% CI: 74 – 78) respectively. At the time of presentation, after adjusting for age, there was an association between the duration of fever and temperature at presentation, and being infected with multidrug-resistant *S.* Typhi. Of 189 isolates 83 were found to be resistant to first-line antimicrobial therapy. There was no statistically significant difference in clinical presentation of blood culture sensitive and resistant *S.* Typhi isolates.

Conclusion: Incidence of *S.* Typhi in children is high in urban squatter settlements of Karachi, Pakistan. Findings from this study identified duration of fever and temperature at the time of presentation as important symptoms associated with blood culture-confirmed typhoid fever. Preventive strategies such as immunization and improvements in water and sanitation conditions should be the focus of typhoid control in urban settlements of Pakistan.

Key words: *S.* Typhi; population-based incidence; drug resistance; Pakistan

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Introduction

Typhoid fever remains a major public health problem in the developing world, especially in developing countries of Asia [1-3]. It is estimated that approximately 21.5 million illnesses and 216,510 deaths worldwide occurred in year 2000 alone, and the majority of reported typhoid fever cases were from South and Southeast Asia, where disease burden was highest with more than 10 million illnesses and 100,000 deaths [4]. However, the current global estimates of typhoid fever are limited. Based on a literature review of 23 published studies from 11 countries, data on typhoid are sparse [5].

Since typhoid fever was first identified as a serious febrile illness in the early nineteenth century, health professionals have been challenged in making an early and correct diagnosis. It was difficult to differentiate typhoid fever from other prolonged febrile illnesses until the discovery that *Salmonella enterica* serovar Typhi (*S.* Typhi) could be identified in tissue culture [6]. Confirmatory diagnosis of typhoid fever was, and is still, based on microbiological identification of the organism from bone marrow, blood, stool, or urine samples. However, blood culture is time-consuming and expensive. In addition, the sensitivity of blood culture is 50% and is variable even within geographic regions, depending largely on the method and setting where the procedure is performed [7,8]. Considering that typhoid is highly endemic in areas where

conditions for food and water sanitation are poor, timely diagnosis is of the utmost importance.

During the early stage, typhoid presentation is non-specific and variable. The major presenting symptom is a high-grade fever (>38^{o}C). Acute non-specific febrile illnesses in endemic settings are managed at outpatient clinics [9]. Due to the non-availability of laboratory diagnostic techniques, attending physicians often rely on clinical presentation for diagnosis [10], and in situations where laboratory support is available, additional out-of-pocket costs for such tests are limiting factors for requesting expensive laboratory tests. Physicians therefore rely on empirical therapy. In the presence of high rates of drug resistance and due to a diverse clinical picture, low predictive value of serological tests, and expensive microbiological techniques, typhoid patients often end up with prolonged fever, resulting in hospitalization, and sometimes fatal outcomes [11,12]. Furthermore, the emergence of multidrug-resistant *S.* Typhi strains has made management of typhoid fever more expensive and difficult [13-15]. Multidrug-resistant (MDR) *S.* Typhi has become a significant issue in clinical management of typhoid fever [16-18].

Due to limitations of national surveillance system for infectious diseases, population-based data on typhoid fever are not available in Pakistan; however, local epidemiological studies have highlighted high typhoid fever incidence rates in young children and antimicrobial-resistant strains as important public health issues [19]. A study of typhoid fever in Pakistan reported an incidence of culture-proven typhoid fever of approximately 170 /100,000 (95% CI: 120, 220) in children younger than sixteen years of age [2]. Based on the World Health Organization (WHO) criterion that a disease incidence of more than100/100,000 persons per year is considered high [4], these estimates show a significant disease burden of typhoid fever in children in Pakistan. The study, however, only enrolled cases through passive surveillance and did not capture febrile episodes visiting private health care providers that cater up to 70% to 85% of health services in Pakistan [20].

In light of the concern to estimate incidence of typhoid fever in the pediatric population, a comprehensive, community-based surveillance study was initiated in three urban slum communities of Karachi, Pakistan, as part of a pilot phase of a Vi polysaccharide typhoid vaccine effectiveness trial. Here we present the results of one year of surveillance data that include incidence rate estimates, differences in clinical presentation for *S.* Typhi, and patterns of antimicrobial resistance in children. The objectives of this paper are to describe the incidence estimates for typhoid fever in children and to compare the clinical presentation of typhoid fever cases with non-Typhi fever episodes and drug resistant *S.* Typhi. Estimates from this study will provide useful information in the management of typhoid fever in settings where diagnosis is primarily clinical. These results can also help to shape policy for setting the priority of public health programs to reduce child mortality due to vaccine-preventable diseases, such as typhoid fever.

Methodology

Study setting

The study was conducted in three low socio-economic settlements of Karachi, Pakistan, namely Sultanabad, Hijrat colony, and Bilal colony. The majority of the study population (116,500) is Muslim, and consists of different ethnicities and immigrants from northern Pakistan. Health care is provided to the population through private physicians, whereas preventive health-care services are administered by government-operated health-care centres.

Disease surveillance

A *de jure* census was conducted prior to the start of surveillance activities to enumerate all individuals within the study setting. The census data was stored in an electronic database, and provided demographic and baseline information at individual and household levels for the project's various activities. Disease surveillance was comprised of domiciliary visits by a team of community health workers (CHWs) and the establishment of three project health centres, one in each setting. The domiciliary visits provided information on the extent of common childhood illnesses in the area. During weekly visits, CHWs collected information on fever, diarrhea, cough, and abdominal pain using a standardized questionnaire. A child having a history of fever in a household was referred to project health centres, or parents were encouraged to see their private physician. Project health centres provided free consultation and medication from 10:00 a.m. to 10:30 pm, six days a week.

To capture all typhoid fever episodes, general practitioners of the study area were included in the surveillance system for the referral of fever episodes to project health centres. There were various meetings with the health care providers of the area in which details of the program were discussed and their help was requested. Cash was offered as an incentive for

referred cases [21]. For enrollment, a child must have had a history of fever for three days or more, be between the ages of 2 and 16 years old, and reside in the study area. Patients with a febrile episode of less than three days were examined by study physicians, provided with empirical therapy, and requested to come for a follow-up visit if the fever did not subside. After obtaining verbal informed consent from a parent or guardian, clinical information was collected on a case report form, and a blood sample of 6 to 10 milliliters was taken from the enrolled child for the microbiological diagnosis of typhoid fever.

A child with a positive growth of *S*. Typhi in blood culture was visited at home for assessment of risk factors and any complications associated with the disease, as well as to confirm the child's residence. All typhoid fever cases were followed up for one year after diagnosis for the assessment of long-term complications associated with typhoid fever. Surveillance periods for Sultanabad and Hijrat colony were from 1 August 2002 to 31 July 2003, whereas surveillance for Bilal colony was from 1 August 2003 to 31 July 2004.

Definitions

The following definitions were established:

- Blood culture-negative fever episode: child presenting with a history of fever for three days or more, and blood culture results showed no micro-organism growth
- Blood culture-positive *S*. Typhi sensitive to first-line antibiotics:child presenting with a history of fever of three days or more, *S.* Typhi was isolated on blood culture, and *S.* Typhi was sensitive to all first-line antimicrobial agents (chloramphenicol, ampicillin, co-trimoxazole)
- *S.* Typhi resistant to first-line antibiotics: child presenting with a history of fever of three days or more, where *S.* Typhi was isolated on blood culture and *S.* Typhi was resistant to all first-line antimicrobial agents (MDR).
- Mono drug resistant *S*. Typhi resistant: child presenting with a history of fever of three days or more, where *S.* Typhi was isolated on blood culture and *S.* Typhi was resistant to at least one of the five tested antimicrobial agents (chloramphenicol, co-trimoxazole, ampicillin, ceftriaxone, nalidixic acid).

Laboratory methods

A venous blood sample was collected from each consenting patient. Blood was later inoculated into enriched soybean-casein digest broth with resins in BACTEC (Becton-Dickinson, New Jersey, USA) bottles. For individuals younger than five years of age, BACTEC PEDS Plus bottles were used. Upon growth as indicated visually or by the BACTEC machine, the blood culture bottles were sub-cultured onto a MacConkey agar plate, irrespective of Gram stain. Also, even in the absence of visible growth, the bottles were sub-cultured onto MacConkey agar on days 1, 2, 4 and 7 (day 1 was the day when the sample was obtained). The bottles were then incubated for 10 days before being discarded. Colonies giving biochemical reactions suggestive of *Salmonellae* were confirmed serologically with specific O and H antisera (BD Laboratories). Bacterial growth from KIA was used for agglutination. Bacterial growth was first tested for slide agglutination in polyvalent O *Salmonella* antiserum. If negative for agglutination, bacterial growth was tested for agglutination in Vi antiserum. If positive for Vi agglutination, then the suspension was boiled to destroy the Vi antigen and tested in polyvalent O antiserum. The boiled suspension was also tested in monovalent O antigen factor sera. If positive for agglutination, the bacterial suspension was tested without boiling in monovalent O antigen factor sera.

Laboratory investigations were performed at the clinical laboratory of Aga Khan University Hospital (AKUH). Consultants from the AKUH visited field site laboratories to assess the quality and correctness of the blood collection techniques and to ensure proper transportation. The AKUH laboratory operates according to laboratory guidelines of international standards (certified by ISO and Joint Commission International Accreditation). Identification of *Salmonella* isolates was externally confirmed (by the University of Oxford-Wellcome Trust Research Unit at The Centre for Tropical Diseases, Ho Chi Minh City, Vietnam). *Salmonella* isolates were tested for antibiotic susceptibility by Kirby Bauer disc diffusion method on Muller-Hinton agar with standard antimicrobial discs (Clinical Laboratory Standards Institute - 2006). *Salmonella* isolates were tested for chloramphenicol, ampicillin, trimethoprim / sulphamethoxazole, fluoroquinolone, ceftriaxone and nalidixic acid.

Table 1. Population-based incidence of typhoid and paratyphoid fever in children, Karachi, Pakistan

***Salmonella* Typhi**		
	Sultanabad	*Hijrat C*
Total number of cases	36	22
Total population aged 2 – 16 years	7008	8424
Incidence rate (/100 000)	513.7	261.2
95% Confidence Interval	(499.6, 527.8)	(252.0,
***Salmonella* Paratyphi A**		
Total number of cases	2	9
Incidence rate (/100 000)	28.5	106.8
95% Confidence Interval	(25.2, 31.9)	(100.1,

Data collection, management, and analysis

Patient information was collected through structured questionnaires. Forms were reviewed and signed by the study physicians prior to shifting to the Data Management Unit (DMU). Data were double-entered into visual FoxPro (Microsoft Corporation, Redmond WA, USA), a generic database management system. Annual incidence rates and respective 95% confidence intervals were calculated per 100,000 children per year. The number of children in the study setting from the baseline census data was used as a denominator based on a fixed cohort approach, assuming in-migration equals out-migration. Numerator (primary case definition) for calculations consisted of all episodes of fever in which *S.* Typhi was isolated by blood culture. Means for continuous and proportions for categorical variables were calculated as a measure of central tendency. Three sets of analyses were performed for assessment of differences in cases with no microbiological growth, antimicrobial-sensitive *S*. Typhi, and multidrug-resistant *S*. Typhi. In addition, stratified analysis was performed to assess differences in typhoid infection (antimicrobial-sensitive and MDR) for variables of age and study site (Sultanabad, Hijrat, Bilal). Univariate analysis was performed to assess the association of covariates with outcome, using an odds ratio and 95% confidence intervals for an effect estimate through logistic regression analysis. Criteria for selection of a variable in the adjusted model were based on biological plausibility, a p-value of less than 0.20 for association with outcome, and face value of variables. Data were analyzed with STATA version SE 10.0 (StataCorp LP, College Station, Texas, USA).

Ethical considerations

The study was approved by the Ethical Review Committee of Aga Khan University, and the Institutional Review Boards (IRB) of the International Vaccine Institute (IVI), Seoul, Korea.

Results

A total of 41,845 children aged 2 to 16 years were followed for one year. The disease surveillance system enrolled 7,401 eligible children as suspected typhoid fever cases in the study. The mean age of the enrolled children was 7.5 years, 71% were below ten years of age, and the mean duration of fever was six days (SD 6.0). A micro-organism was isolated in 960 (13%) cases. One hundred and eighty-nine *S.* Typhi and 32 *S.* Paratyphi A isolates were isolated from blood cultures. There were no *S.* Paratyphi B or C isolates during the surveillance period. Among other microbiologically significant growths were *Streptococcus pneumonia* (5), *Pseudomonasaerugina* (4), *Acinetobactoerlowffi* (4), Enteropathogenic*E. coli* (3), and *Staphylococcusaurus* (1).

Annual culture-positive *S.* Typhi incidences were 513 per 100,000 child years, 261 per 100,000 child years, and 496 per 100,000 child years in Sultanabad, Hijrat, and Bilal colonies, respectively, while *S.* Paratyphi A incidences were 28 per 100,000 child years, 106 per 100,000 child years, and 79 per 100,000 child years in Sultanabad, Hijrat, and Bilal colonies, respectively (Table 1). The incidence was higher in younger children (less than 10 years old) in all three sites (Table 2). Thirty-five (19%) *S.* Typhi cases were sensitive to all six antibiotics tested, and 83 (44%) were multidrug-resistant (Figure 1). Nine other cases of *S.* Typhi were resistant to nalidixic acid only (Figure 2). Two *S*. Paratyphi cases were resistant to

Table 1. Age-specific incidence of typhoid cases in 3 slum areas of Karachi, Pakistan (2002 – 2004)

Age	Target Population	Blood Culture-positive		Annual Incidence/ 100,000	95% CI for Incidence Rate
		S. Typhi Cases	% of Total		
Sultanabad/Hijrat Colony					
2 – 4	3,520	17	29	483.0	(463.7, 502.2)
5 – 9	5,756	23	40	399.5	(385.9, 413.3)
10 – 12	3,461	15	26	433.4	(415.0, 451.8)
13 – 16	2,695	3	5	111.3	(100.7, 121.9)
Overall	15,432	58	100	375.8	(367.7, 384.0)
Bilal Colony					
2 – 4	6,598	41	31	621.4	(605.5, 637.3)
5 – 9	10,141	65	50	640.9	(627.9, 654.0)
10 – 12	5,498	18	14	327.4	(314.7, 340.1)
13 – 16	4,176	7	5	167.6	(157.2, 178.1)
Overall	26,413	131	100	495.9	(488.9, 503.1)

chloramphenicol, and one was resistant to ampicillin. We did not find multidrug-resistant (MDR) *S.* Typhi in the Hijrat colony and Sultanabad sites, and 66% of the MDR *S.* Typhi strains were found in Bilal colony alone.

Clinical comparison of blood culture-negative cases to blood culture- positive S. *Typhi cases*

Age, temperature at the time of presentation, abdominal pain, and abdominal tenderness were independently associated with *S.* Typhi sensitive to first-line antibiotics compared to non-*S.* Typhi fever episodes (Table 3). On stratified analysis, temperature at the time of presentation, duration of fever, and abdominal pain had a statistically significant association with antibiotic-sensitive *S.* Typhi in Hijrat and Sultanabad, whereas in Bilal colony, age, temperature at the time of presentation, headache, and abdominal distention had a statistically significant association with antibiotic-sensitive *S.* Typhi. Upon adjusting for other covariates, temperature at the time of presentation had a statistically significant association with antibiotic-sensitive *S.* Typhi episodes in children (Table 4).

Clinical comparison of blood culture-negative cases to blood culture-positive MDR S. *Typhi cases*

Duration of fever and temperature at the time of presentation were independently associated with MDR *S.* Typhi cases (Table 5). After adjusting for the effect of other variables, temperature at the time of presentation and fever duration of more than 10 days remained significantly associated with MDR *S.* Typhi cases. We did not find a statistically significant difference in the clinical presentation of antibiotic-sensitive *S.* Typhi compared to MDR *S.* Typhi cases.

Discussion

Our study estimated the annual incidence rates of *S.* Typhi to range from 252 to 503 per 100,000 child years in three impoverished areas of Karachi. Typhoid incidence estimates are four times higher than the WHO-defined criterion for high typhoid incidence [4] and two times higher than the incidence (170 per 100,000 persons per year) found in an earlier study conducted in the same setting [2]. However, the previous study in the same setting did not have a referral system for health-care providers in the study area; therefore, the majority of cases of typhoid fever that were seen by private practitioners were missed. Considering the nature of typhoid fever, people's health-seeking behavior, and predictive value of the tests available, we believe these results are, at best, conservative estimates of typhoid incidence in these settings of Pakistan. Typhoid fever incidence varied significantly among the three sites. As surveillance methods were standardized across all three sites and the populations were highly similar, the observed differences in incidence can be attributed to variations in environmental risk factors such as contaminated water and population density. Approximately 35% of the cases detected were seen in the 2- to 5-year-old age group, and 78% of the cases were seen in children ages 2 to 10 years. Data from various countries

Figure 1. Distribution of resistant strains of *S.* Typhi isolated from children 2 to 16 years old in Karachi, Pakistan

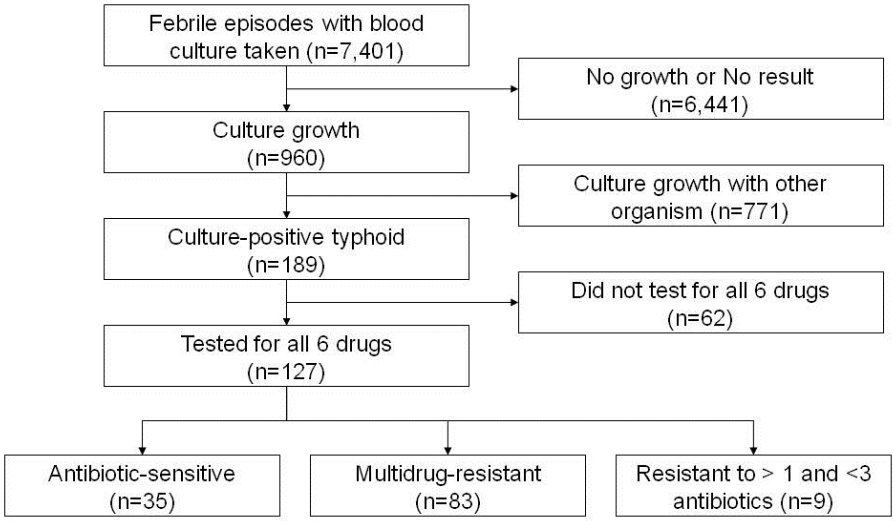

Figure 2. Overall results of fever episodes enrolled in the surveillance study of typhoid fever in Karachi, Pakistan

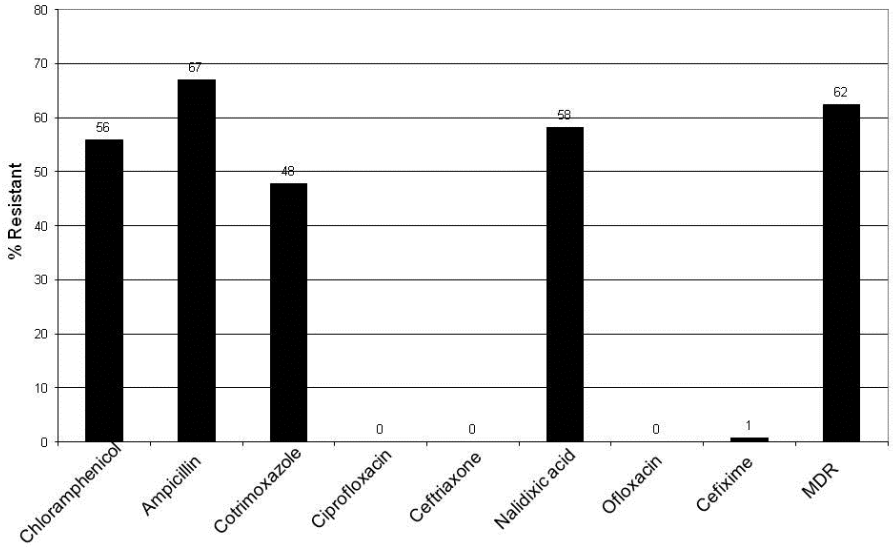

Table 3. Comparison of clinical characteristics between blood culture-negative fever episodes and blood culture-positive *S.* Typhi episodes

	No. (%) of Culture-positive	No. (%) of Culture-negative	Odds Ratio	95% CI for OR
	n = (189)	n = (7,034)		
Age (mean/sd)	(7.0/3.3)	(7.5/3.8)	0.96	0.93 - 1.0
<5	58 (31)	2,139 (30)	1	
5 to 10	88 (47)	2,894 (41)	1.12	0.8 - 1.6
>10	43 (23)	2,001 (28)	0.79	0.53 - 1.2
Male	92 (49)	3,508 (50)	0.95	0.71- 1.3
Duration of fever (mean/sd)	(6.9/4.3)	(6.3/6.0)	1.01	0.99 - 1.0
<5days	65 (34)	2,952 (42)	1	
5-10days	74 (39)	2,933 (42)	1.15	0.82 - 1.6
>10days	50 (26)	1,149 (16)	1.98	1.36 - 2.9
Temperature (mean/sd)	(38.6/0.8)	(38.1/0.8)	2.12	1.77- 2.5
<=37.5	16 (8)	1,406 (20)	1	
37.5-39.0	119 (63)	4,776 (68)	2.19	1.29 - 3.7
>39.0	54 (29)	844 (12)	5.62	3.2 - 9.9
Vomiting	36 (27)	1,318 (23)	1.27	0.86 - 1.9
Constipation	6 (5)	321 (6)	0.81	0.35 - 1.9
Diarrhea	13 (10)	491 (9)	1.17	0.66 - 2.1
Bloody diarrhea	1 (1)	93 (2)	0.47	0.06 - 3.4
Seizure	1 (1)	11 (0)	3.99	0.51 - 31.2
Headache	76 (58)	3,363 (59)	0.97	0.68 - 1.4
Abdominal distention	4 (3)	458 (8)	0.36	0.13 - 0.9
Abdominal pain/cramps	100 (76)	4,328 (76)	1.04	0.69 - 1.6
Abdominal tenderness	5 (4)	135 (2)	1.64	0.66 - 4.1

support the evidence that typhoid fever infection is more common in younger children than in older children [1,3,22].

Availability of specific antimicrobial agents for typhoid treatment has reduced typhoid mortality and to some extent complications associated with disease. However, increased urbanization and migration to major cities, especially in south and southeast Asia, has increased the population density of these settings, resulting in higher risk of enteric infections such as typhoid.

Typhoid has challenged health-care providers, especially in developing countries, with non-specific clinical presentation at an early stage. We assessed the association of clinical symptoms and signs of children presenting to study clinics with their blood culture results. Our results show that duration of fever, temperature at the time of presentation, and abdominal distention are statistically significantly associated with antibiotic sensitive *S.* Typhi infection. In the absence of a laboratory support, typhoid is easier to diagnose for health-care providers based on duration of fever, and clinical severity. We did not find any statistical differences in the clinical presentation of blood culture sensitive and MDR *S.* Typhi. Diagnostic sensitivity for typhoid fever increases with duration of fever. Fever duration, however, affects a child's health in addition to direct and indirect economic cost to the family. Prevention strategies for controlling typhoid could overcome the diagnostic challenge typhoid fever poses to health-care providers.

Our incidence estimates are based on one year of surveillance; therefore, we may not have captured the year-to-year variations in typhoid fever epidemiology. This is reflected also in the *S.* Typhi antimicrobial sensitivity patterns.

We did not find any typhoid-associated complications during the study. This may have been due to early diagnosis and management of febrile illnesses. Our results, therefore, may not represent the true burden of typhoid fever complications in Pakistan. There is a need to assess complication rates associated

Table 4. Adjusted odds ratio of factors association with *S.* Typhi infection compared to blood culture-negative fever episodes using logistic regression

	Adj Odds Ratio	95% Confidence Interval	p-value
Age (years)	0.97	0.93 – 1.02	0.27
Duration of fever			
<5 days	1.0		
5 – 10 days	1.35	0.89 – 2.05	0.16
>10 days	3.09	1.96 – 4.86	<.01
Temperature at the time of presentation (°C)	2.49	2.03 – 3.07	<.01
Abdominal distention	0.26	0.09 – 0.72	0.01

Table 5. Comparison of clinical characteristics between blood culture-negative fever episodes and MDR *S.* Typhi

	MDR No. (%)	Culture- negative No. (%)	Odds Ratio	95% CI for OR
	N = (83)	N = (7034)		
Age (mean/sd)	(7.0/3.2)	(7.5/3.8)	0.96	0.91 – 1.02
<5	26 (31)	2139 (30)	1	
5 – 10	39 (47)	2894 (41)	1.11	0.67 – 1.83
>10	18 (22)	2001 (28)	0.74	0.4 – 1.35
Sex (Male)	41 (49)	3508 (50)	0.98	0.64 – 1.51
Duration of fever (mean/sd)	(7.1/4.2)	(6.3/6.0)	1.02	0.99 – 1.04
<5days	25 (30)	2952 (42)	1	
5-10days	34 (41)	2933 (42)	1.37	0.81 – 2.3
>10days	24 (29)	1149 (16)	2.47	1.4 – 4.34
Temperature (mean/sd)	(38.5/0.7)	(38.1/0.8)	2.03	1.56 – 2.64
<=37.5	5 (6)	1406 (20)	1	
37.5 – 39.0	56 (67)	4776 (68)	3.3	1.32 – 8.25
>39.0	22 (27)	844 (12)	7.33	2.77 – 19.43
Vomiting	15 (28)	1318 (23)	1.28	0.71 – 2.34
Constipation	4 (7)	321 (6)	1.35	0.48 – 3.75
Diarrhea	5 (9)	491 (9)	1.09	0.43 – 2.74
Bloody diarrhea	0 (0)	93 (2)	-	
Seizure	0 (0)	11 (0)	-	
Drowsy	0 (0)	38 (1)	-	
Headache	26 (48)	3363 (59)	0.65	0.38 – 1.11
Abdominal distention	2 (4)	458 (8)	0.44	0.11 – 1.82
Abdominal pain/cramps	43 (80)	4328 (76)	1.26	0.65 – 2.45
Abdominal tenderness	3 (6)	135 (2)	2.43	0.75 – 7.9

with typhoid fever cases managed at outpatient clinics and to compare them to rates from hospital-based studies [9,10,12]. Such studies will help in assessing the cost effectiveness of intervention programs for the prevention of typhoid fever.

Since this study enrolled fever cases with a duration of three days or more and consisted of a surveillance targeting typhoidal illnesses, the majority of other organisms that can cause illness in children may have been overlooked. Nevertheless, incidence estimates of typhoid underscore the importance of a public health strategy to contend with complicated childhood illnesses such as typhoid fever. Emergence of antibiotic-resistant strains due to irrational use of advanced antimicrobials by health-care providers is validated by the fact that one-fourth of enrolled individuals gave a history of prior antibiotic use as treatment for fever. Prior antibiotic use may have resulted in culture-negative results for actual typhoid cases [7]. Prior antibiotic therapy was recorded at the time of enrolment. There was no statistically significant association between history of antibiotic use and typhoid fever incidence. However the local population has a lack of knowledge and awareness about the difference between various medicines which made it difficult for us to identify the different antimicrobials previously used. This may explain these unexpected results.

We also found a high rate of antimicrobial resistance in *S.* Typhi as more than 50% of the isolates were resistant to first-line therapy. This high rate of resistance to first-line therapy poses a major problem both in terms of treatment and cost of managing typhoid, as diagnosed cases of typhoid fever are managed as outpatient cases in developing countries. A high resistance pattern against first-line drugs indicates more frequent use of newer generation antimicrobials. Frequent use of cephalosporin can result in the increased cost of treatment, and in turn may affect patient compliance with treatment that subsequently may increase the chances of reduced susceptibility of *S.* Typhi to cephalosporin due to inappropriate dosage.

Although much care was taken to detect cases of *S.* Typhi infection, our study has some limitations worth mentioning. We could not estimate the disease burden for populations younger than two years old and older than 16 years old. The age limitation was partly due to the fact that children younger than two years old were not the target population for the Vi vaccine effectiveness trial that this study was nested into. The study also utilized a passive surveillance system, whereby only patients who visited the project health centres or collaborating private practicing physicians were captured. It is possible that a proportion of patients with febrile illness visited health-care providers outside of the study setting and thus were missed by our surveillance. In addition, the number of *S.* Typhi isolates was low during the early phase of the study mainly because the surveillance system was in the process of being established at that time. A major component of the surveillance system was the participation of private practicing physicians in the study area. The number of *S.* Typhi isolates almost doubled after the inclusion of referrals from private practicing physicians [21]. Therefore, in the initial phase of data collection, we might have missed prolonged fever cases from the private sector. Finally, blood culture confirmation for *S.* Typhi has a sensitivity of only about 50%. In this study, we considered blood culture negatives as true negatives. Hence, our comparison of clinical presentation may have diluted the actual differences in clinical presentation between groups.

The findings from this study highlighted duration of fever and temperature at the time of presentation as important symptoms associated with blood culture-confirmed typhoid fever. Typhoid fever cases present with fever only at the initial stage. Diagnostic clinical sensitivity increases with the duration of fever. To avoid complications associated with poorly managed typhoid fever cases, we recommend that in areas of high endemicity, health-care providers must rule out the possibility of typhoid fever, especially in younger children, by taking into account the history of duration of fever and fever pattern, along with other symptoms and signs.

S. Typhi infection is common in Pakistan. The most affected age group is children younger than ten years of age. Improved sanitation and food hygiene are long-term solutions for reducing or eliminating typhoid fever [23] but these approaches are linked to socio-economic progress, which tends to be slow in typhoid-endemic areas, including Pakistan. While immunization against typhoid has not been widely used in endemic settings, large-scale immunization schemes are being recommended by the WHO as an effective short- to medium-term public health measure against typhoid [24]. Due to the high incidence of typhoid infection and alarming rates of antimicrobial resistance, there is an urgent need for the introduction of typhoid vaccines to control typhoid fever in Pakistan. Two typhoid vaccines are licensed and available in the market but mainly used for travellers

from developed nations to typhoid endemic countries. These are the oral live-attenuated Ty21a vaccine, which requires at least a three-dose regimen, and the parenterally-administered, single-dose Vi polysaccharide (Vi) vaccine. The protective efficacy of the enteric-coated capsule formulation of Ty21a is 62% at seven years after the last dose in areas where the disease is endemic [25]. The efficacy of Vi polysaccharide vaccine was 72% for 17 months [26]. The emergence of multidrug resistance is making therapy increasingly difficult. Preventive programs based on cost-effective, safe, and highly protective vaccines should be utilized d to combat typhoid fever that still affects children in the developing world, especially in South and Southeast Asia.

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