Effectiveness of typhoid conjugate vaccine against culture-confirmed typhoid in a peri-urban setting in Karachi: A case-control study

Introduction

Salmonella Typhi causes a high burden of enteric fever in South Asia, Southeast Asia and sub-Saharan Africa, where it is a major source of morbidity and mortality[1, 2]. *S.* Typhi is responsible for 60% to 80% of typhoid infections in humans, and though mainly endemic, presents epidemic potential [3]. While access to clean water, improved sanitation and hygiene (WASH) is the most effective measure for widespread typhoid control, the high cost is a hindrance to executing these measures in resource-scarce regions [4, 5]. A 2017 global burden of typhoid and paratyphoid fever study estimates 14·3 million cases and 135·9 thousand deaths worldwide annually [6]. In Pakistan, typhoid is the most common bacteremic illness in children, with rates over 400 cases per 100,000 child-years reported from Karachi [3].

While typhoid can normally be treated with antibiotics, strains of *S.* Typhi have exhibited increasing resistance to most of the available antibiotic treatment options due to selective pressure from overuse of antibiotics [2, 7]. In Pakistan, more than 50% of *S.* Typhi strains are multidrug-resistant (MDR), defined as resistant to the first-line antimicrobials (chloramphenicol, trimethoprim-sulfamethoxazole, and ampicillin/amoxicillin), 90% are resistant to fluoroquinolones [8, 9] and 68% are extensively drug resistant (XDR)[10], exhibiting resistance to ceftriaxone along with resistance to first-line antimicrobials and fluoroquinolones [11]. The high number of cases combined with the limited treatment options relying on expensive antibiotics are a great cost on the country's public health system [12] and there is an urgency to expand primary preventive measures such as vaccination to prevent the spread of drug resistant typhoid [2].

Two previously licensed typhoid fever vaccines, a live oral Ty21a vaccine and a parenteral Vi polysaccharide (ViPS) vaccine, are not recommended for use in children less than 2 years old, are relatively poorly immunogenic, cannot produce T-cell mediated immunity and require multiple doses for a good response [4]. Typhoid Conjugate Vaccine (Typbar-TCV®; manufactured by Baharat Biotech International Limited) is a recently pre-qualified vaccine consisting of 25µg of Vi polysaccharide from *S.* Typhi conjugated to tetanus toxoid carrier protein in isotonic saline, licensed as a single intramuscular shot (0·5 mL) that can be safely used from the age 6 months and above [2, 5, 13]. A human challenge study using TCV in a population of immunologically naïve adult volunteers from the UK reported 52% efficacy of TCV against *S.* Typhi bacteremia [14]. Preliminary data from a population-based Phase 3 clinical trial in Nepal comparing TCV with meningococcal vaccine among children aged 9 months to 16 years old reported 85% efficacy of TCV against culture-confirmed *S.* Typhi [15]. Data on post-implementation effectiveness of TCV from campaign settings is limited. Herein, we report the result of TCV effectiveness against culture-confirmed *S.* Typhi in the context of an outbreak of XDR typhoid in Lyari Town, Karachi, Pakistan. Further, we also report the factors that affect the performance of TCV in the real-world setting.

Methods

Setting

Lyari Town, with a population of 846,434, is a densely populated slum settlement in a peri-urban area of Karachi. Lyari is sub-divided into 11 smaller administrative units, called union councils. Although the town has a rich political and ethnic culture, poverty, political turmoil, and social and economic inequalities among different ethnicities have hindered development. Lyari Town often faces acute water shortages, lack of adequate sanitation amenities, wastewater flowing

through municipal sewer systems or household septic tanks, frequent power failures, substandard housing conditions, and poorly maintained roadways. These conditions facilitate transmission of infections, such as typhoid fever, in the area. It was one of towns in Karachi hit badly by the XDR typhoid outbreak that started in November, 2016 [16].

TCV was introduced into the Lyari Town population through a hospital-, school- and community-based mass immunization campaign from 10th April 2019 through 25th October 2019, 87993 children age 6 months to 15 years were vaccinated with a single dose of TCV. The TCV immunization campaign was followed by an age-matched case-control study. Three key healthcare facilities serving Lyari Town, Lyari General Hospital, Aga Khan Jan Bai Secondary Care Hospital and Kharadar General Hospital, were included in the study. These sites were established based on the availability of pediatric outpatient and inpatient care, including diagnosis, treatment, and management of typhoid fever, the availability of laboratory facilities for blood culture, and the willingness of the hospital administration to participate in the study. Previous hospital-based immunization campaigns for TCV had been held at these healthcare facilities.

Kharadar General Hospital is a 210-bed not-for-profit tertiary care and a post-graduate teaching hospital serving more than three million underprivileged residents of Lyari Town. Services are provided at highly subsidized rates, adjusted by donations / Zakat and Patient Welfare Department provides free services to patients who cannot pay. Aga Khan Jan Bai Secondary Care Hospital is a private health care facility providing high quality services to predominantly upper and middle-income residents of Lyari Town. It is a well-equipped 48-bedded hospital with a delivery room, operation theatre, pharmacy, outpatient, inpatient, nursery and a laboratory. It maintains electronic health record system and supports system of referral to the main campus,

Aga Khan University Hospital, diagnostic centers and medical centers. The welfare department provides services to needy patients at minimal or no-charges.

Lyari General Hospital is 525 bedded public tertiary care teaching hospital in Lyari. The hospital has various departments including adult and pediatric emergency, gynecology, institute of cardiology and operating theater complex. The hospital laboratory is outsourced to Dow University and there is a laboratory and collection point within the hospital.

Study Design and Enrollment

We conducted an age-matched case-control study. Blood culture-confirmed typhoid fever patients were identified from pediatric outpatient departments, inpatient departments, and laboratory registers of collaborating health facilities from August 2019 to December 2019. Age-matched controls were enrolled in the control-to case ratio of 1:1 for facility-based controls to case and 2:1 for community-based controls to case.

A case was defined as six months to 15 years old resident of Lyari, visiting any of three sentinel hospitals with culture-confirmed typhoid during the period when the TCV vaccination campaign was conducted, who provided assent and/or their caretakers provided consent for participation in this study. Case-patients were identified through active surveillance for typhoid fever at the participating sentinel hospitals and laboratories. Dedicated trained research associates screened all children with suspected typhoid fever visiting the study sites on a daily basis. Parents of the children meeting the eligibility criteria were offered enrollment and written informed consent was sought from parent as well as child (if child was older than 7 years of age) before data collection.

Hospital controls were eligible if they were at least seven months of age and born within ± six months of the date of birth of a case 6-36 months old or born within ± three years of the date of birth of a case 3-15 years old.

Health facility controls were afebrile children who sought care or were admitted to the same hospital as the case-patient for surgical procedures unrelated to vaccine preventable diseases, as close to the time of the case-patient's onset of symptoms as possible (within seven days). Patients presenting to the emergency department, pediatric ward, orthopedic ward, surgical ward or outpatient department who had had no febrile illness in the four weeks prior to enrollment, who had undergone elective surgery or were admitted for trauma were eligible to be enrolled as hospital controls.

Community controls were defined as afebrile children living in the catchment area, Lyari Town, for at least one month prior to the case-patient's onset of illness and who had no febrile illness in the four weeks prior to enrollment. After enrollment of a case, a member of the study team visited the case-patient's neighborhood to enroll two age-matched controls per case. Once at the case-patient's house, the investigator either went to the left or to the right (determined by a coin toss) and interviewed the fourth neighbor to find an eligible child. If no child met the inclusion criteria or the guardian/s refused to participate, then the investigator went to the fifth house and proceeded sequentially until a control was enrolled. The process continued until all neighborhood controls were enrolled.

Cases and controls were excluded if they were found to be guests or non-residents of Lyari Town or children <6 months or otherwise ineligible to receive TCV.

Sample Size

The sample size was calculated using NCSS PASS version 12. Assuming 5% level of significance, 40% coverage of TCV among controls, 20% correlation between cases and controls (due to matched case-control design), and 55% [17] (matched odds ratio of 0·45) vaccine effectiveness against culture confirmed typhoid fever. Allowing for 15% non-response rate, the required minimum sample size was 82 cases, 82 matched hospital controls and 164 matched community controls to achieve the objective of this study.

Data collection

Parents/caregivers of all enrolled children (cases and controls) were interviewed by trained research associates using a structured questionnaire in the local language. Information was collected on the history of vaccination with TCV, as well as demographic and socioeconomic characteristics and risk factors for typhoid including water, hygiene and sanitation practices. We also inquired about the food exposures during two weeks prior to the onset of symptoms for the cases, and in the two weeks preceding the interview for controls. At enrollment each study subject was assigned a unique identification number and this number was used for future identification. The geospatial location of all case and control households was marked.

Ascertainment of exposure

Vaccination history was collected on all case-patients and control children. During the interview, the parent or guardian was asked for each child's vaccination card. If he/she didn't have the child's vaccination record in the hospital, a home visit was scheduled to review the vaccination record and document dates of vaccination. If the vaccination card was not available or only a

verbal report of vaccination was available, for either case-patients or controls, vaccination status was verified from the local health unit register where the vaccine was administered. If TCV vaccination was verbally reported by parents/guardians, but not proven by vaccination card or vaccination records, the child was not considered vaccinated.

A dose of TCV was defined as relevant (the child was considered vaccinated) if it was administered at least four weeks before the onset of illness in cases or at least four weeks before the enrollment date for controls.

Data management and analysis

All data was collected electronically using e-questionnaires on tablets and data were synced in a secure central server at Aga Khan University Data Management Unit at the end of the day. There were built-in logical checks applied in the questionnaire to avoid illogical responses and missing information. A dedicated data coordinator at the data management unit also screened the data on daily basis to flag any queries or issues in the collected data and respective research associates were informed for necessary corrections. Weekly quality assurance/quality control reports were generated and provided to the research supervisor and principal investigators.

The data were cleaned and imported into STATA version 16 for analysis. For all analyses, p values <0·05 were considered statistically significant. Demographic, socioeconomic and other characteristics of cases and controls were compared using conditional logistic regression to identify significant risk factors for culture-positive S. Typhi.

To determine TCV effectiveness, matched analyses were performed comparing children with typhoid to controls (neighborhood- and age-matched children and healthcare facility- and age-matched children). Vaccine effectiveness (VE) was calculated using the formula:

$$\text{Vaccine effectiveness} = (1-\text{OR}) \times 100$$

We estimated the VE in three groups: cases versus community controls, cases versus hospital controls and cases versus all controls. The primary analysis included all verified reports of vaccine status for children who had received typhoid vaccine versus no vaccine. Multivariable conditional logistic regression was performed to incorporate the effects of potential confounders and effect modifiers. We evaluated the difference in demographics, housing and food and personal hygiene risk factors exposure between cases and controls.

Ethical considerations

The study was approved by the Ethical Review Committee of Aga Khan University Hospital. A child was enrolled into the study if their parent/legal guardian was willing and capable to provide informed consent. If the participant was 7 years of age or older, child assent was also sought.

Results

Out of 82 cases, 28 (34·1%) each were enrolled from Lyari General and Kharadar General Hospitals and the remaining 26 (31·7%) were enrolled from Jan Bai Aga Khan Secondary Care Hospital. A total of 82 hospital and 164 community age-matched controls were also enrolled. Participant ages varied from 6 months to 15 years. The mean age of culture confirmed typhoid case patients was 54·6 months and 68·3% of the cases were males. The mean age of community controls was 58·9 months and 51·8% were male. The mean age of hospital controls was 55·1 months and 56·1% of them were males. Out of 82 case patients, 8 (9·8%) were vaccinated with TCV. Within the control groups, there was higher percentage of vaccination; 38 (23·2%) community controls and 27 (32·9%) hospital controls were vaccinated with TCV. A higher proportion of participants were enrolled in the older age-group, 61-180 months old, as a higher

proportion of the cases were identified in elder age group and age-matched controls were enrolled accordingly (Table 1).

Table 1. Demographic characteristics of Community Controls, Hospital Controls and Typhoid Cases and their domestic hygiene practices

	Community Controls n(%)	**Hospital Controls n(%)**	**Case Patients n(%)**	**Total n(%)**
Male	85 (51·8%)	46 (56·1%)	56 (68·3%)	187 (57·0%)
Age in months (±SD)	58·9 (41·6)	55·1 (36·7)	54·6 (39·4)	56·9 (39·8)
Age groups				
6 - 24 months	47 (28·7%)	21 (25·6%)	24 (29·3%)	92 (28·0%)
25-60 months	41 (25·0%)	26 (31·7%)	26 (31·7%)	93 (28·4%)
61-180 months	76 (46·3%)	35 (42·7%)	32 (39·0%)	143 (43·6%)
Health Facility				
Lyari General Hospital	56 (34·1%)	28 (34·1%)	28 (34·1%)	112 (34·1%)
Khadar Hospital	56 (34·1)	28 (34·1%)	28 (34·1%)	112 (34·1%)
Jan Bai Aga Khan Secondary Care Hospital	52 (31·7)	26 (31·7%)	26 (31·7%)	104 (31·7%)
Vaccinated against typhoid fever	38 (23·2%)	27 (32·9%)	8 (9·8%)	73 (22·3%)
Type of household				
Pucca*	157(95·7%)	80 (97·6%)	80 (97·6%)	317 (96·6%)
Semi-Pucca/Kaccha**	7 (4·3%)	2 (2·4%)	2 (2·4%)	11 (3·4%)
Source of drinking water				

Municipal supply within the house (running water)	101(61·6%)	61 (74·4%)	44 (53·7%)	206 (62·8%)
Bought water	45 (27·4%)	18 (22·0%)	28 (34·1%)	91 (27·7%)
Community tap/ Underground Well / Other	18 (11·0%)	3 (3·7%)	10 (12·2%)	31 (9·5%)
Main food preparer ever been diagnosed with typhoid fever	8 (4·9%)	2 (2·4%)	8 (9·8%)	18 (5·5%)
Times a day is food prepared at home				
Once a day	90 (54·9%)	41 (50·0%)	37 (45·1%)	168 (51·2%)
More than once a day	74 (45·1%)	41 (50·0%)	45 (54·9%)	160 (48·8%)
Food stored in the refrigerator				
Always	95 (57·9%)	42 (51·2%)	45 (54·9%)	182 (55·5%)
Sometimes /Never	69 (42·1%)	40 (48·8%)	37 (45·1%)	146 (44·5%)

*Pucca household refers to a dwelling that is designed to be solid and permanent usually built of stone, brick, cement, concrete, or timber.

** A semi pucca refers to a dwelling of which either the roof or the walls but not both is made of substantial materials like burnt bricks, stone, cement, concrete or timber· Kaccha household is built of mud brick, none of roof or walls is made of solid material.

The study participants (cases, community controls and household controls) had similar food hygiene practices· However, eating outside home was observed to be more frequent in cases as compared to controls (Table 2).

Table 2. Food hygiene practices of Community Controls, Hospital Controls and Typhoid Cases

	Community Controls; n(%)	**Hospital Controls; n(%)**	**Case patients; n(%)**	**Total; n(%)**

Frequency of meals outside home in last four weeks				
Never	65 (39·6%)	25 (30·5%)	14 (17·1%)	104 (31·7%)
Once per month	51 (31·1%)	23 (28·0%)	22 (26·8%)	96 (29·3%)
More than once per month	21 (12·8%)	16 (19·5%)	27 (32·9%)	64 (19·5%)
Once or more per week	27 (16·5%)	18 (22·0%)	19 (23·2%)	64 (19·5)
Consumption of un-boiled/untreated water in the last four weeks				
School	39 (23·8%)	19 (23·2%)	17 (20·7%)	75 (22·9%)
Roadside restaurant	23 (14·0%)	9 (11·0%)	9 (11·0%)	41 (12·5%)
Mosque	112 (68·3%)	58 (70·7%)	55 (67·1%)	225 (68·6%)
Friends/Neighbors house /Relatives	47 (28·7%)	21 (25·6%)	26 (31·7%)	94 (28·7%)
Food items consumed outside home in the last four weeks				
Roadside Cabin / Thaila	69 (42·0%)	38 (46·3%)	35 (42·7%)	142 (43·3%)
Restaurant	64 (39·0%)	38 (46·3%)	32 (39·0%)	134 (40·9%)
School/Office canteen	41 (25·0%)	20 (24·4%)	14 (17·1%)	75 (22·9%)
Friends/Neighbors House/Relatives/Marriage Party	115 (70·1%)	56 (68·3%)	56 (68·3%)	227 (69·2%)
Meals from outside home in last four weeks				
Aloo chat/ Fruit chat	75 (45·7%)	40 (48·8%)	35v (42·7%)	150 (45·7%)
Sugar cane / Fresh squeezed juice	81 (49·4%)	40 (48·8%)	45 (54·9%)	166 (50·6)
Mithai / sweets	80 (48·8%)	46 (56·1%)	36 (43·9%)	162 (49·4%)
Fried fish	65 (39·6%)	40 (48·8%)	31 (37·8%)	136 (41·5%)
Kulfi/ Ice cream	114 (69·5%)	59 (72·0%)	57 (69·5%)	230 (70·1%)

Vaccine Effectiveness (VE)

Using all typhoid negative subjects as controls, the age and gender-adjusted VE was found to be 72% (95% CI: 34% - 88%). Corresponding adjusted VE estimates using only community was found to be 71% (95% CI: 25% - 89%) and in case and hospital controls it was found to be 77% (95% CI: 42% - 91%) (Table 3).

Table 3· Vaccine Effectiveness of TCV among children under 6 months to 15 years of age in Lyari Town, Karachi

	No. of children vaccinated in cases	**No. of children vaccinated in controls**	**OR**	**Unadjusted VE**	**aOR ***	**Adjusted VE**
All Controls	8/82 (9·8%)	65/246 (26·4%)	0·25 (0·10 - 0·59)	75% (41%-90%)	0·28 (0·11 - 0·66)	72% (34% - 88%)
Community controls	8/82 (9·8%)	38/164 (23·2%)	0·27 (0·10 -0·70)	73% (30% - 90%)	0·29 (0·11 - 0·75)	71% (25% - 89%)
Hospital controls	8/82 (9·8%)	27/82 (32·9%)	0·24 (0·09 -0·58)	76% (42% - 91%)	0·21 (0·08 - 0·57)	77% (42% - 91%)

*Adjusted for age, gender

Assessment of Risk Factors for Typhoid Fever

The reported consumption of meals prepared outside the home more than once per month was significantly associated with the development of culture confirmed typhoid fever as compared to

no consumption of food outside: (aOR 3·66; 95% CI: 1·53 - 8·75). Positive history of vaccination with TCV (aOR 0·36 ; 95% CI: 0·14 - 0·90) was significantly associated with decreased risk of typhoid fever (Table 4). Gender, food preparer diagnosed with typhoid fever and eating at a restaurant in last four weeks showed statistically significant association with the risk of typhoid fever in univariate analysis. However, these associations were not statistically significant after adjustment in multivariate analysis.

Table 4. Risk factors for Salmonella typhi disease among cases and age-matched controls in Karachi, Pakistan

	All Controls			
	Univariate Analysis		**Multivariate Analysis**	
Risk Factors	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Gender				
Female	Ref·		Ref	
Male	1·86 (1·10 - 3·15)	0·020	1·61 (0·91 - 2·83)	0·097
Age in years	0·98 (0·96 - 1·00)	0·086	0·98 (0·96 - 1·00)	0·205
Vaccinated against typhoid fever				
No	Ref·		Ref	
Yes	0·21 (0·09 - 0·53)	0·001	0·29 (0·11 - 0·72)	0·008
Source of drinking water				
Municipal supply within the house (running water)	Ref·		Ref	
Bought water**	1·91 (0·99 - 3·69)	0·055	1·67 (0·80 - 3·48)	0·165
Community tap/ Underground Well / Other	1·87 (0·75 - 4·63)	0·178	1·45 (0·54 - 3·90)	0·452
Main food preparer ever been diagnosed with typhoid fever				

No	Ref·		Ref	
Yes	3 (1·01 - 8·90)	0·048	3·25 (0·98 - 10·77)	0·053
Times a day is food prepared at home				
Once	Ref·			
More than once	1·42 (0·84 - 2·37)	0·187		
Frequency of meals outside home				
Never	Ref·		Ref	
Once per month	2·52 (1·09-5·79)	0·029	2·24 (0·90 - 5·57)	0·082
More than once per month	6·75 (2·82-16·18)	<0·001	3·72 (1·55- 8·94)	0·003
Consumption of un-boiled/untreated water in the last four weeks				
School	0·8 (0·39 - 1·62)	0·532		
Roadside restaurant	0·81 (0·35 - 1·84)	0·612		
Mosque	1 (0·45 - 2·21)	1·000		
Friends/Neighbors house /Relatives	0·87 (0·46 - 1·67)	0·676		
Food items consumed outside home in the last four weeks				
Roadside cabin / Thaila	0·94 (0·48 - 1·82)	0·867		
Restaurant	2·16 (1·14 - 4·11)	0·019	1·56 (0·71 - 3·45)	0·262
Friends/Neighbors house /Relatives/ Marriage party	0·90 (0·44 - 1·85)	0·783		
Meals from outside home in last four weeks				
Aloo chat / Fruit chat	0·77 (0·40 - 1·45)	0·424		
Sugar cane / Fresh squeezed juice	1·57 (0·76 - 3·24)	0·215		
Mithai/ Sweets	0·65 (0·36 - 1·19)	0·164		
Fried fish	0·72 (0·38 - 1·38)	0·322		
Kulfi/ Ice cream	0·93 (0·44 - 1·98)	0·847		

* Adjusted for sex, age, vaccination history, source of drinking water, disease history of food preparer, frequency of meals outside home, meals from outside home in last four weeks and food consumed at restaurant in last four weeks

**Bought Water is water that is bought from vendor for drinking or other household purposes but it's not mineral water or not bottled water.

Discussion

This study showed that TCV is effective (VE: 72%) against culture-confirmed typhoid among children aged 6 months to 15 years old in the setting of an outbreak of XDR typhoid in a peri-urban community of Karachi, Pakistan. We found reported consumption of food prepared outside the home as a significant risk factor for the development of culture-confirmed typhoid in our study population.

The confidence intervals for our VE estimates are generally wide, but excluded the null, indicating a statistically significant inverse relationship between single shot of TCV and infection with *S.* Typhi. The wide confidence intervals can be attributed to our small sample size. The overlapping confidence intervals for the VE estimates of the community control and hospital controls suggest that the combined adjusted VE estimate is a justifiably precise estimate of the true VE.

Unpublished data from a cohort and nested case-cohort conducted in Hyderabad Sindh has reported the VE of TCV as 55%, 95% and 97% against suspected, culture confirmed and XDR *S.* Typhi. A recent Phase 3 efficacy trial of TCV in Nepal reported a vaccine efficacy of 81·6% (95% CI: 58·8 - 91·8) among children 9 months to 16 years old. The VE estimates from this

randomized, controlled trial are higher than what we found in our effectiveness study [15]. Vaccine efficacy reported by the randomized control trial differs from post-introduction vaccine effectiveness studies as clinical trials are conducted under optimal conditions and program implementation may vary in the context of routine, real-world use of the vaccine [18]. Moreover, the Nepalese efficacy study was a randomized control trial conducted in a region which has a lower burden of typhoid and a non-outbreak setting.

In Zimbabwe, a TCV vaccination campaign was conducted for three months among children 6 months to 15 years of age as a response to a local outbreak. The TCV vaccination campaign was reported to be highly effective and sharp decline was observed in the incidence of typhoid cases three months following the vaccination campaign. The study did not report VE and the data presented were limited as few blood cultures were performed [19].

A human challenge, single center, phase 2b trial in UK enrolled 112 healthy volunteers aged 18-60 years old with no history of *S.* Typhi infection, typhoid vaccination, or prolonged exposure in typhoid endemic region. Volunteers were either administered TCV, Typbar polysaccharide or Meningococcal vaccine. One month after vaccine administration, they were exposed to the oral ingestion of *S.* Typhi and closely assessed for the development of typhoid infection (persistent fever ≥38°C for at least 12 hours or S. Typhi bacteremia) with blood culture. The study reported vaccine efficacy of 55% (95% CI: 27% -72%) among the volunteers who received TCV as compared to the meningococcal vaccine. Unpublished data from a cohort and nested case-cohort conducted in Hyderabad, Sindh has reported the VE of TCV as 55%, 95% and 97% against suspected, culture-confirmed and XDR *S.* Typhi. The VE of TCV is higher than that reported for Vi capsular polysaccharide typhoid vaccine; 31% among children 5–16 years of age in Karachi [20].

We conducted this case-control study following the introduction of mass immunization campaign with TCV in epidemic region with the predominant strain type being XDR. VE estimates are expected to be lower during outbreak periods as compared to non-outbreak periods. During outbreaks, cases and controls are more likely to have uniform exposure to infection, and diagnosis of milder infections is better because of enhanced public awareness and decreased provider and patient bias against testing vaccinated cases [21].

We found that eating food prepared outside the home was significantly associated with the risk of typhoid fever. Our results are concordant to the findings of studies conducted in Karachi and Indonesia in which eating from an outside food cabin has been reported to be significantly associated with higher risk of typhoid infection [22, 23]. In Pakistan, food stalls and cheap restaurants do not apply for licensing or registration and food authorities do not inspect their food or beverage on regular basis. Some food stalls use un-boiled, unchlorinated water or tap-water, contaminated containers, and may have poor hygiene and sanitation. Eating food prepared outside the home exposes children to wide variety of food preparers, some of whom can be chronic carriers of *S.* Typhi. The street vendors and cheap restaurants buy ice from ice-factories that is made of un-clean water not safe for human consumption. As *S.* Typhi survives for prolonged periods in ice, ice used by the street vendors and small restaurants is a potential source of infection. Consumption of cold beverages in Santiago, Chile [24] and the Philippines [25] or ice cream in Pakistan [22] has been previously reported to be associated with developing typhoid fever.

The results of this study should encourage policymakers to consider the use of TCV vaccine as a public health tool in outbreak settings, particularly when immediate protection is crucial, as in the prevention of typhoid outbreaks in slum dwellings. Poor food hygiene practices among adult

food handlers is a leading cause of spread of typhoid. Effective strategies to provide health education on food safety and hygiene of food handlers should be considered to avoid food contamination risks. Access to the safe water should be assured in the impoverished areas of urban slums to avoid further outbreaks.

More evidence is still needed with respect to long-term protection of this TCV vaccine. It is uncertain whether the vaccine induces indirect protection, “herd immunity”, in addition to direct protection of those vaccinated. It will be important for future studies to evaluate more comprehensively the combined direct and indirect effects of the TCV on AMR. Furthermore, the effect of TCV on long term carriage of *S.* Typhi is of concern and requires future research.

WHO has granted prequalification to a second TCV, TYPHIBEV, in December 2020. TYPHIBEV is also manufactured in Bharat, by Biological E, India. TYPHIBEV is a TCV where the Vi polysaccharide is conjugated to the CRM197 protein, a single shot can be given to children over 6 months of age and up to 45 years old adults. Its safety and immune response profile are similar to Typbar and it can be given concomitantly with measles vaccine at 9 months. There are many TCVs in the different stages of development , which in future would provide more alternatives, formulations, and manufacturers. The expanding and bolstering TCV market is promising against vaccine supply bottlenecks [26].

It should be noted that these vaccines are monovalent and designed to protect against disease due to *S.* Typhi. There will be an attributable level of enteric fever that will not be affected by the conjugate vaccines, to the extent that *S.* Paratyphi A contributes to the overall incidence of enteric fever in a particular setting. The change in epidemiology after TCV introduction needs to be assessed through long term surveillance in areas of TCV introduction. The burden of *Salmonella* paratyphi along with *S.* typhi is becoming more evident, predominantly in Asia,

which leads to an increased interest in *S.* typhi and paratyphi A bivalent vaccines. A number of bivalent vaccine (*S.* typhi and *S.* paratyphi A) candidates are in the stage of preclinical development.

Limitations

This case-control study, like all observational studies, could be subject to selection and information biases. Confounding was minimized because controls were matched with cases by age and information bias was minimized because data were obtained in the same way from cases, hospital and community controls. It seems unlikely that information bias may invalidate our results because information on the disease status was obtained from blood culture reports in health records from participating hospitals. Information on exposure (vaccination status) was verified by vaccination card. In addition, variables that could confound the results were adjusted in the conditional regression analysis. However, the effect of some residual confounding cannot be ruled out.

A single dose of TCV is effective against culture confirmed typhoid among children aged 6 months to 15 years old in an XDR typhoid outbreak setting of a peri-urban community in Karachi, Pakistan. Eating food prepared outside the home more than once during a month was significant risk factor for contracting typhoid among the study population. Many queries remain, including the duration of sero-protection, financing schemes for sustainable supply and delivery of the vaccine, and direct and indirect protective effects of the vaccine to control typhoid fever. Considering the potential impact of TCV in control of typhoid, policymakers, donors, manufacturers and financing bodies at the global level should consider working towards

strengthening capacity to ensure sufficient quantities of vaccine are produced, accelerate early WHO prequalification, continue Gavi financing support, define vaccination strategies, and facilitate support for policy and access.

Credit Author Statement

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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