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Risk factors for typhoid fever in an endemic setting, Karachi, Pakistan

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SUMMARY

We conducted a study to evaluate risk factors for developing typhoid fever in a setting where the disease is endemic in Karachi, Pakistan. We enrolled 100 cases with blood culture-confirmed *Salmonella typhi* between July and October 1994 and 200 age-matched neighbourhood controls. Cases had a median age of 5·8 years. In a conditional logistic regression model, eating ice cream (Odds ratio [OR] = 2·3; 95% confidence interval [CI] 1·2–4·2, attributable risk [AR] = 36%), eating food from a roadside cabin during the summer months (OR = 4·6, 95% CI 1·6–13·0; AR = 18%), taking antimicrobials in the 2 weeks preceding the onset of symptoms (OR = 5·7, 95% CI 2·3–13·9, AR = 21%), and drinking water at the work-site (OR = 44·0, 95% CI 2·8–680, AR = 8%) were all independently associated with typhoid fever. There was no difference in the microbiological water quality of home drinking water between cases and controls. Typhoid fever in Karachi resulted from high-dose exposures from multiple sources with individual susceptibility increased by young age and prior antimicrobial use. Improving commercial food hygiene and decreasing unnecessary antimicrobial use would be expected to decrease the burden of typhoid fever.

INTRODUCTION

Worldwide, an estimated 16 million episodes of typhoid fever causing 600000 deaths occur each year [1]. The overwhelming majority of infections and deaths occur in developing countries where typhoid fever is endemic [1]. Recently the situation has worsened with the emergence and wide dissemination of multiply drug resistant organisms in several countries throughout Asia [2–4] and Africa [5–7]. The spread of these organisms has been accompanied by case-fatality rates approaching those reported from the pre-antimicrobial treatment era [8]. At the Aga Khan University Hospital Laboratory in Karachi, Pakistan, *Salmonella typhi* is the organism most commonly isolated from blood cultures. Six percent (1166/19342) of all blood and bone marrow cultures grew *S. typhi* in 1994; 53% of these isolates were resistant to ampicillin, co-trimoxazole, and chloramphenicol (S. Fisher-Hoch, Aga Khan University Hospital Laboratory, personal communication).

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Despite this marked public health burden, little is known about *S. typhi* transmission or risk factors for infection or disease in Karachi or other typhoid-endemic settings. The vast majority of epidemiologic investigations of *S. typhi* transmission have been conducted during acute typhoid fever outbreaks [9, 10]. It is unclear how valid the conclusions from these investigations are to a city such as Karachi, where there are over a thousand [11] culture-confirmed cases of *S. typhi* each year and presumably many times more cases that are not culture confirmed, all occurring without an identified source. Black and colleagues evaluated endemic *S. typhi* transmission in Santiago, Chile, in 1981 and found that purchasing lunch at school rather than bringing lunch from home, sharing food items with friends, and eating flavored ice purchased outside the home were all associated with typhoid fever [12]. Compared to Santiago in 1981, Karachi in 1994 apparently has a much higher incidence of typhoid fever, a substantially different social setting, and a less complete water and sanitary infrastructure, and so routes of transmission for *S. typhi* may well differ. To better direct public health interventions, we conducted a study to identify the risk factors for developing typhoid fever in Karachi.

METHODS

Setting

Karachi, the largest city in Pakistan, is a developing country mega-city with an estimated population of 10 million people. The Aga Khan University Hospital Clinical Laboratory in Karachi is a private university-affiliated laboratory which has collection points throughout the city, and so draws users from all regions, including a substantial number of low-income persons who use the laboratory in times of serious illness.

Design

We conducted a neighbourhood and age-matched case-control study. We identified consecutive cases from among Karachi residents who had *S. typhi* isolated from a blood culture at the Aga Khan University Hospital Laboratory between 3 July and 9 October 1994, except for the first 10 days in September when the team of interviewers was unavailable. We excluded persons who had travelled overnight outside of Karachi in the previous month. We interviewed consenting cases, or a parent, if the case was under age 12, in their homes. To recruit controls we first identified the front door of the household nearest the case's household. Secondly, we avoided back-tracking and identified the next closest household. We repeated this process until we identified the household six doors away from the case household. We then inquired if there was someone in the household close in age to the case (If age < 12 months, ± 3 months; 12–24 months, ± 6 months; 2–5 years, ± 1 year; 5–10 years, ± 2 years; 10–20 years, ± 3 years; 20–30 years, ± 5 years; > 30 years, ± 10 years). We excluded potential controls who were either currently febrile or had 5 days of consecutive fever within the preceding 4 weeks or who had travelled outside of Karachi in the preceding 4 weeks. If no eligible controls were found or if persons in the household refused to participate, we went to the next closest front door. We repeated this process until we had two controls for each case.

We inquired about food exposures in the 2 weeks prior to onset of symptoms for the cases, and in the 2 weeks preceding the interview for controls. We chose a 2-week time frame rather than including the incubation period of up to 4 weeks or longer demonstrated for *S. typhi* because volunteer studies suggest that 80% of typhoid fever cases have incubation periods of 14 days or less [13], and because during questionnaire pre-testing, attempts to evaluate exposures of cases in the 28 days preceding onset of symptoms yielded too many uncertain responses. We asked how food was prepared and stored in the home and what the study subject's sources of food were outside the home. We asked whether they consumed specific food items we considered likely to be vehicles for *S. typhi* transmission based on locally available foods and on a review of foods identified in previous published investigations of *S. typhi* outbreaks. We collected detailed information on the study subject's drinking water.

Interviewers asked cases to identify the initial symptoms associated with their illness and the date when this symptom occurred. Interviewers next asked if the cases had taken any antimicrobials in the 2 weeks prior to the onset of their first symptom. If cases answered yes, interviewers questioned them to confirm that the antimicrobials were taken for a different reason than the symptoms that led to a blood culture.

Because reliable estimates of household income are difficult to obtain in Karachi and nearly impossible to

verify, we constructed two indices to compare the relative wealth of case and neighbourhood-matched control households. We calculated the person-per-bedroom ratio, that is, the number of persons living in the household divided by the number of bedrooms; and the person-per-toilet ratio, i.e., the number of persons living in the household divided by the number of toilets.

Laboratory

To culture *S. typhi* from blood, 5 ml of venous blood from adults, and 1–2 ml from children, was inoculated into 45 ml each of brain–heart infusion and thioglycolate broth, and incubated at 37 °C for 7 days. Each bottle was examined daily for visual evidence of growth and routinely subcultured to blood agar and MacConkey's agar plates (Oxoid, Basingstoke, United Kingdom). Non-lactose fermenting colonies on MacConkey's agar were biochemically identified as *S. typhi* by using API 20E (BioMerieux, Marcy l'Étoile, France). Serological identification of *S. typhi* was performed by slide agglutination using *Salmonella* species specific antisera. Antimicrobial sensitivities for ampicillin (10 μg), chloramphenicol (30 μg) and co-trimoxazole (25 μg) were determined by standard disk diffusion techniques, using the method according to Kirby Bauer [14].

The field team asked the study respondents, to identify their usual source of drinking water, and collected 40 cc of drinking water directly from that source into sterile sampling bottles containing sodium thiosulphate to neutralize residual chlorine. The water was kept in a cool box and transported to the Aga Khan University Hospital Laboratory and tested within 6 h of collection. The water samples were examined for the presence of coliform bacteria with the multiple tube fermentation technique [15]. Initially, equal volumes of drinking water were added to single-strength MacConkey broth (40 g/l); however, because of the high levels of contamination encountered, we ultimately elected to add 50, 10 and 1 ml volumes of water to 50-ml volumes of double strength (80 g/l) MacConkey broth. Using a standard nomogram, we derived the most probable number which is a statistical estimate of the number of coliform bacteria per 100 ml of water.

To establish whether the main food preparer in the home was a chronic carrier of *S. typhi* we collected a blood sample from him or her. Serum was separated and assayed for antibody to the *Salmonella typhi* Vi antibody (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) [16, 17].

Statistical analysis

Since *S. typhi* infection is endemic in Karachi we assumed that there would be multiple ways to acquire infection, and that the risk from any single route might therefore be relatively small. We modelled a broad range of exposure rates, odds ratios, and degrees of discordance between cases and controls. Ultimately, we chose to enroll 100 cases and 200 controls because we judged these would provide sufficient statistical power to identify risk factors over a wide range of prevalences and strengths of association.

For univariate analysis we evaluated the difference in categorical exposures between cases and controls by calculating the matched odds ratio (OR) and *P* values using maximum likelihood estimates. We calculated exact confidence limits. We evaluated the difference in exposures measured by continuous variables by calculating the difference between the value for each control versus the value for the matched case, and then performing a *t* test to see if the distribution of the differences was significantly different from zero [18].

We expected to identify multiple routes of transmission. Because specific foods and other exposures would be expected to be closely associated with each other, we controlled for confounding through a multivariate analysis. We placed all of the exposures with a *P*-value $\leqslant 0{\cdot}05$ on univariate analysis into a conditional logistic regression model. For the one continuous variable that was significantly different between cases and controls, the person to bedroom ratio, we recoded it as a categorical variable, based upon whether or not the value was above the median for the combined case and control distribution, and entered it into the model. We then removed variables one at a time that did not contribute significantly to the fit of the model. We calculated attributable risk and the summary attributable risk by using the multivariate odds ratio and percentage of exposed among the cases [19].

RESULTS

The median age of our 100 cases was 5·8 years (range 4 months–70 years); 71 % were under 10 years of age.

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Table 1. *Univariate analysis of risk factors for* Salmonella typhi *disease among 100 cases and 200 age- and neighbourhood-matched controls, Karachi, Pakistan, 1994*

Risk factor	Number and (%) of cases with this risk factor	Number and (%) of controls with this risk factor	Matched odds ratio (Maximum likelihood estimate)	Exact 95% confidence limit	*P*-value
Water purity					
Home water					
Grossly contaminated	50 (63)	97 (63)	1·1	0·6, 2·1	0·46
Clean home drinking water†	16 (16)	32 (17)	0·9	0·4, 1·9	0·48
Drinking water outside home					
Anywhere outside a home	45 (45)	75 (38)	1·5	0·8, 2·7	0·10
Work	8 (8)	3 (2)	13·1	1·7, 591·5	0·003
School	16 (16)	42 (21)	0·6	0·3, 1·4	0·15
Restaurant	19 (19)	25 (13)	1·8	0·8, 3·8	0·08
Mosque	5 (5)	11 (6)	0·9	0·2, 3·2	0·55
Community tap	1 (1)	14 (7)	0·1	0·0, 0·9	0·02
Eating outside the home					
During the whole study period					
Eating out > 1 week	36 (36)	73 (37)	1·0	0·6, 1·7	0·52
Eating at a roadside cabin	17 (17)	20 (10)	1·8	0·8, 3·6	0·07
Eating at a restaurant	36 (36)	60 (30)	1·4	0·8, 2·6	0·15
Eating at school	11 (11)	23 (12)	0·9	0·4, 2·2	0·51
Eating from a street vendor	13 (13)	26 (13)	1·0	0·4, 2·0	0·56
Eating at a friend's home	11 (11)	20 (10)	1·1	0·5, 2·6	0·47
Eating at a wedding	24 (24)	40 (20)	1·3	0·7, 2·3	0·26
During July and August‡					
Eating out > 1 per week	28 (49)	37 (32)	2·3	1·0, 5·2	0·02
Eating at a roadside cabin	13 (23)	12 (11)	2·4	1·0, 5·6	0·03
Eating at a restaurant	26 (46)	33 (29)	2·7	1·1, 6·6	0·01
Eating food prepared by a street vendor	7 (12)	14 (12)	1·0	0·3, 2·7	0·58
Eating from a friend's home	9 (16)	11 (10)	1·7	0·6, 5·0	0·18
Eating at a wedding	12 (21)	20 (18)	1·2	0·5, 2·9	0·36
Home cooking practices					
Cooking food > once per day	57 (57)	113 (57)	1·0	0·6, 1·8	0·54
Storing food in a refrigerator	63 (63)	133 (67)	0·8	0·4, 1·5	0·29
Always reheating old food before serving	67 (85)	152 (91)	0·6	0·2, 1·6	0·17
Food items					
Lassi (a mixture of yogurt, ice, and water)	24 (24)	46 (23)	1·1	0·6, 2·1	0·48
Aloo Chola (mixed fresh vegetables)	18 (18)	40 (20)	0·9	0·5, 1·7	0·40
Ice lolly (frozen fruit juice)	16 (16)	39 (20)	0·8	0·4, 1·6	0·28
Sugar cane juice	8 (8)	21 (11)	0·7	0·3, 1·9	0·31
Fruit *chaat* (fruit salad)	2 (2)	10 (5)	0·4	0·0, 1·9	0·17
Mithai (a sweet with dairy products)	47 (47)	98 (49)	0·9	0·5, 1·6	0·42
Fried fish	23 (23)	68 (34)	0·6	0·3, 1·0	0·03
Kulfi (a cream based desert)	12 (12)	48 (24)	0·4	0·2, 0·9	0·01
Ice cream	64 (64)	104 (52)	1·7	1·0, 3·1	0·03
Commercial brand of ice cream	51 (51)	80 (40)	1·6	1·0, 2·9	0·04
Non-branded ice cream	15 (15)	23 (12)	1·4	0·6, 3·1	0·24
Fresh squeezed fruit juice	29 (29)	43 (22)	1·6	0·9, 3·0	0·07
Antimicrobial use					
Antimicrobials in the 2 weeks prior to illness	25 (25)	22 (11)	3·0	1·4, 6·5	0·001

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Table 1. (*cont.*)

Risk factor	Number and (%) of cases with this risk factor	Number and (%) of controls with this risk factor	Matched odds ratio (Maximum likelihood estimate)	Exact 95% confidence limit	*P*-value
Antimicrobials in the 2 weeks prior to illness, restricted to sensitive cases§	8 (24)	22 (11)	3·0	0·8, 14·3	0·06
Antimicrobials in the 2 weeks prior to illness, restricted to resistant cases**	17 (27)	22 (11)	3·0	1·2, 7·8	0·007
Contact with typhoid patients					
Contact with persons diagnosed with typhoid in the preceding 4 weeks	18 (18)	30 (15)	1·3	0·6, 2·6	0·30
Food preparer ever having a diagnosis of typhoid	29 (29)	43 (22)	1·5	0·9, 2·5	0·11
Vaccine					
Ever received typhoid vaccine	8 (8)	20 (10)	0·7	0·2, 2·1	0·35
Received typhoid vaccine in the preceding 2 years	6 (6)	19 (10)	0·5	0·1, 1·7	0·16

* Most probable number of coliforms > 50 CFU per 100 ml for the 79 cases and 156 control water samples analysed.
† Most probable number of coliforms = 0 CFU per 100 ml among the 94 cases and 188 control water samples analysed.
‡ Restricted to the 57 cases and 114 controls who were interviewed for exposures during July and August.
§ Restricted to the 34 cases whose isolates were sensitive to ampicillin, chloramphenicol and cotrimoxazole.
** Restricted to the 63 cases whose isolates were resistant to ampicillin, chloramphenicol and cotrimoxazole and their 124 matched controls.

Cases below 10 years were similar in age to controls (54·7 *vs.* 54·4 months, $P = 0{\cdot}95$). Older cases were slightly older, by a mean 12 months, than controls (19 years 11 months *vs.* 18 years 11 months, $P = 0{\cdot}01$). Cases and controls did not differ significantly by sex distribution (56% males *vs.* 48%, $P = 0{\cdot}11$). There were a mean 2·7 persons per bedroom living in case households, which was a mean 0·3 persons per bedroom more than their matched controls ($P = 0{\cdot}01$). There was not a significant difference in the number of persons per toilet between case and control households (case mean = 3·3, mean difference = 0·05, $P = 0{\cdot}83$).

Overall, cases and controls were equally likely to eat meals outside the home during the entire study period (Table 1). However, when analysis was restricted to when the study subject's evaluated exposure occurred in July and August, corresponding to the time that most children are out of school for summer break, cases were 2·3 times more likely to eat outside the home at least once per week (49% *vs.* 32%, Matched Odds Ratio [OR] = 2·3, 95% confidence interval [CI] 1·0–5·2), 2·4 times more likely to eat from a roadside cabin (23% *vs.* 11%, OR = 2·4, 95% CI 1·0–5·6), and 2·7 times more likely to eat at a restaurant (46% *vs.* 29%, OR = 2·7, 95% CI 1·1–6·6).

Ice cream was the only queried food item that was more commonly eaten by cases at any time during the study than controls (64% *vs.* 52%, OR = 1·7, 95% CI 1·0–3·1). When evaluated separately, commercially branded ice cream was associated with a significantly increased risk. The risk associated with non-branded ice cream was of similar magnitude, but it was a less common exposure, and the difference was not statistically significant (Table 1). There was no difference between case and matched control households in the number of times they prepared food daily or whether leftover food was refrigerated or reheated before serving.

Cases and controls drank a similar amount of water during the 2-week recall period (mean 66·5 glasses for cases, 5·5 glasses more for controls, $P = 0{\cdot}12$). Drinking water from home was the most common source, accounting for 87% of the study subject's reported water consumption. One hundred cases (100%) and 193 controls (97%) provided a home drinking water sample for analysis. Only 48 (16%) of these samples were clean, i.e., they had a most

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Table 2. *Multivariate analysis using conditional logistic regression of risk factors for* Salmonella typhi *infection among 100 cases and 200 age and neighbourhood-matched controls, Karachi, Pakistan, 1994*

Exposure in the 2 weeks preceding illness	Conditional odds ratio	95% confidence limits	*P* value	Attributable risk
Eating ice cream	2·3	1·2, 4·2	0·008	0·36
Taking antimicrobials previously	5·7	2·3, 13·9	0·0001	0·21
Eating food from a roadside cabin during the summer	4·6	1·6, 13·0	0·004	0·18
Drinking water at work	44·0	2·8, 680·2	0·007	0·08
Drinking water from a community tap	0·03	0·003, 0·331	0·004	

probable number of 0 coliform bacteria [20]. Cases were no less likely to have clean household drinking water than controls (16% *vs.* 17%, OR = 0·9, $P = 0·48$). The mean level of bacterial contamination of water among cases was 708 CFU/100 ml; cases had a mean 99 fewer CFU/100 ml than their matched control ($P = 0·24$).

Cases were significantly more likely than controls to report drinking water at work (8% *vs.* 2%, OR 13·1, 95% CI 1·7–591) (Table 1). Water drunk at work for both cases and controls was less likely to be treated by any method, e.g., boiling or filtering, than water drunk at home (26% *vs.* 73%, $P = 0·002$). Subjects who did drink water at work reported drinking a median one glass per day. Because of the young age of the study population this was an infrequent exposure ($n = 11$) among cases and controls.

Cases were three times more likely to report taking antimicrobials in the 2 weeks preceding the initial symptom of their illness, compared to controls in the 2 weeks preceding their interview (25% *vs.* 11%, OR 3·0, 95% CI 1·4, 6·5). Sixty-two (62%) of *S. typhi* strains from cases were resistant to all three first-line antimicrobials (ampicillin, co-trimoxazole and chloramphenicol); 34 (34%) were sensitive to all three. This risk of prior antimicrobial use was of similar magnitude when the analysis was restricted to those cases whose isolates were sensitive to all first line antimicrobials, or to those cases whose isolates were resistant to them (Table 1). Fifteen (60%) of the 25 *S. typhi* cases who reported prior antimicrobial use took an antimicrobial that contained either ampicillin or amoxicillin. Cases reported taking a median 15 doses of antimicrobial medication.

The primary food preparer was a family member in 95% of households. We collected a serum sample from the food preparer in 78 case households (78%) and 130 (65%) control households. The V_i antibody titer for each of the 208 specimens was $< 1:40$, suggesting that none of these food preparers was a typhoid carrier.

Typhoid fever cases did not report contact with persons who had typhoid fever in the 4 weeks preceding their illness significantly more frequently than controls in the preceding 4 weeks (18% *vs.* 15%, OR = 1·3, $P = 0·30$), nor were cases more likely to report that the main food preparer in the home had ever been diagnosed with typhoid fever (29% *vs.* 21%, OR = 1·5, $P = 0·11$). Whole killed typhoid vaccine was available in Karachi, but its use was equally infrequent among cases (8%) and controls (10%) (Table 1).

On multivariate analysis, using conditional logistic regression, five exposures remained independently associated with *S. typhi* infection (Table 2). Four of these exposures, eating ice cream, taking antimicrobials, eating food from a roadside cabin during the summer, and drinking water at work, increased the risk of illness. The summary attributable risk for these four exposures was 77%. Eating ice cream accounted for 36% of the illness and taking antimicrobials for 21% (Table 2). Drinking any water from a community tap was associated with a lower frequency of *S. typhi* infection. Fourteen controls reported drinking water from a community tap. They drank a mean 4·9 glasses from this source, which accounted for a mean 5·4% of their 2-week water consumption. By contrast the 8 cases who reported drinking water at work, drank a mean 16 glasses or 23% of their 2-week consumption from this source.

We evaluated whether exposures would have a different effect among different age-groups by stratifying on age (categories were $\leqslant 12$ months, 13–24 months, 25–60 months, 5–10 years, and > 10 years) and evaluating the matched odds ratios for each of the univariate exposures looking for marked differences

in stratum specific estimates. We found no strong interaction between exposures and age group.

DISCUSSION

Endemic typhoid is different from epidemic typhoid. In a typical typhoid fever outbreak, there is a large susceptible population with one primary source of *S. typhi* [9, 21]. The central epidemiologic question is, 'What is the vehicle of transmission?' In endemic typhoid, by contrast, there is widespread exposure to the pathogen through multiple vehicles; the central question becomes, 'Why do some people develop disease and others do not?' One reason clearly relates to immunity. Children who have less developed immunity to *S. typhi* than adults, have increased susceptibility to disease, as illustrated by the median age of 5·8 years for our cases. We matched on age in this study so that we could evaluate other risks.

Two of the risk factors identified in this study, eating commercially packaged ice cream (AR 36%) and eating food from a roadside cabin during the summer (AR 18%), probably represent high-dose exposure to *S. typhi*. Among human volunteers, disease attack rates increased with increasing ingested dose of *S. typhi* [21]. Dairy products may be a particularly effective media for *S. typhi* growth. In our study eating ice cream accounted for the greatest proportion of the infections. In a recent review of 68 epidemiologic investigations of typhoid outbreaks, the vehicle of transmission was milk in 11 (16%) and ice cream in 3 (4%) [9]. Within Pakistan, a 1989 study of 50 samples of commercially prepared ice cream from four different manufactures found 42 (84%) were contaminated with faecal coliforms at a geometric mean concentration of $1{\cdot}1 \times 10^3$ CFU/gm [22]. The association between eating ice cream and typhoid fever in Karachi presumably resulted from contamination during manufacture of branded ice cream and conditions that permitted rapid multiplication of *S. typhi*.

Roadside cabins in Karachi are small, open-air eating establishments typically without refrigerators and with very limited facilities for cleaning foodstuffs and utensils. The mean daily maximum temperature in Karachi during July and August 1994 was 31·6 °C (Karachi Regional Meteorological Centre, personal communication), which is sufficiently warm to permit rapid doubling of a human enteric pathogen. The fact that eating food from a roadside cabin was associated with *S. typhi* infection during the early weeks of the study, but not in the later weeks, may reflect changing sources of infection over time. Another possible explanation is that the association between eating from roadside cabins and infection with *S. typhi* was due to chance. The study evaluated a large number of exposures, and separate evaluation of July and August exposures was not an *a priori* hypothesis. However, there was a break in the sample collection after August, and an interim analysis suggested that roadside cabins were sources of *S. typhi* infection during the first 2 months of the study.

Taking antimicrobials in the 2 weeks preceding the onset of symptoms accounted for 21% of *S. typhi* infections and likely reflects altered gut susceptibility to replication and/or invasion by *S. typhi*. Although to our knowledge, antimicrobial use has not been previously reported to increase risk of *S. typhi* disease, prior antimicrobial use has been associated with disease from various serotypes of antimicrobial-resistant Salmonella [23–27] and in one investigation, with an antimicrobial sensitive strain [28]. The epidemiologic association between Salmonella infection and antimicrobial exposure is supported by animal studies. The number of Salmonella organisms required to cause systemic infection in mice pretreated with antibiotics is 100000 fold lower than the dose required to cause illness in untreated mice [29, 30]. Antimicrobials are available without a prescription throughout Pakistan, as is the case in many developing countries. Misuse of antimicrobials is common [31, 32]. In the setting of diminishing therapeutic options to treat this deadly infection, preventing infections by limiting antimicrobials to clinical situations where they are indicated is an important, albeit difficult, public health measure.

Although 50% of typhoid fever outbreaks were associated with contaminated water [9], the quality of home drinking water, which represented the bulk of water consumed by study subjects, was not associated with *S. typhi* infection in Karachi. The most likely explanation is that home drinking water, if contaminated, presented a dose of *S. typhi* below the threshold for infection for most partially immune Karachiites. Water contaminated with *S. typhi* is believed to have lower concentrations of organisms than contaminated foods [33]. Black, in his study of endemic typhoid in Santiago also reported no association with water source and typhoid fever [12]. Indeed, Black noted that the incidence of typhoid fever increased in Santiago between the 1940s and 1970s, during which time access to potable water

became almost universal [12]. Drinking water at work in Karachi was associated with typhoid fever (OR = 44, AR = 8%). This may represent more heavily contaminated water at work with little effort to purify it, or it may be a marker for persons who either have little choice or are careless in choosing their sources of both water and food. Clearly, Karachi drinking water is not microbiologically safe, but this study suggests that improving water quality alone in Karachi homes would not be expected to substantially decrease the number of persons developing typhoid fever.

Drinking water from a community tap, a source of water in some low-income communities, was associated with a lower frequency of infection, though it accounted for only 5% of reported water consumption by controls. This counter-intuitive finding probably represents some difference in socio-economic status between cases and controls that was incompletely controlled through neighbourhood matching. Obtaining a blood culture from the Aga Khan University Hospital Laboratory involves some additional cost and implies that an ill child's family sought out an above average health care provider in that the care provider had enough expertise in clinical medicine to understood the value of a blood culture. We attempted to control for differences in socio-economic status through a neighbourhood matched design and measuring differences in bedroom and toilet crowding. Notably, there were significantly fewer persons per bedroom among case households than controls, suggesting cases did have somewhat larger homes, and presumably higher incomes. On multivariate analysis, however, it was not bedroom crowding but a particular water source that apparently best captured and controlled this difference in socio-economic status.

Seventy-seven percent of typhoid fever in this study was attributable to the four identified risk factors. One fourth of cases acquired *S. typhi* through means that the study did not identify. While some of these persons may have acquired *S. typhi* through the identified sources, but failed to recall them during the interview, others presumably acquired *S. typhi* either from a source that occurred too infrequently to be statistically significant in a study of this size or from sources that we failed to inquire about.

The validity of a non-blinded case-control study can be compromized by interviewer and recall bias. Since the interviewers in this study knew whether a study subject was a case or control it is possible they might have elicited exposure information more aggressively from controls than from cases, and thus introduced bias. However, the questionnaire was standardized so that questions were asked of cases and controls in the same way, and the interviewers were specifically instructed to avoid differentially seeking exposure information from cases. It is also possible that respondents in case households reflected upon their exposures prior to illness more carefully than controls, and so may have more fully reported exposures than controls. The time frame of the case and control questionnaire, however, was different. Cases were asked about exposures in the 2 weeks prior to the onset of their first symptom, while controls, were asked to recall exposures in the 2 weeks immediately preceding the interview. We expected respondents to remember meals they ate outside the home during this time frame and whether or not they took medication. The individual food items and amount and sources of water might be more difficult to recall. However, the mean number of glasses of water which cases and controls recalled drinking during the 2 week recall was similar and the percentage of recall on consuming food items listed in Table 1 are also quite similar suggesting that there was no marked difference in recall between cases and controls.

The picture that emerges from this analysis is that of a pathogen widely dispersed throughout the city which is primarily acquired outside of the home. Home cooking and food storage practices were similar in the houses of cases and controls, but there was an increased risk associated with eating foods outside of the home in the summertime. Microbiological quality of home drinking water was not associated with *S. typhi* infection, but the practice of drinking water at work was. None of the 208 home food preparers had serological evidence of being chronic *S. typhi* carriers. The one specific food associated with *S. typhi* was ice cream, which was manufactured and packaged outside the home. Thus, efforts to improve commercial food hygiene and sanitation are more likely to be effective in preventing *S. typhi* infections in Karachi than are efforts directed at improving hygiene in homes.

As multiply drug-resistant *S. typhi* continues to spread, clinicians in endemic countries caring for patients with presumed typhoid fever are often forced to choose between drugs that may be ineffective, or fluoroquinolones or third-generation cephalosporins [8], the cost of which is beyond the means of many persons at highest risk for this disease. Clearly, the best approach is prevention. Infrastructure and economic development is most effective [34] and

should be encouraged, but is, at best, decades away for many typhoid fever endemic countries. Continued efforts to develop and distribute low-cost vaccines that provide earlier immunity to children as well as a better and longer duration of immunity may help alleviate the problem in the intermediate term. While awaiting these developments, immediate efforts to improve commercial food hygiene in Karachi and to limit unnecessary antimicrobial use can help prevent morbidity and mortality from typhoid fever.

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