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Burden of antibiotic resistance among children with typhoid in Gadap Town, Karachi, Pakistan

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ABSTRACT

Background: Increasing antibiotic resistance by pathogenic bacteria is observed in poor sanitary conditions. The peak incidence of typhoid occurs between 5–15 years of age. This is the most common bacteraemic illness of children in Pakistan. The aim of this study was to investigate the frequency of drug-resistant *Salmonella* Typhi and *S. Paratyphi A* in children hospitalized or treated as outpatients at a tertiary care centre that serves Gadap Town, an extensive slum district of Karachi. **Methods:** A total of 275 peripheral blood samples were collected from children up to 14 years old who presented with clinical features of typhoid to Fatima Hospital, Baqai Medical University, over a two-year period. Samples were cultured for growth of aerobic and facultative anaerobic bacteria, identified by biochemical reactions. Antimicrobial susceptibility was tested by Kirby-Bauer disc diffusion using eight different antibiotics. **Results:** Among all samples, 30 (10.9%) were positive for *S. Typhi* by blood culture. The rate of positivity was 23 (76.7%) cases for ages 5–14 years, three (10.0%) in each of age groups 2.0–2.9 and 4.0–4.9 years, and one patient (3.3%) aged 3.0–3.9 years. The majority of *S. Typhi* isolates were resistant to co-trimoxazole (66.7%), ampicillin (63.3%), nalidixic acid (60.0%), chloramphenicol (50.0%) and aztreonam (50.0%). However, most isolates were susceptible to ceftriaxone (76.7%) and ciprofloxacin (66.7%). There were 15 multidrug-resistant isolates but no typhoid-related deaths. **Conclusion:** Our findings show evidence of antimicrobial resistance by *S. Typhi* isolated from Karachiite children living in a poverty-stricken setting where water quality and sanitation are both unsatisfactory. Currently, Pakistan's most populated city is recognized as a focus of typhoid cases. Therefore, this first report of the emergence of confirmed cases of multidrug-resistant *S. Typhi* from the only public hospital in its largest neighbourhood identifies a grave public health concern.

Introduction

The rod-shaped, flagellated, aerobic, Gram-negative bacterium

Salmonella

enterica subspecies *enterica* serovar Typhi (*S. Typhi*) is the main cause of typhoid fever, also

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known simply as typhoid. Other closely related pathogenic subspecies serovars *S. Paratyphi* A, B and C are the causative agents of paratyphoid fever [1], and collectively these two diseases of broadly similar clinical manifestation are historically also referred to as enteric fever. It is estimated that *S. Typhi* is responsible for almost 80% of enteric fever incidence worldwide. *S. Paratyphi* A accounts for most remaining cases although serovar distribution is highly geographically variable [1]. Typhoid is a potentially fatal, acute multisystemic infection characterized by fever and abdominal pain that is spread by eating or drinking food or water contaminated with the faeces of an infected person [2]. As the dissemination of *S. Typhi* is associated with poor hygiene and inadequate sewage systems, clinical case reports from developed countries are rare except in instances of travel-associated typhoid [3]. However, in regions where provision of clean water is not a reality of everyday life, countries remain prone to diseases of poverty including typhoid. Here, communities remain reliant on antimicrobial agents to control the spread of enteric pathogens [4].

The global incidence of typhoid exceeds 21 million confirmed cases annually, leading to an estimated 75,000 to 208,000 deaths [5-10]. The actual burden of disease by country for both typhoid and paratyphoid remains uncertain because many cases are unrecognized, particularly in young children who may have a non-specific illness [11]. Overall, typhoid ranks among the most frequently occurring infectious diseases, particularly in south Asia – the region that includes Pakistan [12]. While persons of any age may be infected with *S. Typhi*, the disproportionately high incidence in children suggests active community transmission of the disease [13]. In Pakistan, the two provinces most affected by typhoid are Punjab to the east [14] and Sindh to the southeast. Approximately 1,000 typhoid cases per 100,000 children have been reported annually over several years in Karachi, the country's most populous city and provincial capital of Sindh, located on the Arabian Sea coast [15,16].

In recent years there has been an alarming rise in worldwide rates of antimicrobial resistance by pathogenic enteric bacteria [17], largely attributed to poor stewardship leading to the misuse of once highly-effective drugs [18]. Multidrug-resistant (MDR) *S. Typhi*, defined as resistance to the three first-line antibiotics used to treat typhoid – ampicillin, chloramphenicol and cotrimoxazole,

appeared in the 1970s and is now widespread globally [19]. This is becoming extensively drug-resistant (XDR) *S. Typhi* (defined as MDR plus resistance to fluoroquinolones and third-generation cephalosporins), mainly through acquiring plasmids conferring antibiotic resistance via bacterial conjugation (commonly associated with the H-58 haplotype) [19]. While there is increasing emergence of XDR *S. Typhi* strains in Pakistan [20], previous findings found no more than 1% resistance to ceftriaxone, making this the antimicrobial of choice for the management of typhoid in many Karachi hospitals [21]. However, it was also reported that first-line drugs can be reused after their discontinuation for many years markedly lowering resistance among isolates of *S. Typhi* in Pakistan [22]. The purpose of our study was to investigate the frequency of drug-resistant *S. Typhi* and *S. Paratyphi* A in children hospitalized or treated as outpatients in a tertiary care centre that serves an impoverished district of Karachi.

Materials and Methods

Study design

This cross-sectional study was conducted in the Department of Paediatrics, Fatima Hospital, which is the principal teaching hospital affiliated to and situated within the premises of Baqai Medical University, Karachi. This hospital serves Gadap Town, where all patients were resident. This is the largest but least developed area of the Malir administrative district, where families lack basic health and municipal amenities. Prior to commencing the project the research protocol was reviewed and approved by the Human Ethics Committee of Baqai Medical University.

Study cohort

There were a total of 275 children registered during the two-year study period, with an age range of 1 month to 14 years. All patients were of low socioeconomic status and had poor access to safe drinking water. In fact, residents of this area are extremely poor, with limited health care options. All patients were unvaccinated against typhoid at the time of the study.

Patient enrollment

Informed written consent was obtained from their parents prior to study enrollment. Each febrile case was screened by the attending physician for symptoms of typhoid, mainly presenting with fever $\geq 37.5^{\circ}\text{C}$ for at least three consecutive day prior to hospital admission. Peripheral blood samples were

collected from each febrile patient with three exclusion categories: those who were taking antimicrobials of any kind, showed a positive blood smear for malaria parasites or declined to participate. In addition to blood collection and hospital admission details, demographic data including age, sex, family socioeconomic status, residential area and medical history were also recorded for each patient.

Collection and culturing of blood samples

Following previously published procedures [23,24] an aliquot of 3-5 ml of peripheral blood was obtained from each child by an experienced phlebotomist. All samples were inoculated immediately into brain heart infusion broth and incubated at 37°C for up to 7 days. During this period, bottles were examined daily and those which showed visible signs of growth were sub-cultured onto each of MacConkey's agar, chocolate agar and blood agar. Sub-culturing was also performed on any sample that showed no signs of growth on day 7 prior to it being considered negative.

Standard microbiological tests

Further to blood culturing, growth of both Gram-positive and Gram-negative bacteria was attained. Biochemical reactions and colonial morphology were examined and interpreted according to standard microbiological procedures [25-28]. For Gram-negative bacilli, different biochemical tests, selective or differential media were used; namely, Simmons' citrate agar, triple sugar iron agar, urea broth, sulphide-indole-motility medium and oxidase test. In addition, *S. Typhi* cultures were selected and identified by serotyping with antisera.

Antimicrobial susceptibility

All identified *Salmonella* isolates were characterized for antimicrobial susceptibility by the Kirby-Bauer disc diffusion method [29] and interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines [30] as either sensitive (S), intermediate (I) or resistant (R). Each bacterial suspension was adjusted to the 0.5 McFarland turbidity standard and inoculated onto Mueller-Hinton agar plates onto which were placed discs, each containing one of eight different antibiotics (Oxoid™, Musaji Adam & Sons, Karachi): nalidixic acid (30 µg); ampicillin (10 µg); cotrimoxazole (25 µg/ml); cefixime (5 µg); chloramphenicol (30 µg); ciprofloxacin (5 µg); aztreonam (30 µg/ml); and ceftriaxone (30 µg/ml). Zones of inhibition (mm) were measured and

compared with CLSI guidelines [30]. Resistance to each of three classes of antibiotic – ampicillin, chloramphenicol and cotrimoxazole – was considered to indicate the presence of MDR bacteria. Moreover, XDR bacteria were defined as additionally exhibiting resistance to fluoroquinolones and third-generation cephalosporins.

Statistical analysis

A descriptive analysis of validated and compiled data was performed using statistical package for social science (SPSS, version 17). Numerical variables such as age were expressed as mean ± standard deviation. Counts and percentages are reported for all qualitative data such as gender, age group, patient type and bacterial isolate. Cross-tabulation of identified bacteria was performed with respect to age group in order to explore the descriptor in more detail.

Results

A total of 275 children of different demographic characteristics and who presented with clinically suspected typhoid were registered for the study and consented to phlebotomy by venepuncture. Blood samples were collected from all categories of patient at the Fatima Hospital, Karachi, either admitted to a paediatric ward (50.2%) or intensive care unit (16.0%), or as an outpatient (33.8%) (Table 1). The proportion of male patients (161; 58.5%) was greater than that of females (114; 41.5%). The mean age of registered patients with typhoid bacteraemia was 3.8 years. The overall frequency of children by age group is shown in table (2).

Of 164 culture-positive samples, *S. Typhi* was isolated in 30 cases (10.9% of total samples). Only one case showed growth of *S. Paratyphi A* – in an eight-year-old male outpatient – and there were no *S. Paratyphi B*, *S. Paratyphi C* or non-typhoidal isolates. By gender distribution, 17 positive cases of *S. Typhi* were from males (56.7%) and 13 (43.3%) were from females. Of the 30 *S. Typhi* cases 23 (76.7%) were from the 5–14 years age group, followed by three (10.0%) in each of the 2.0–2.9 and 4.0–4.9 years age groups and a single case (3.3%) for a patient of 3.0–3.9 years of age. During the study period, *S. Typhi* was not isolated from any children younger than two years.

Aside from the combined total of 31 *Salmonella* isolates, 133 cases (48.4 % of total samples) tested positive for other bacterial species.

These included *Staphylococcus aureus*, *Enterobacter spp.*, coagulase-negative *Staphylococcus*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterococcus spp.*, *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Serratia spp.*. In the highest number of cases *S. aureus* was the cause of bacteraemia (34; 12.4%), followed by *S. Typhi* (30; 10.9%). There was no growth of any bacteria in 111 of the 275 tested samples (40.4 %).

In regard to antibiotic susceptibility patterns of *S. Typhi*, as determined by Kirby-Bauer disc diffusion, most of the 30 isolates of *S. Typhi* were found to be resistant to all first-line drugs (**Figure 1**). For instance, there was a high level of resistance to both co-trimoxazole (20; 66.7%) and

ampicillin (19; 63.3%), with half (15; 50.0%) lacking sensitivity to chloramphenicol. In contrast, 24 (80.0%) of *S. Typhi* cases showed complete or partial sensitivity to ceftriaxone; only six isolates (20.0%) showed resistance to this third-generation antibiotic (**Table 3**). The single isolate of *S. Paratyphi A* demonstrated resistance to ciprofloxacin while being sensitive to all other antibiotics tested. Among all patients with typhoid bacteraemia enrolled in this study, 15 cases (50.0%) were found to be caused by MDR *S. Typhi* but no XDR strain was found. Overall, the data presented in **figure (1)** and **table (3)** are suggestive of the emerging resistance of *S. Typhi* to multiple antibiotic classes in Karachi.

Table 1. Studied patients visiting or admitted to the Fatima Hospital, Karachi.

Paediatric Ward	Frequency	Percentage
Inpatient	138	50.2
Intensive Care Unit	44	16.0
Outpatient	93	33.8
Total	275	100.0

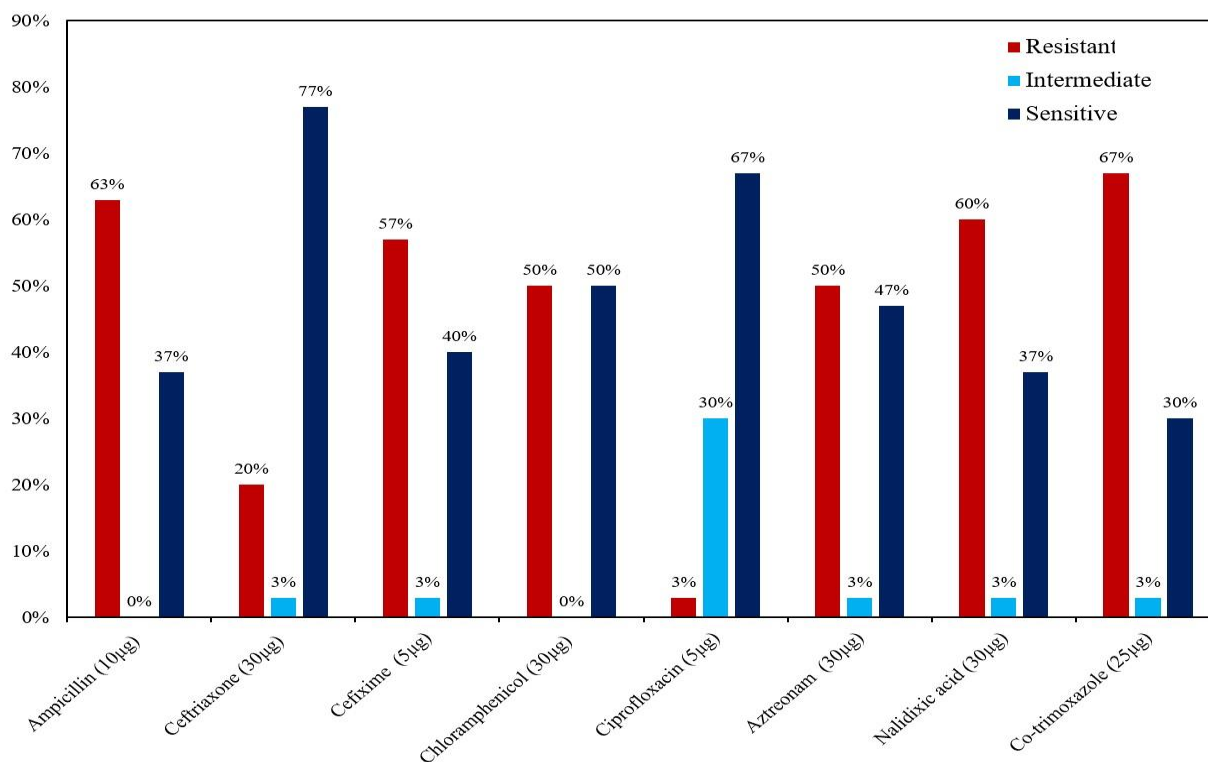
Table 2. Age group distribution of studied patients.

Age group	Frequency	Percentage
< 1 year	82	29.8
1 to < 2 years	47	17.1
2 to < 3 years	28	10.2
3 to < 4 years	15	5.4
4 to < 5 years	17	6.2
≥ 5 years	86	31.3

Table 3. Sensitivity of *S. Typhi* to selected antibiotics by the Kirby-Bauer disc diffusion method. Interpretation followed CLSI guidelines [30]. Case numbers showing resistance, partial or total sensitivity to each antibiotic are indicated in italics.

Antimicrobial agent (concentration, µg) <i>Number of cases (of 30 total)</i>	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Ampicillin (10)	≤ 13 <i>19</i>	14–16 <i>0</i>	≥ 17 <i>11</i>
Ceftriaxone (30)	≤ 19 <i>6</i>	20–22 <i>1</i>	≥ 23 <i>23</i>
Cefixime (5)	≤ 15 <i>17</i>	16–18 <i>1</i>	≥ 19 <i>12</i>
Chloramphenicol (30)	≤ 12 <i>15</i>	13–17 <i>0</i>	≥ 18 <i>15</i>
Ciprofloxacin (5)	≤ 20 <i>1</i>	21–30 <i>9</i>	≥ 31 <i>20</i>
Aztreonam (30)	≤ 17 <i>15</i>	18–20 <i>1</i>	≥ 21 <i>14</i>
Nalidixic acid (30)	≤ 13 <i>18</i>	14–18 <i>1</i>	≥ 19 <i>11</i>
Co-trimoxazole (25)	≤ 10 <i>20</i>	11–15 <i>1</i>	≥ 16 <i>9</i>

Figure 1. Susceptibility to first-line antibiotics of *S. Typhi* isolates collected from children in Gadap Town, Karachi, as determined by Kirby-Bauer disc diffusion.



Discussion

The findings of the present investigation undertaken in Gadap Town, northwestern Karachi, indicate that children in the age group 5–14 years are prone to suffer from infection with *S. Typhi* when compared to *S. Paratyphi A*. This observation is in agreement with that of a retrospective surveillance study of enteric fever cases conducted across all regions of Pakistan [22]. A recently published study from Karachi also showed that adults are less susceptible to typhoid than are children aged 5–15 years [31]. We did not detect any case of typhoid or paratyphoid in children under 2 years of age in this location.

Herein, more boys than girls were infected with *S. Typhi* but the sample size was insufficient to enable a statistically relevant analysis. However, the trend for greater susceptibility of male children to typhoid is consistent with other studies in which gender was considered as a risk factor for contracting enteric fever [31-33]. This may be associated with gender differences relating to level of exposure, wherein boys spend more time playing and eating outdoors. Our study is hospital-based so does not report mild cases; hence, it may underrepresent the extent of typhoid among Gadap Town children.

The clinical manifestations reported for patients with culture-confirmed typhoid included high fever over several days, weakness, abdominal pain, constipation, headaches and mild vomiting. However, no severe complications or deaths occurred, and all patients made a full recovery. There were no clear clinical differences observed between *S. Typhi* and the one recorded *S. Paratyphi A* infection. More than half of the culture-confirmed cases were infected with MDR *S. Typhi*, which showed resistance to all first-line drugs prescribed for empiric treatment of uncomplicated typhoid.

The analysis demonstrates a pattern of increasing resistance of *S. Typhi* to third-generation cephalosporins, as 56.7% of isolates (17/30) showed complete resistance to cefixime and 20.0% (6/30) did so to ceftriaxone. This greatly limits options available to effectively treat typhoid and thereby to control its spread. This is because cephalosporin therapy is currently the preferred choice for treating typhoid in south Asia [34]. Ongoing sporadic cases of typhoid in two districts of Hyderabad, the second-largest city in Sindh and situated 160 Km to the northeast of Karachi, are caused by ceftriaxone-resistant XDR strains that show reduced

susceptibility to fluoroquinolones [21,32]. Most cases were of young children (under 15 years) who contracted infection due to ingestion of contaminated water. The current study reveals the existence of *S. Typhi* resistant to ceftriaxone in a second region of Sindh. Yet, we found the lowest resistance of *S. Typhi* to ceftriaxone and ciprofloxacin among all antibiotics tested. Nevertheless, the results from these two cities underline the fallibility of reliance on these drugs to treat typhoid in Pakistan. There is now serious concern that *S. Typhi* XDR strains originating from Pakistan that have been detected recently in North America and Europe may become more widespread through intercontinental travel, which poses a significant global public health threat [35-37]. It is advised for international visitors to Pakistan to be vaccinated against typhoid prior to arrival and to follow safe hygiene practices when travelling to identified endemic hot spots [38].

As part of an integrated approach to counter the burden of typhoid in endemic areas there is a pressing need to consider the introduction into routine public health programmes of new generation typhoid vaccines [39]. Among the several commercially available preparations the typhoid conjugate vaccine (TCV) is preferred at all ages in view of its improved immunological properties, suitability for younger children and anticipated longer duration of protection [40]. The World Health Organization now recommends the use of the safe and clinically efficacious TCV [41] and in late 2019 Pakistan became the first country worldwide to instigate its use, launched in Sindh, as a routine immunization for children from 9 months to 15 years old [42]. This is an ongoing, collective effort of the Pakistan Government, non-government organizations and educational institutes to roll out vaccination in parallel with a nationwide community engagement initiative to disseminate knowledge and awareness of typhoid prevention and control measures. These public information sessions explain the benefits of immunization against typhoid but also highlight the importance of early diagnosis and proper clinical management of disease. Equally importantly, the irrational and inappropriate use of drugs that has led to the existence of MDR and XDR *S. Typhi* is strongly discouraged, which is pivotal to suppressing the spread of enteric fever among the Pakistani population [43].

This study was performed in Gadap Town, a predominantly slum district of Karachi that is

known for its poor water quality and sanitation, poverty and social disorganization [44]. There is a high proportion of families with several siblings, together with a low rate of schooling. Each of these environmental characteristics is a noted risk factor associated with typhoid in children living under conditions of high population density and lack of access to safe drinking water, where hand washing is not a routine practice before eating or after using the toilet [45]. Almost all residents of the unplanned settlements are of low socioeconomic status [46], so cannot afford the cost of physician consultations, drugs and vaccines for treatment or prevention of typhoid. Malnutrition is also a common contributing factor for bacterial neglected tropical diseases among children in this area [43,47].

Health care providers must remain vigilant to the risk of the ongoing spread of drug resistance in all *S. enterica* serovars that cause enteric fever. A reduction in case numbers may be achieved through community engagement initiatives including improved awareness of the importance of hand washing, with soap when available, and the provision of a healthy, balanced diet. Moreover, filtration or chlorination of drinking water, with facility for safe storage, is a public health priority [45]. Surveillance monitoring should continue, not only for *S. Typhi* but also *S. Paratyphi A*, for which there is no licensed vaccine [48]. Furthermore, as part of a multi-collaborative One Health initiative, drug discovery research should aim to develop novel antimicrobials for empiric treatment of typhoid patients.

Conclusion

A rise in prevalence of MDR *S. Typhi* was observed among children of an impoverished Karachi community. Developing resistance to numerous antibiotics will require careful monitoring to limit escalation of this public health threat. Typhoid case treatment necessarily often starts before antimicrobial sensitivity test results are available. Hence, these findings highlight the importance of considering possible resistance before prescribing an antibiotic regimen.

Author contribution statement

All authors contributed substantially to the development and the writing of this article as indicated. Study conception, SM and QA; study design, SM, QA and AWT-R; study supervision, QA and AWT-R; funding acquisition, SM; data collection, SM; statistical analysis, SM and AWT-

R; interpretation of data, all authors; writing — original draft preparation, SM; writing — review and editing critically for important intellectual content, QA, OTM and AWT-R. All authors read and approved the final version of the manuscript.

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Ethics statement

This study was approved by the Human Ethics Committee of Baqai Medical University and conformed with the ethical regulations of the World Medical Association and the Declaration of Helsinki.

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Competing interest statement

The authors declare no conflicts of interest.

Financial disclosures statement

The authors report no relevant financial or non-financial competing interests.

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