ORIGINAL ARTICLE

Isolation of Extensively Drug Resistant S*almonella Typhi* in Blood Culture from Tertiary Care University Hospital

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Abstract

Background
Typhoid fever is a serious systemic infection which affects all age group from children to elderly. It has multiple systemic effects as untreated infections may cause prolonged morbidity and hospitalization. The treatment modalities range multiple antibiotics like co- trimoxazole, ciprofloxacin, chloramphenicol and ampicillin. The treatment of choice in multidrug resistant *Salmonella* has been third generation cephalosporins like Ceftriaxone.

Methodology
The total number of Blood culture received was 464, collected from January to December in year 2018 at Jinnah Sindh Medical University laboratory. The sample was received in BACTEC blood culture bottle. When a culture bottle signaled positive, the bottle was removed and an aliquot of the broth was Gram stain and processed for culture for organism identification and antimicrobial susceptibility testing

Results
During 2018 from January till December 464 blood cultures came to JSMU Diagnostic laboratory and blood bank out of which 31 cases of enteric fever were diagnosed in automated blood culture Bactec. All the isolated *Salmonella* belonged to *Salmonella typhi* subgroup and there was no case of *salmonella paratyphi* A or B. out of 31, 25 *Salmonella* were resistant to Ceftriaxone sensitive only to azithromycin and Meropenem. All the 31 positive *Salmonella* cases were from patient of pediatric age group with range of 11 months to 14 years.

Conclusion
The rise of extensively drug resistant *Salmonella* is alarming and leaves limited treatment options with carbapenems which are costly as well as require administration by injectable route. Preventive measures like improved sanitation and vaccination may be adapted in order to curtail spread of such resistant strains of typhoid.

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Key Words
XDR**,** *Salmonella typhi*, extremely drug resistant enteric fever

Background
Typhoid fever caused by *Salmonella typhi* and *paratyphi* is one of the several diseases causing disease burden of developing countries. In 2000, over 2 million people were diagnosed to have this preventable infection worldwide. In 2004, typhoid fever caused over 200,000 deaths globally, of which majority of cases occurred in Asia.[1] Despite advancement in this modern era, recent statistics shows that typhoid fever is very common in developing countries accounting for an estimated 120 million infections and 700,000 annual deaths occurring worldwide.[2] Enhanced water quality and proper sanitation constitute cornerstone solutions to this preventable infectious disease, vaccination in high-risk areas is a possible control strategy recommended by the World Health Organization (WHO) for the short-to medium-term management of high risk group like children.[3]

Typhoid has been historically treated by antibiotics like ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol; hence these are termed as first line treatment antibiotics.[4] Those strains of *Salmonella typhi*, which show resistance to these three antibiotics, are considered multidrug resistant (MDR), and resistant isolates were first observed in the late 1970s to early 1980s. These resistant strains were treated with fluoroquinolones (ciprofloxacin, ofloxacin) which constitute the second line treatment. Non-susceptibility to the second-line antibiotics (the fluoroquinolones) or fluoroquinolones resistance has also been frequently reported since these became the treatment of choice in regions with MDR infections. Ceftriaxone, a third-generation injectable cephalosporin, and azithromycin, a macrolide, are employed as the treatment for typhoid fever when other options cannot be used as typhoid strains show resistance to first and second line antibiotics.[4] However, periodic cases of ceftriaxone- or azithromycin resistant typhoidal *salmonella* infection have recently been reported. Over the last twenty years, a dominant, commonly MDR, haplotype of *S. Typhi* called H58 has been scattering all over the world.[5] It is rampant across South and Southeast Asia. Several local outbreaks of typhoid have been linked to various sublineages of H58.[6-8]

Fluoroquinolones like ciprofloxacin are considered treatment

of choice in treating typhoid fever caused by multidrug resistant strains of *Salmonella typhi* but with increasing antibiotic resistance there are numerous reports of treatment failures with fluoroquinolones.[9] Multidrug resistance (MDR), defined as resistance to the three first-line classes of antimicrobial agents (chloramphenicol, ampicillin, and trimethoprim/ sulfamethoxazole [TMP/SMX]), has become prevalent in most of South Asia, with figures reaching 15% in India and 45% in Pakistan.[10] There has been previous research in Pakistan demonstrating increase in resistance against Ciprofloxacin. The trend of antibiotic resistance was already on the rise with emerging MDR strains of *Salmonella*.[11] Extensively drug resistant *Salmonella* is defined as in addition to being Multi drug resistant isolates are resistant to third generation cephalosporins (cefixime / ceftriaxone). Previously reports of emerging resistance in *Salmonella typhi* and *paratyphi* against third generation cephalosporins which is termed as XDR strains was seen first reported by outbreak in Hyderabad Sindh. Subsequently it was sporadically reported by cases seen in Karachi followed by widespread uprising of reported cases of XDR *Salmonella typhi*.

Materials and Methods

This cross-sectional study was conducted in Jinnah Sindh Medical University laboratory Karachi. Ethical permission to use lab data was taken from JSMU laboratory as no patient confidential data was used and privacy was maintained. The total number of Blood culture received was 464 from January 1st to December 31 2018.The samples were received in BACTEC blood culture bottle. When a Bactec bottle gave a positive fluorescent signal, it was removed and an aliquot of the broth was Gram Stained and processed by sub culturing on blood agar and Mac conkey agar, antibiotic sensitivity was done on Mueller Hinton agar (MHA). After 24 hours of incubation, the grown bacterial colonies were identified by serological identification, done by Remel 9-0 antisera and biochemical identification was done by TSI, urea, SIM and citrate. Antibiotic sensitivity done on MHA was interpreted using CLSI 2018 break points for *Salmonella*.

Results

During 2018 from January till December 464 blood cultures came to JSMU Diagnostic laboratory and blood bank out of which 31 cases of enteric fever were diagnosed in automated blood culture Bactec 9050. All the isolated *Salmonella* belonged to *Salmonella typhi* subgroup. Out of 31 isolates 26 *Salmonella* were resistant to Ampicillin, chloramphenicol, co- trimoxazole, Cefixime and Ceftriaxone, with showing sensitivities only to azithromycin and meropenem. The antibiotic susceptibility of the xdr isolates showed, all but five were resistant to ampicillin, chloramphenicol, co-trimoxazole and third generation cephalosporin (cefixime and ceftriaxone). Remaining five *Salmonella* were resistant to cefixime but sensitive to ceftriaxone. None of the isolates were resistant to azithromycin, meropenem and imipenem.

The age group of all patients with *Salmonella* infection ranged from 6 months to 14 years. Out of thirty-one cases, 18 were male and 12 were female.

All but five patients with XDR *Salmonella* were treated with azithromycin for 5 days with complete recovery on follow up. The remaining five pediatric patients were treated with meropenem as they were clinical unstable to be treated with oral antibiotics.

Discussion

Typhoid fever is a life-threatening infectious disease caused by *Salmonella* enterica serovar *Typhi*. *Salmonella typhi* colonizes only humans, is transmitted through the fecal-oral route. Typhoid fever can be cured invariably by adequate and timely antimicrobial treatment. The rise of extremely drug resistant *Salmonella* is alarming and leaves limited treatment options with carbapenems which are costly as well as require administration by injectable. Preventive measures like improved potable water quality sanitation and vaccination in high-risk areas are means of potential control strategy recommended by the World Health Organization (WHO) for the short-to medium-term management.

Typhoid fever is a notifiable illness in the Sindh province of Pakistan. The cases of *Salmonella typhi* and para*typhi* identified in blood are reported to the Sindh health authorities with a special note to indicate the emergence of ceftriaxone resistance. Swift emergence and brisk spread of resistant isolates underline the significance of AMR surveillance for typhoid and other enteric Gram-negative bacteria and draw attention to the inadequacy of relying solely on non-culture-based serological methods for diagnosis of typhoid (such as Widal and *Typhi*dot tests), which do not provide susceptibility results. In view of the emergence of ceftriaxone resistance in *Salmonella typhi*, culture- and sensitivity-guided treatment becomes imperative as empirical treatment with ceftriaxone is no longer dependable in the region.[12] Following antibiotic resistance testing, cases were effectively treated with azithromycin and meropenem, resulting in recovery by all patients. Immediate control measures include education of the patients and household members and emphasis on hygiene and food safety.

The emergence and spread of XDR *S. Typhi* in Sindh, Pakistan, is a disquieting demonstration of how an omnipresent antibiotic resistance can be acquired by MDR *S. Typhi*, rendering it XDR and further narrowing treatment options. Antibiotics save millions of lives annually, but the evident ease and rapidity by which life-threatening bacteria such as *S. Typhi* can develop resistance severely limit their efficacy.

Small data from tertiary care hospital university laboratory propose that better strategies against typhoid are warranted, such as awareness of treating clinicians to send blood cultures when suspecting enteric fever and not rely on serological

typhoidal tests as they are not specific and cannot give information regarding antibiotic susceptibility pattern.

However, vaccination could not immediately be undertaken to prevent the spread of this highly resistant clone to patients and their household contacts due to unavailability free of cost vaccine and inability of these poor patients with limited resources to afford it privately. Patients' families were counseled to increase awareness about ease of spread among house hold contacts and preventive measures like hand washing, boiling drinking water, consumption of hygienic food and drinks. Handouts about awareness of proper sanitation and proper disposal of human wastes was also given to patients' attendants in order to drive knowledge and attention to take steps to break the chain of transmission of these infections.

Limitations

1. This study included only bacteremic isolates of XDR *Salmonella*. 21 of the patients were lost to follow up after reporting of positive culture and treatment history could not be taken via telephonic communication and only advice for treatment was sent through a comment in the blood culture report.

Table 1: Age group and resistance patterns of XDR *Salmonella*

Age group	ceftriaxone resistant	ceftriaxone sensitive	Male	Female
6month-14 years	26	--	16	10
1year-10years	--	05	3	2

Isolation of *Salmonella typhi* in different age group and gender

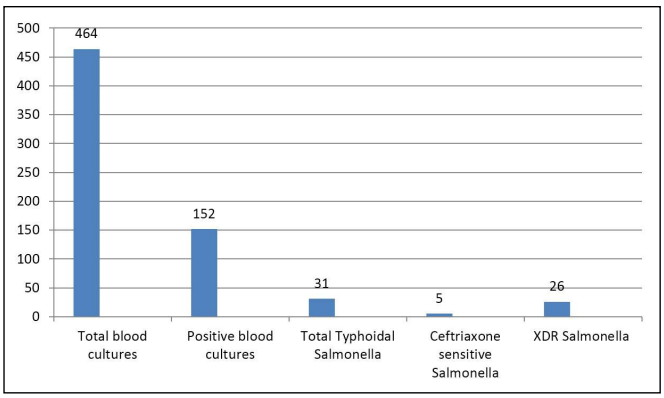

Figure 1. Graphical distribution of 464 blood cultures (total blood cultures during year 2018 from JSMU laboratory)

2. The study participants who were hospitalized in JPMC and NICH Provided relevant clinical history, but clinical details of 14 outpatients from various parts of Karachi could not be obtained.

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