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Original Article

Emerging Trends of Antibiotic Resistance pattern of Salmonella Typhi

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Abstract:
Introduction: Salmonella typhi is the major serotype of Salmonella accountable for enteric fever. Emergence of antimicrobial resistance limits the available treatment options for enteric fever and is one of the foremost contributors to the increase in morbidity and mortality rates.
Materials and Methods: This was cross-sectional study conducted at Dow University of Health Sciences over a period of 6 years, between January 2010 to December 2016. Blood samples were collected and incubated in Bact/Alert automated system (bio-Merieux). The isolates were identified and characterized by standard and specific methods. Antibiotic susceptibility of all isolates was assessed by the Kirby-Bauer disc diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI).
Results: During the period of 2010 to 2013, the highest frequency of S. typhi was observed in pediatric age group and males. Ampicillin (57.1%), Trimethoprim/sulfamethoxazole (60.1%), and Chloramphenicol (57.1%) showed highest resistance. From 2014 to 2016, the incidence of infection was greatest in children and females. Ciprofloxacin (7.6%), Ampicillin (54.7%), Trimethoprim/sulfamethoxazole (60.1%), and Azithromycin (4.6%) were observed to have increasing pattern of resistance.
Conclusion: Our study showed emergence of multi drug resistant isolates of S. typhi. Therefore, Antibiotic steward ship program, vaccination and local surveillance are highly recommended to control the spread of multi drug resistance among isolates of S. typhi.
Key Words: S.typhi, antibiotic susceptibility, multidrug resistance, emerging trends

Introduction

Enteric fever is a systemic infection with high morbidity and mortality worldwide.[1] Salmonella typhi is the major serotype of Salmonella accountable for enteric fever in Pakistan. The global incidence of 120 million infections and 700,000 annual deaths has been reported.[2] The major factors that help in the spread of enteric fever are contaminated water, food, over populated environment, poor ventilation, poverty and illiteracy. However, good sanitary conditions, facility of clean drinking water and use of antibiotics have distinctly reduced the occurrence of typhoid fever in the developed world, but its prevalence remains high in the developing countries.[3] Most of the reported cases from the developed countries have been observed to be linked with history of travelling from endemic areas.[4]

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In different parts of the world, variance in the presentation of disease and emergence of antimicrobial resistance have been responsible for the difficulty in diagnosis and treatment strategies. Misuse and overuse of drugs, malpractice by physicians, over the counter sale, faulty formulations of drugs etc. are few contributing factors in the spread of MDR S. typhi.[5] The prevalence of MDR S. typhi is high in South Asian countries, accounting for 14% in India and 44% in Pakistan.[5] Fluoroquinolones are the drug of choice after the emergence of MDR strains but Fluoroquinolone-resistant S. typhi has also been isolated.[6-11] Cephalosporin (Ceftriaxone, Cefixime) and Azithromycin have been recommended as alternative drugs for MDR S. typhi after the development of resistance to Fluroquinolones.[12-14]

Epidemics of Typhoid fever caused by MDR S. typhi impose therapeutic challenges and further complicate health problems. Therefore, there is an urgent need of strict surveillance programme regarding unjudicial use of antibiotics, emerging trends of resistance and improvement in management strategies that could be

helpful in reducing the development of resistance against cephalosporin group. Furthermore, there is no agreement among health care professionals concerning the choice of antimicrobial for the management of Enteric fever. Therefore, this study was conducted with the aim to closely evaluate the changing pattern of sensitivity of S. typhi from 2010 to 2016 from tertiary care hospital of Karachi

Materials and Methods

This was a cross-sectional study conducted in the microbiology section of clinical laboratories at Dow University of Health Sciences. 5-10 ml blood samples of adults were collected in FA Bact/Alert blood culture bottles while 1-2 ml blood samples from children were taken in PF bottles. Duration of the study was from January 2010 to December 2016. The age of the patients was categorized into 10 groups at 10 years of intervals.

Blood culture bottles were incubated in the Bact/Alert automated system (bio-Merieux) for 7 days. A Gram-staining of the smear was performed from the positive broth of blood bottles and was sub-cultured onto chocolate agar, 5% sheep blood agar and macConkey agar plates. Blood and chocolate plates were incubated in a 5% CO2 incubator and macConkey agar plate incubated at 37oC for 18-24 hours. The isolates were identified by colony morphology, Gram stain and biochemical reaction such as sulfide indole motility (SIM) test, triple sugar iron (TSI) agar test citrate agar slant and urease test. The isolates of Salmonella were further confirmed by agglutination with polyvalent 9-0 for Salmonella typhi.

Antibiotic susceptibility of all isolates was assessed by the Kirby-Bauer disc diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI).[15]

The antibiotics, including Ciprofloxacin (CIP), Ampicillin (AMP), Trimethoprim/sulfamethoxazole (SXT), Chloramphenicol (C), Cefixime (CFM), Ceftriaxone (CRO) and Azithromycin (AZM) were dispensed on plates and incubated for 18-24 hrs at 37°C. Zones of inhibition around the antibiotics were measured after incubation. The isolates were considered as multidrug resistant if they were resistant to more than one class of antibiotics.

Data analysis was accomplished by using Statistical Package for Social Science (SPSS version 17.0) for frequencies of age group, gender, and antibiotic resistance pattern.

Results

A total of 557 S. typhi strains were isolated from blood cultures registered in three hospital. The resistance pattern of antimicrobial agents during 2010 to 2013 included Ciprofloxacin (1.6%), Ampicillin (57.1%), Trimethoprim/sulfamethoxazole (60.1), Chloramphenicol (57.1%), Cefixime (7.1%), Ceftriaxone (2.3%) (Figure #1).

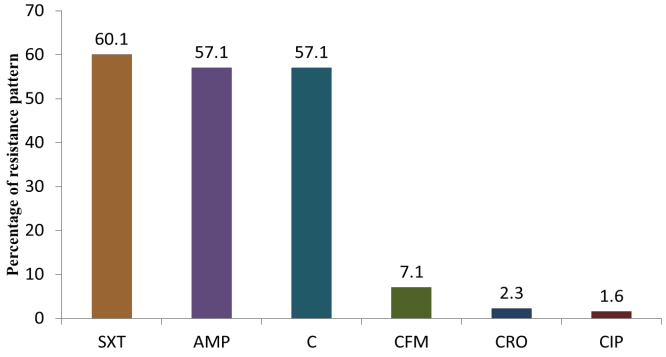

Figure1: Antimicrobial resistance pattern in percentage during 2010 to 2013.

The highest frequency of S.typhi isolates was found in children (age group-1) followed by age group 2 and 3. S.typhi isolates were predominantly recovered from males as compared with females from all age groups (Figure # 2).

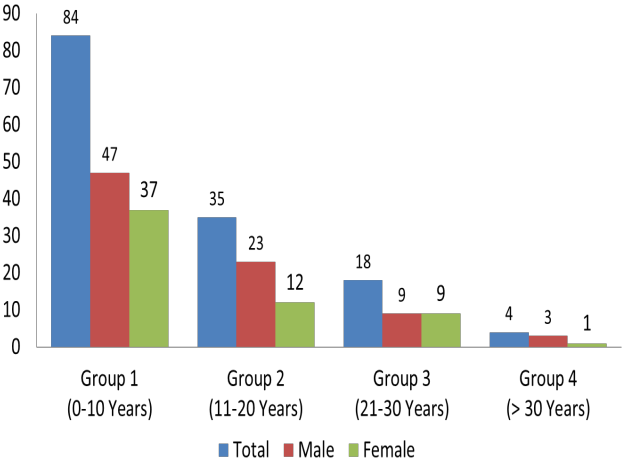

Figure2: Gender wise distribution of S.typhi infection among different age groups during 2010 to 2013.

From 2014 to 2016, resistance pattern included Ciprofloxacin (6%), Ampicillin (54.1%), Trimethoprim/sulfamethoxazole (60.1%), Chloramphenicol (49%), Cefixime (6.95 %), Ceftriaxone (5.9%) and Azithromycin (4.9%) (Figure3).

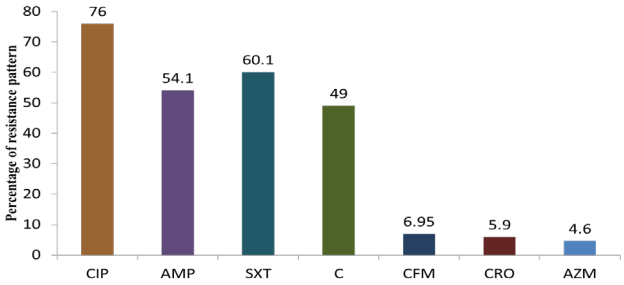

Figure3: Antimicrobial resistance pattern in percentage during 2014 to 2016.

The incidence of infection was highest in age group 1. S. typhi isolates were found more in females in age groups 1 and 2 as compared with age group 3 and 4 (Figure 4).

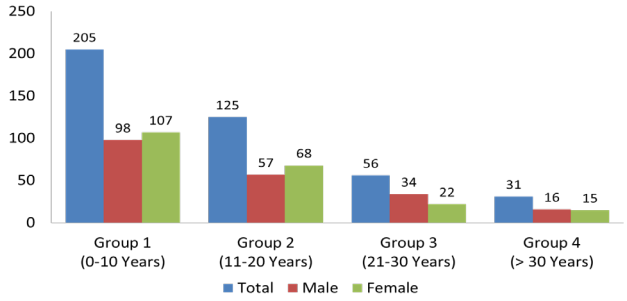

Figure 4: Gender wise distribution of S. typhi infection among different age groups during 2014 to 2016.

From 2010 to 2016, the prevalence MDR S. typhi raised from 54.1% to 60.91%. However, Flouroquinolone resistance drastically increased from 1.6% to 76%. Moreover, resistance of ceftriaxone shifted from 2.3% to 5.9 %. Azithromycin was included during 2014 to 2016 but it started showing increasing pattern of resistance (4.6%) (Figure 5).

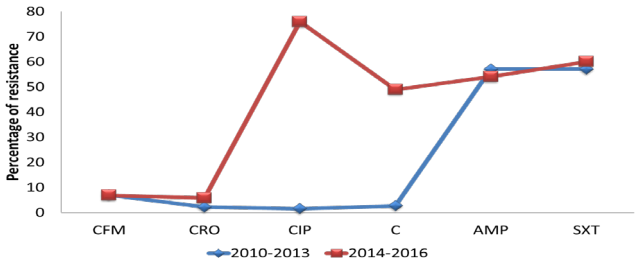

Figure 5: Trends of S. typhi resistance pattern of different antibiotics over 2010-2016

Discussion

First outbreak of resistant strains of S. typhi was reported in 1980. Later, it became endemic in South East Asian countries.[16] Antimicrobial resistance is mainly caused by the selective pressure of unjustified usage of antibiotics. In the developing countries, people want immediate relief from infections; therefore, health professionals are forced to prescribe antibiotics for speedy recovery. The consequences of infection with MDR bacteria are longer stay at the hospital, expensive treatments, cross-infections with other MDR bacteria and eventually high mortality rate[17-19] In the past 20 years, emergence of resistance against standard antimicrobials has become the biggest challenge for the medical community for patients in endemic areas as well as for travellers who are not vaccinated and visit these areas.

Fluoroquinolones were the treatment of choice after the resistance against first line conventional antibiotics but due to its excessive use they turned out to be ineffective.[20] The development of MDR S. typhi with resistance to Fluoroquinolones, Cephalosporins and Azithromycin is quite distressing, leading to narrowing down the therapeutic choices.

Typhoid can affect any age group but our study showed high occurrence in children. Children are the most vulnerable group exposed to poor hygienic conditions and unavailability of medical support. The incidence of enteric fever among children aged 2–5 years was 573.2 and 340.1 per years in Pakistan and India, respectively[2] Moreover, report from Delhi has mentioned the incidence of typhoid fever to be higher in the children of preschool stage.[21]

Our study claimed male predominance, which is in accordance with other studies.[22, 23]

Males are more prone to be infected because of their exposure to external environment. In countries like Pakistan, where male dominant culture exists, male child gets rapid attention to health problems than the female child. Our study reported high prevalence of MDR S. typhi over the period of last seven years. The trend represents the widespread occurrence of resistant strains in South Asian countries. Our results, however, are justified by the report of Yan et al.[16] However, the declining pattern of antibiotic resistance of S. typhi was also reported. It could be due to variation in genetic profile of an organism and selection of alternative antimicrobials.[24,25]

Chloramphenicol was first introduced in 1948 for the treatment of Typhoid along with Ampicillin and Cotrimoxazole but became ineffective after 2 years of its usage.[26] Mirza et al claimed that antimicrobial resistant genes were exchangeable between Salmonella and other Enterobacteriacea.[27] These resistant genes reside on R-plasmid, which is mainly responsible for their spread by conjugation.[28]

In 1987, emergence of MDR S. typhi was identified for the first time in Pakistan.[29] It was due to the

acquisition of 98 MDa plasmid activating resistance to Chloramphenicol, Aminoglycosides, Tetracyclin, Sulphonamides and Ampicillin. By 1994, 77% of MDR S. typhi was isolated from the northern areas of Pakistan.[30]

Our study showed decline in resistance against Chloramphenicol from 57.1% to 49%, confirmed by the reports of Chand H G et al. which showed 100% susceptibility to Chloramphenicol.[31]This decreasing trend is due to the shifting to other therapeutic options. However, the chances of re-occurrence of resistance are still high because MDR strains are capable of disseminating their R-plasmids, encoding resistance determinants to the sensitive strains of S. typhi. [32]

Considering increasing resistance against first line therapy, Flourquinolones emerged as an ideal choice for the treatment of MDR S. typhi.[33] A broad antimicrobial profile, cost effectiveness, tolerance level and oral consumption led to their excessive use which resulted in failure of their efficacy, hence encouraged physicians to prescribe Cephalosporin. The resistance of Fluroquinolones has raised from 1.6 % to 76% in the past 7 years as per this current study and justified from other reports. Resistance to Fluroquinolones is the acquisition of mutations that result in increase in efflux pumps of bacteria and substitution of amino acids in the specific regions of DNA gyrase (encoded by gyrA and gyrB), and topoisomerase IV (parC and parE) which are important targets of Fluroquinolones.[34] Mutations between amino acids 67 and 106 are known quinolone resistance determining regions (QRDR).[35] Point mutations in QRDR of gyrA lead to nalidixic acid resistance, however, additional mutations are responsible for Ciprofloxacin resistance.[36]

Emergence of Fluoroquinolone resistance and increasing susceptibility of first line drugs may impose on health authorities to re-evaluate the policy of antibiotics usage for empirical treatment. Moreover, the reemergence of susceptibility of old drugs has several benefits such as their cost effectiveness, easy availability and recognized clinical competency.[37] In the current scenario of Flouroquinolone resistance, the effectiveness of the Cephalosporins has been studied. The expanded-spectrum cephalosporins, such as Ceftriaxone and Cefixime, have presented promising results for the treatment of enteric fever. However, only cefixime can be given orally, whereas, Ceftriaxone has parenteral routes of administration. The unjudicial use of these antimicrobials has led to the progress of Extended-Spectrum b-Lactamases (ESBLs) production in S. typhi.[38]

Azithromycin is a broad-spectrum azilide, which can be used as an alternative to Ceftriaxone, Ofloxacin and Chloramphenicol because of insignificant relapse rate and a satisfactory compliance in patients. It has good penetration in tissues as compared to serum and found to be highly effective in eradicating the intracellular S. typhi. IT is a potential substitute in children for whom quinolones are contraindicated. Our study demonstrated 4.6 % resistance in the past three years. Kalonji et al, reported resistant strains of S.typhi to Azithromycin.[39] Plasmid mediated mph A gene is one of the important reasons of high level resistance to Azithromycin. However, there are other mechanisms such as mutations in rlpD and rlpV gene that are also involved in mediating resistance. Azithromycin achieved dominance over other drugs due to its cost effectiveness, single dosage and short duration of therapy.[40] Vaccination is quite helpful in precluding enteric fever in travelers from endemic areas, monitoring epidemics and taking care of children from endemic areas. The high burden of disease is found in children; therefore, typhoid vaccines should be included in immunization programs.

Conclusions

Our study reported increasing trend of MDR, Fluoroquinolone and Azithromycin resistant isolates of S. typhi, which is of serious concern for health care community. Antibiotic steward ship program, vaccination and detection of mechanism of resistance are highly recommended to control the spread of multi drug resistance among isolates of S. typhi.

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Microbiology section of clinical laboratories at Dow University of Health Sciences.

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E. Critical Review
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