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EMERGING ANTIMICROBIAL RESISTANCE IN TYPHOID FEVER: CHALLENGES IN DIAGNOSIS AND TREATMENT

Iqra Jamil[1], Dr Asad Ur Rehman[2], Dr M. Fayyaz Ur Rehman[3], Dr Farhan Rasheed[4]

[1]PhD Microbiology Scholar, Dr. Ikraam Ul Haq Institute of Industrial Biotechnology Government College University Lahore, Email: Iqra.jameel@hotmail.com
[2]Associate Professor Biotechnology/Chairperson of Medical Laboratory Technology Dr. Ikraam Ul Haq Institute of Industrial Biotechnology, Government College University Lahore, Email: Dr.asadurrehman@gcu.edu.pk
[3]Associate Professor Institute of Chemistry, University of Sargodha, Sargodha Email: Fayyaz9@gmail.com
[4]Professor of Pathology, Ammer ud Din Medical College/Post Graduate Medical Institute, Lahore, Email: Dr.farhanrasheed@gmail.com

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Corresponding Author: Iqra Jamil, PhD Microbiology Scholar, Dr. Ikraam Ul Haq Institute of Industrial Biotechnology, Government College University, Lahore Email: Iqra.jameel@hotmail.com

ABSTRACT

Typhoid fever is one of the serious blood stream infections in Pakistan with the most affected group of individuals are less than 10 years of age. Typhoid fever has a morbidity rate of over 21 million cases annually. *Salmonella typhi*, the causative agent of typhoid fever is primarily transmitted through oral-fecal route. Presence of toxins such as endotoxin (LPS), enterotoxin, and cytotoxin are all associated with virulence of *S. typhi*. Currently, laboratories diagnose typhoid fever through blood and stool cultures, which are considered the gold standard. Initially, the primary medications for treating typhoid were ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. But the development of antimicrobial resistance with the multi-drug and extensively drug-resistant phenotype limited the treatment options. Resistance in *S. typhi* isolates more frequently associated with the acquisition of resistance genes, various types of plasmids specially those of Incompatibility (Inc) plasmids and chromosomal mutations. By ensuring access to immunizations, clean water, and proper hygiene practices, the likelihood of contracting typhoid can be reduced.

Introduction:

Typhoid fever can be a serious, even fatal, bacterial infection in the bloodstream. According to Jabeen *et al*. (2023), countries with lower and moderate incomes (LMICs), such as

Pakistan, India, and Bangladesh, enteric fever poses a significant threat to public health (1). S. Karl Eberth identified typhi as the pathogen that caused typhoid fever in 1880, despite reports of the disease dating back to the early 1800s.

Incidence and Prevalence of typhoid fever:

The incidence, mechanisms of transmission, and consequences of typhoid fever differ significantly between industrialized and underdeveloped nations. In developed countries, the disease's prevalence has significantly decreased. About 400 clinical cases of enteric fever are reported in the US each year, which means that there are less than 0.2% new cases per 100,000 persons annually, similar to the rates in Japan and Western Europe (2). Between 2008 and 2015, the Centers for Disease Control and Prevention (CDC) in the United States received reports of just 350 culture-confirmed cases of typhoid fever, or less than 0.5 cases per 100,000 persons (3). According to a report by the World Health Organization (WHO) in 2014 on the annual prevalence of typhoid worldwide, out of an estimated 21 million cases of the disease, there are around 222,000 typhoid-related deaths worldwide each year. A thorough examination of the morbidity and mortality rates from typhoid and paratyphoid fever around the world revealed that the incidence of typhoid fever was less than 0.1/100,000 in Central and Eastern Europe and Central Asia, and 724.6/100,000 in sub-Saharan Africa (4). Asia is said to have the highest recorded disease burden. With about 110 cases per 100,000 individuals, Southeast Asia has the third-highest prevalence of enteric fever, which accounts for 93% of all cases globally. Typhoid incidence reports for five Asian countries were estimated to be 24.2 cases per 100,000 people in Vietnam, 29.3 cases in China, 180.3 cases in Indonesia, and 412.9 and 493.5 cases in Pakistan and India, respectively (5).

Typhoid: Pakistan Context:

Over the past few decades, typhoid disease has remained quite prevalent in Pakistan. A study carried out in low-socioeconomic regions of Karachi between 2002 and 2003 found that the annual incidence of typhoid fever cases was four times higher than the WHO-defined criterion, ranging from 252 to 503 cases per 100,000 child years and the incidence of typhoid was greater in children in age group of 2–10 years which was 78% as compared with children aged 2–5 years (35%) (6, 7). October (the post-monsoon season) had the largest number of typhoid cases, followed by May and June (8). According to a 1990–1993 study on children under the age of 12, 67.2% of *S. typhi* strains were multidrug-resistant (MDR) (9). *S. typhi* was resistant to fluoroquinolone in 88.2% of the cases, followed by ampicillin, trimethoprim/sulfamethoxazole, and chloramphenicol at 66.8%, 66.5%, and 66.1%, respectively, indicating a significant incidence of antimicrobial-resistant strains in a 3-year review (2008–2011) (10). The high incidence of typhoid in this area is caused by a number of risk factors, such as inadequate water supplies, overcrowding, poor sanitation, and substandard living circumstances (11). Although there are vaccines available in Pakistan, their low coverage is caused by a number of issues, such as limited access to immunization and a lack of knowledge and awareness (12). While a more recent study indicated that the conjugate vaccine (Vi-CRM197), created by Novartis Vaccine for Global Health, can be safely used in endemic populations of all ages, the results of a vaccination trial carried out in Pakistan demonstrated that the Vi polysaccharide vaccination was an effective strategy for the control of typhoid fever (13).

Morbidity and mortality rate of typhoid fever:

In many underdeveloped nations, typhoid fever is a major source of illness and mortality. Up to 33% of typhoid fever patients in hospital and community settings from developing nations died before the introduction of antibiotics, whereas up to 10% of those in rich countries did the same (14). Typhoid fever has a morbidity rate of over 21 million cases annually, with some of these cases resulting in mortality (2). Over 2.16 million typhoid episodes and 216

000 deaths were predicted to have occurred globally in 2000, with Asia accounting for over 90% of these cases (15).

Association of typhoid with age:

Although typhoid fever can strike anyone at any age, it is thought to primarily affect children and young people. Children ages 8 to 13 have the greatest prevalence in endemic areas. Contrary to popular assumption, the disease affects youngsters as young as one to five years old, according to a recent study conducted in Delhi's slums. Approximately 20–30% of typhoid fever cases occur in youngsters under the age of ten (16). Typhoid fever affects 26% of children under five, according to the Indonesia Demographic and Health Survey (2002–03) (17). Moreover, it was observed that the incidence of typhoid fever in children aged 2 to 5 was equal to that of children aged 5 to 15 in countries with higher burdens, such as Pakistan, India, and Indonesia (18).

***Salmonella typhi* and its transmission routes:**

Salmonella typhi is the bacterium that causes typhoid fever, an infectious disease that is transmitted through oral-fecal route. It is typically brought on by consuming contaminated water and food. The main cause of typhoid is contamination of surface water, including sewage, fresh water, and ground water, since *S. typhi* bacteria can live in water for days. Within subspecies, 1 of the major species of S. enterica, *S. typhi* is a highly preserved serovar (19). With O-antigen type O9-12, phase 1 flagellin type H:d, and Vi capsule positivity, *S. typhi* is categorized as Group D according to the Kaufmann-White classification. *S. typhi* is typically monophasic as a result. Rare isolates of *S. typhi* are Vi- negative, although the majority belong to the Kaufmann-White classification (20). *S. typhi* is rarely found in environments with extensive access to clean water, sanitation, and hygiene infrastructure. Instead, it is mainly transferred by the fecal-oral pathway, which is typically contaminated food and drink. Typhoid fever is spread by urine or the fecal-oral route. This can happen directly through dirty hands that are contaminated with the urine or feces of carriers or cases, or indirectly through consuming tainted food, milk, or water, or through flies (2). The transmission routes for typhoid fever in 43 outbreak cases were determined in a survey carried out in 2020. Of these, direct contact accounted for 5%, food-borne transmission for 40%, and water-borne transmission for 56%. Compared to outbreaks spread by food or direct contact, waterborne outbreaks typically have a greater average number of episodes in each epidemic. Low-pressure water distribution systems, broken pipes, a lack of water chlorination, and the close proximity of drinking water sources to restrooms were among the factors that contributed to water contamination (21).

Virulence factors of *S. typhi*:

The presence of toxins such as (LPS) endotoxin, enterotoxin, and cytotoxin, colonization, adhesion, and invasion, as well as survival inside the host cells, are all stages of infection that are associated with virulence factors of *Salmonella typhi* (22).

Surface K antigens, which are heat-sensitive polysaccharides found on the surface of the bacterial capsule, are the smallest common antigens found in Salmonella species (23). Alpha 1-4 linear homopolymers make up the capsular Vi antigen. They are connected to galactose aminouronic acid, which has varying degrees of acetylation at the C3 position. One of the primary characteristics that sets *S. typhi* apart from nontyphoid Salmonella (NTS) is the synthesis of the Vi antigen, a polysaccharide capsule. By preventing antibodies from targeting the O-antigen, the Vi capsule decreases phagocytosis while increasing serum resistance. By protecting the O-antigen from antibodies, the Vi capsule prevents phagocytosis and provides serum resistance. (24, 25). The viaB locus of Salmonella pathogenicity island (SPI)-7 contains the genes that produce the Vi capsule. It also encodes a type IVB pilus and the type III secretion system (T3SS) effector SopE (26).

Two pathogenicity-island encoded type III secretion systems (T3SS) that are critical for Salmonella virulence—the SPI-1 and SPI-2 T3SS—are shared by both typhoidal and NTS. The SPI-1 T3SS is also necessary for *S. typhi* to invade nonphagocytic cells (27). The lipopolysaccharide (LPS) known as the "0" antigen is present in the outer L-layer beneath the capsular material. Additionally, this "L" layer contains antigenic proteins known as outer membrane proteins (OMP). Both porin (OMP F and OMP C) and non-porin compounds are included in these OMPs. Non-porin proteins are structural proteins, while porins are pore-forming channels that aid in solute uptake. Patients with typhoid fever exhibit a good antibody response to all of these antigens, which are highly immunogenic. For serological diagnostics, it is employed (28). By detecting monomeric flagellin through the TLR5 and NAIP receptors, flagella not only aid in virulence but also significantly activate innate immune responses (29). There are two variants of H antigen, referred to as phase 1 and phase 2, which can be present in one or both forms. The organism tends to switch between these phases. Furthermore, the H antigen serves as a useful epidemiological tool for tracing the source and transmission mechanism of infections. Most *S. typhi* strains directly express the FliC antigen H: d and are monophasic (30). Fimbriae and pili serve as crucial adhesion factors for *S. typhi*. This pathogen employs these virulence factors for various cellular interactions during infection and colonization in the host. Among the six fimbrial operons identified in *S. typhi*, Stg is one of them. Certain Salmonella species carry a large, low copy number plasmid that houses virulence genes. Virulence plasmids must be responsible for systemic disease; they range in size from 50 to 90 kb, yet all contain a common 7.8 kb segment, known as SPV, that is vital for bacterial growth in the reticuloendothelial system (31).*S. typhi* utilizes a sophisticated method known as bacterial mediated endocytosis, unlike most bacteria that rely on receptor-mediated endocytosis to penetrate a target cell. Bacterial proteins invade the host cell and modulate signaling pathways that govern the dynamics of cytoskeletal membrane structure and gene expression, both of which compel *S. typhi* into the host. The specific cell that *S. typhi* aims for is the macrophage (32). A significant portion of the outer membrane (OM) of Gram-negative bacteria contains endotoxin. Research has shown that endotoxins play a critical role in the pathogenicity of infections caused by Gram-negative bacteria. In humans, it serves as a powerful mediator of various pathological effects, primarily within the gastrointestinal tract. Therefore, these substances are also referred to as enterotoxins (33).

Table 1: Virulence factors of *S.typhi*

Virulence Factor	Encoding genes	Functions	Reference
Vi antigen	viaB locus present in SPI-7	Inhibits phagocytosis, confers serum resistance	(24)
Type-III secretion system	SPI-1 and SPI-2	Invasion of non-phagocytic cells	(27)
Somatic O antigen	Outer L-layer	Highly immunogenic OMP F and OMP C which form pore forming channels	(28)
Flagella (H) antigen	Flagellar proteins	Activators of innate immune responses, epidemiologic tool	(29)
Fimbriae and pili	Operons Fimbriae	Invasion and colonization	(34)

Virulence plasmids	-	Trigger systemic disease	(31)
Invasiveness	-	Mediate bacterial endocytosis	(32)
Biofilm	-	Allows synergic growth and protection from unfavorable environment	(35)
Endotoxin	-	Mediates pathophysiological effects	(36)

The typhoid toxin, which is encoded by SPI-11, is exclusively expressed in *S. typhi* (37). It is a distinctive AB toxin composed of a homopentamer that includes one binding (B) subunit (PltB) and two active (A) subunits (CdtB and PltA) (38). The SCV generates vesicles that harbor the typhoid toxin, subsequently releasing it into the extracellular environment. Typhoid toxin targets various cells through PltB-mediated attachment to glycans, mainly those terminating in N-acetylneuraminic acid (Neu5Ac) (38). Receptors for typhoid toxin have been identified on the glycosylated podocalyxin-like protein 1 (PODXL) of human epithelial cells and on CD45 of immune cells such as macrophages. Notably, Neu5Ac is found exclusively in humans, highlighting the host specificity and adaptation of *S. typhi* (39).

Pathogenesis of *S. typhi*:

S. typhi infections in humans generally arise from the ingestion of food or water that has been contaminated (40, 41). Human challenge studies indicate that the infectious dose is around 10,000 organisms; however, this amount likely varies between individuals and environments, with recent research suggesting it could be even lower (40, 42). The bacteria can use microfold (M) cells to penetrate the intestinal mucosa, leading to an infection that may initially go unnoticed but can swiftly spread systemically and result in a brief primary bacteremia (43). As a result, despite the pathogen's invasive nature, it usually does not trigger an immediate inflammatory or diarrheal response. A notable feature of *S. typhi* infection that sets it apart from many diseases caused by nontyphoidal Salmonella (NTS) serovars is the lack of a mucosal inflammatory reaction. After infection, clinical symptoms might not always manifest during the incubation phase. Patients with typhoid fever often experience fatigue, and their fever typically increases in a gradual stepwise manner. *S. typhi* produces a unique array of chemicals that influence how it invades and adheres to human cells. The Vi capsule, a homopolymer of α(1→4)-D-GalpANAc that is variably acetylated at the C-3 position, plays a role in attachment, potentially by shielding *S. typhi*'s surface components from host receptors and complement in vivo. Though this connection remains undiscovered by others, a Type IVB pilus has been associated with attachment to human cells through the cystic fibrosis conductance regulator (44). The 27-kDa outer membrane protein T2544 of *S. typhi*, which targets laminin, has been associated with virulence (45). Pathogenic Salmonella species engage non-phagocytic gut epithelium by utilizing a specific set of effectors through a finely tuned mechanism involving the Type 3 secretion system (T3SS), a crucial element for Salmonella pathogenesis (46). *S. typhi* employs two T3SS known as Salmonella pathogenicity island 1 (SPI-1) and Salmonella pathogenicity island 2 (SPI-2). The 40 kb gene cluster referred to as SPI-1 contains 39 genes that code for T3SS-1, its chaperones, effector proteins, and transcriptional regulators that control the expression of various virulence genes both within and outside of SPI-1 (47).

Clinical observations indicate that after ingestion, *S. typhi* disseminates to systemic sites such as the liver, spleen, bone marrow, and gall bladder following passage through the intestinal epithelium. Symptoms of typhoid fever generally manifest 10–14 days post-ingestion, and include fever, headache, muscle pain, abdominal discomfort, and either constipation or diarrhea (48). Approximately 3–5% of those infected may continue to shed *S. typhi* for months to years after recovering from severe illness (49). One of the most feared complications of typhoid fever is intestinal perforation. Additionally, typhoid fever may lead to pneumonia, meningitis, endocarditis, osteomyelitis, and arthritis, among other complications (50).

The carrier state:

The carrier state is a well-established feature of typhoid fever. One potential source of typhoid is human carriers, such as Mary Mallon, famously referred to as "Typhoid Mary," who can excrete large amounts of *S. typhi* while showing no symptoms and leading a typical life. Although the molecular mechanisms that lead to the carrier state are not well understood, colonization of the gallbladder is crucial for *S. typhi* to persist in the human host for extended periods (51). Research indicates that in endemic regions, approximately 2% to 4% of individuals may act as carriers. Carriers often have unusually elevated levels of anti-Vi antibodies, likely due to the prolonged expression of the T cell-independent antigen Vi (51). Recent studies suggest that being a carrier can enhance the production of antibodies against various *S. typhi* antigens (52). The presence of gallstones and gallbladder disease may worsen carrier status (25).

Diagnosis of typhoid fever:

Clinical guidelines are generally utilized to identify typhoid in developed countries. In areas where typhoid is common, any fever persisting for more than a week without an obvious cause should be assumed to be typhoid until other clinical conditions are ruled out. Currently, laboratories diagnose typhoid fever through blood and stool cultures, which are considered the gold standard. However, in resource-limited settings, especially those lacking basic laboratory facilities, this presents a considerable challenge (53). Moreover, the culture method is lengthy, as it takes several days to isolate and identify the pathogens involved. The Widal test, which is the established serological approach for diagnosing typhoid, depends on O and H antigens that exhibit relatively low specificity due to interference from other bacterial infections (54). The test measures concentrations through serial dilutions of serum in a long test tube. According to the manufacturer, it has a sensitivity range of 70 to 80 percent and a specificity range of 80 to 95 percent. Furthermore, due to diminished antibody responses caused by prior antibiotic treatment, it can yield negative results in as many as 30% of cases where typhoid fever is confirmed by culture. Therefore, an efficient and sensitive test for detecting *S. typhi* would benefit both clinical diagnoses and disease containment. Several commercially available serology-based rapid diagnostic tests for typhoid fever, such as Typhidot (Malaysia), TUBEX (Sweden), and Multi-Test Dip-S-Tics (USA), have undergone global performance evaluations. Nonetheless, none of these tests achieved satisfactory results when validated in various endemic environments (55). The Widal agglutination test functions by detecting antibodies in serum. While it remains in use in resource-constrained settings, the Widal test is less effective than blood culture in terms of both sensitivity and specificity (56). Bone marrow bacterial culture provides the highest sensitivity, exceeding 80%. However, due to its expense and invasiveness, bone marrow aspiration and culture are not commonly employed in clinical practice. Thus, despite being less sensitive, blood culture remains the practical benchmark for diagnosing typhoid fever (48). A recent systematic review of ten studies assessed blood cultures' sensitivity compared to bone marrow cultures from a cohort of 529 confirmed *Salmonella typhi* cases (using the positivity of both blood and bone marrow as a composite measure) and found that bone marrow cultures were positive in 96% of

typhoid fever cases, whereas blood cultures were positive in only 61% (57). Research by Lee et al. indicates that to accurately identify bloodstream infections like *S. typhi*, two to three 20 mL samples are needed for adults (57). Blood culture sensitivity is highest during the early stages of infection and, with sufficient sampling, can approach the sensitivity levels of bone marrow culture (58-60). Although stool cultures are widely used in many endemic areas, a positive result may occur during acute illness, convalescent shedding, or chronic carriage (13). Further diagnostic obstacles in low-resource environments involve capacity constraints (such as shortages of trained personnel, diagnostic equipment, quality assurance, etc.) that hinder the differentiation between typhoid and paratyphoid fevers as well as other sources of acute febrile illness (60).

Treatment of typhoid fever:

In the 1950s, effective antimicrobial treatments were introduced, leading to a significant reduction in the case fatality rate of typhoid fever from 30% to 0.5% (61). Initially, the primary medications for treating typhoid were ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol (6, 48). Strains of *S. typhi* that have developed resistance to these three antibiotics are classified as multidrug resistant (MDR), with such cases first reported in the late 1970s to early 1980s. Since fluoroquinolones became the preferred treatment in areas with MDR infections, resistance to these second-line antibiotics has also been frequently noted. Ceftriaxone, a third-generation cephalosporin, and azithromycin, a macrolide, are currently utilized to treat typhoid fever when other treatments are unfeasible (48). However, there have been recent reports of ceftriaxone- or azithromycin-resistant *S. typhi* cases.

Antimicrobial resistance in *S. typhi*: Timely and appropriate antimicrobial treatment reliably cures typhoid fever. Nonetheless, the rise of antimicrobial resistance (AMR) in *S. typhi* constrains available treatment options. Despite indications of regional declines in antibiotic resistance levels (55, 62), the first *S. typhi* isolates exhibiting multidrug resistance (MDR)—defined as resistance to ampicillin (amp), chloramphenicol (chl), and co-trimoxazole (sxt)—were recorded in the early 1970s (Smith, PALUMBO, & EDELSON, 1984). Resistance to ciprofloxacin (cip) emerged in the early 1990s, and currently, more than 90% of clinical isolates from endemic regions demonstrate reduced susceptibility to ciprofloxacin (55). Initially, this resistance was linked to IncHI1 plasmids, which are small DNA molecules known to carry multiple resistance traits and have been found in *S. typhi* worldwide. A notable feature of a particular subtype, 4.3.1.1, is its capacity to incorporate multidrug resistance genes directly into its chromosome at certain specific locations known as IS1 sites. This method of integration aids the bacterium's resistance to various antibiotics. Multidrug-resistant *S. typhi* possesses genes associated with antibiotic resistance, such as blaTEM-1 (conferring ampicillin resistance), dhfR7 and sul1 (imparting co-trimoxazole resistance), and catA1 (bestowing resistance to chloramphenicol). The plasmid or chromosomal segment harboring the IncHI1 region is found in all identified MDR *S. typhi* (63). Resistance to macrolides can result from modifications to target sites by methylases, deactivation by enzymes like esterases and phosphotransferases, and efflux through pumps encoded by genes such as mef and msr (64). These developments have led to a shift in first-line and empirical treatments toward other classes of antimicrobial agents, including ceftriaxone (cro) and azithromycin. Alarmingly, there have been recent reports of resistance to these medications as well (65, 66).

Extensively Drug-Resistant *S. typhi*:

In Pakistan, the rise of extensively drug-resistant *S. typhi* (XDR *S. typhi*) has become a significant health concern. *S. typhi* bacteria are designated as XDR when they show resistance to third-line medications including cephalosporins, ampicillin, trimethoprim-sulfamethoxazole, and fluoroquinolones (67). This sublineage is characterized by the gyrA-S83F chromosomal point mutation and has incorporated yidA along with an IncY plasmid

(p60006) through a multi-drug-resistant composite transposon that carries the ESBL blaCTX-M-15 and the ciprofloxacin resistance gene qnrS1, which contribute to their extensive drug resistance (68, 69). The World Health Organization (WHO) indicated that from 2016 until December 2018, the Provincial Disease Surveillance and Response Unit (PDSRU) documented 5,274 cases of XDR *S. typhi* across 14 districts in Sindh, with 76% of those originating from Karachi, 27% from Hyderabad, and the remaining 4% from other districts. Despite local government efforts to control the outbreak, there was a noteworthy rise in reported cases between 2017 and 2018 (70). International surveillance revealed one case in the UK and five cases in the USA linked to XDR *S. typhi*. From November 2019 to October 2020, 9 XDR typhoid cases were detected in the US among individuals with no recent international travel history; genomic testing confirmed these isolates matched the 4.3.1.1.P1 genotype (a subclade of H58 genotype 4.3.1) that was prevalent in Pakistan (71). In February 2022, an outbreak of 23 XDR *S. typhi* cases tied to contaminated water was reported by the Chinese Center for Disease Control in a suburban apartment complex in Beijing, with genomic analysis once again pointing to the 4.3.1.1.P1 genotype (72). That same month, a case of XDR typhoid linked to genotype 4.3.1.1.P1 was confirmed in Hong Kong; it remains unclear if this case, which had no reported recent travel outside of Hong Kong, is related to the Beijing incident (73). The first widespread epidemic of XDR typhoid was documented in southeast Pakistan in 2016. The incidence of XDR typhoid fever in certain regions of Pakistan has increased from 7 to 15 cases per 100,000 people in areas where the conditions are conducive (1). According to WHO data, the PDSRU reported a total of 5,274 cases of XDR *S. typhi* in 14 districts within Sindh between 2016 and December 2018, with 76% from Karachi city, 27% from Hyderabad district, and 4% from other parts of Sindh (Organization, 2018). Following the spread of extensively drug-resistant *S. typhi* in Hyderabad, numerous similar cases have been documented. The WHO noted over 5,000 instances of XDR *S. typhi* in Karachi from November 2016 to December 2019. Currently, the only remaining effective treatments for XDR *S. typhi* are azithromycin, an oral antibiotic with broad-spectrum efficacy, and carbapenems, which require intravenous administration in more advanced clinical settings, thereby placing a significant burden on patients and healthcare infrastructure. There is a looming possibility of untreatable typhoid: azithromycin-resistant strains have arisen in several countries (74), and resistance to carbapenems has been identified in non-typhoidal Salmonella species (75).

Fluoroquinolones resistance:

Reduced sensitivity to fluoroquinolones is linked to chromosomal mutations and the intake of antimicrobial resistance (AMR) genes. In at least six cases, the genes responsible for fluoroquinolone and cephalosporin resistance have been incorporated from the IncY plasmid into the chromosome of *S. typhi*, which removes the fitness disadvantages associated with plasmid maintenance and enhances the likelihood of resistance being preserved even without ongoing exposure to these antibiotics (76). An examination conducted from 2001 to 2006 at Pakistan's Aga Khan University reveals that during this period, resistance to quinolones escalated dramatically from 1.6% to 64.1%, alongside a significant rise in the prevalence of multidrug-resistant *S. typhi* strains, increasing from 34.2% to 48.5% (77). A single variant resistant to azithromycin found in the AcrB efflux pump has been observed to emerge in multiple *S. typhi* lineages in the early 2020s. This presents a threat to the effectiveness of all oral antibiotics currently prescribed for treating typhoid. Following the discovery in Bangladesh in 2019, at least six cases of AzmR *S. typhi* have been identified in Nepal, India, and Pakistan, stemming from this mutation, which is at the center of the XDR typhoid crisis (74, 78). Ceftriaxone and azithromycin are recommended as the first-line antibiotics in situations of fluoroquinolone resistance. In *S. typhi* H58 lineages, alterations within the quinolone resistance-determining region (QRDR), which includes the DNA gyrase (gyrA and

gyrB) and topoisomerase IV (parC and parE) genes, are increasingly prevalent. The acquisition of plasmid-mediated quinolone resistance (PMQR) genes, such as qnr, oqxAB, or aac(6′)Ib-cr, may also play a role in developing fluoroquinolone resistance. The presence of multiple single nucleotide polymorphisms (SNPs) in the QRDR or a combination of QRDR SNPs along with PMQR genes leads to fluoroquinolone resistance. Recently, the failure of fluoroquinolone treatment in typhoid patients was linked to three specific QRDR SNPs in Nepal (79).

Strategies to overcome typhoid fever:

Preventing the spread of typhoid can be achieved through ensuring access to safe drinking water, maintaining hygiene, and providing uncontaminated food supplies (80). Moreover, enhancing the microbial quality of drinking water has been found to be more effective than improving sanitation. In 2008, the World Health Organization (WHO) suggested the importance of administering typhoid vaccinations. However, vaccination-based management strategies have seen limited adoption. Vaccines can effectively prevent typhoid fever, with two options currently available: the Ty21a vaccine, which is a live attenuated oral version, and the Vi parenteral vaccine (6). Both vaccines have comparable efficacy and are suitable for children aged two years and older (81). The Typhoid conjugate vaccine (Typbar-TCV), an innovative option, demonstrated promising survivability in phase 2b and phase 3 trials. Moreover, it has received prequalification from the WHO and can be given to infants starting at six months of age (82, 83). By ensuring access to immunizations, clean water, and proper hygiene practices, the likelihood of contracting typhoid can be reduced.

Conclusion:

Typhoid fever is a life-threatening disease caused by *Salmonella typhi*. The prevalence of typhoid fever is high in developing countries as compared to the developed countries. Due to the emergence of resistance, MDR and XDR typhoid strains resulted that makes the treatment of typhoid fever more challenging. It is important to develop and implement new strategies to control and prevent typhoid fever. It is important to ensure the proper management, treatment plan, and healthcare management of typhoid fever to prevent and control the incidence of resistant strains.

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