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Antibiotic Resistance: Targeting Extensively Drug Resistant (XDR) *Salmonella typhi*

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ABSTRACT
Typhoid fever is one of the most life-threatening bacterial infections and is caused by the gram-negative rod-shaped bacteria *Salmonella enterica serovar Typhi* (*S. typhi*). It is a significant worldwide health hazard and an important cause of fatalities in growing nations. Salmonella is spread through consuming contaminated food and water. Once the infection occurs, its symptoms, like high fever, abdominal pain, rash, constipation, weakness, headache and poor appetite etc. Particularly seen in Pakistan is the alarming rise in the burden of typhoid due to the multi-drug-resistant (MDR) and extensive drug-resistant (XDR) Strains. In Pakistan, the current reports of XDR strain outbreaks are not just a significant central issue in Pakistan but additionally affect public health across the world. The MDR *S. typhi* Strains consist of the H58 (Haplotype) lineage and are associated with the IncHI1 plasmid, which also resists chloramphenicol, ampicillin, trimethoprim, and sulfamethoxazole and also shows resistance against fluoroquinolones. The XDR *S. typhi* Strains carry the IncHI1 and IncY plasmids, which encode several antibiotic resistance genes. The IncY plasmid carries genes that resist the genes qnrS1 for quinolone resistance and blaCTX-M-15, an extended-spectrum beta-lactamase (ESBL). The point mutation that occurred on the AcrB R717Q/L gene also showed resistance against azithromycin. Azithromycin and carbapenems are helpful medications that are generally used for typhoid treatment. The increasing fluoroquinolone non-susceptibility rates and medical shortcomings brought on by azithromycin. The XDR *S. typhi* Strains also show resistance to azithromycin and carbapenems due to the irregular use of medication, which creates big problems for public health. In this review, we also observed that XDR *S. typhi* strains are reported in other countries, with a history of patients traveling from Pakistan. In some areas, the XDR *S. typhi* has not emerged before its exposure, we need to take some precautions, and the health authorities are responsible for controlling the XDR *S. typhi* exposure. Formulating specific SOPs, raising community awareness about XDR *S. typhi*, and executing prevention strategies are vital steps, especially in Pakistan because where drug resistance is common, due to self-medication and improper antibiotic practices.
Keywords: *Salmonella, S. typhi,* XDR, Antibiotic Resistance, ESBL

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INTRODUCTION

Salmonella enterica serotype typhi

Typhoid fever is a human illness or infection of the system induced by *the S. typhi* (*S. typhi*).[1] *Salmonella enterica serotype typhi* (*Salmonella typhi*). The bacteria are flagellated, shaped like rods, and gram-negative. It has a polysaccharide capsule which protects the bacterium from phagocytosis, and also contributing in its pathogenicity.[2] The *Salmonella enterica serotype typhi* (*S. typhi*) is a facultative anaerobe that belongs to the family *enterobacteriaceae*.[3] Worldwide rates of death and morbidity are increased by typhoid fever, an infectious disease brought on by *Salmonella enterica* serovar typhi. The Typhoid fever is a life threatening illness mainly effect children and elders.[4]

Types of *S. typhi*

The *Salmonella* are Gram-negative bacteria, and harmful to human beings. *Salmonella* are typically separated into one type of typhoidal *Salmonella* and another type of non-typhoidal *Salmonella* (NTS) based on their clinical appearance. The typhoidal *Salmonella*, involving the *Salmonella enterica* subspecies *enterica serovars typhi* and *paratyphi A, paratyphi B and paratyphi C*, induce enteric fever, which is a systemic illness.The broad class of non-typhoidal *Salmonella* (NTS) strains includes in excess of 2500 serovars, each of which generally has different animals as hosts and causes milder gastro-intestinal infections in humans that result in an estimated prevalence of 93.8 million illnesses and 155,000 fatalities throughout the year.[5] Because certain strains of non-typhoidal *Salmonella* (NTS) cause infections in the circulatory system along with the invasion of other organs, they are also known as invasive non-typhoidal *Salmonella* (iNTS). An estimated 3.4 million cases are recorded worldwide, and 6, 81,316 people die from iNTS each year. In sub-Saharan Africa, HIV, malaria, and malnutrition are prevalent health challenges, where invasive non-typhoidal *Salmonella* (iNTS) infections are often observed. Both humans and animals can be infected by the broad host-range serovars of NTS, but the typhoidal *Salmonella* are limited to humans.[5,6]

The polysaccharide capsular antigen, protein flagellar antigen Hd, and lipopolysaccharide antigens O9 and O12 are all serologically positive for *S. typhi*.[7]

Typhoid Fever or Enteric Fever

Typhoid fever is a potentially severe and sometimes life-threatening bacterial bloodstream infection. It is becoming more prevalent in many developing countries with contaminated food and water sources and insufficient sterilization.[8] Enteric fever is a momentous community health problem in countries with low and medium incomes like Pakistan, Bangladesh, and India.[9]

Out of all the Southeast Asian countries, Pakistani residents have been determined to possess the greatest risk of *S. typhi* infection.[10] In typhoid-endemic regions, such as Pakistan, pediatric sepsis is mostly caused by the Salmonella enterica serovar typhi (*S. typhi*) bacteria, which causes typhoid fever. The public health sector is increasingly at risk from sepsis, a syndromic reaction to infections that is more common in developing nations.[11]

Only humans are the hosts of *S. typhi,* which can result in typhoid fever, which is marked by a high fever that rises steadily and becomes possibly fatal along with a number of various diagnostic indicators. Though most research has improved our understanding of the disease in general, there are still unknowns. *S. typhi* is monophyletic structure indicates that the illness is very recent.[2]

Epidemiology

Although there have been reports of typhoid fever since the early 1800s, *S. typhi* was identified as the causative pathogen for typhoid fever by Karl Eberth in 1880. Before the advent of antibiotics, mortality rates for typhoid fever were 15% or even higher. However, since antibiotics were developed, the death rate has decreased to less than 1%.[12] At last, typhoid illness has become uncommon in most developed countries. The Centers for Disease Control and Prevention (CDC) only received evidence of 350 cases of typhoid fever per year that were confirmed through culture, in the USA between 2008 and 2015, which is a prevalence of less than 0.5 cases per 100,000 people.[13]

Typhoid fever is still a serious medical condition that causes a great deal of suffering for people all over the world. It is estimated that 21.6–26.9 million illnesses and 216,000 deaths occur each year.[14] In the year 2010, it was estimated that there were 12 million cases of typhoid fever worldwide, with 130,000 deaths. Additionally, there were more than 100 cases per 100,000 individuals each year in Southeast Asian countries.[15] An estimate is that there were 11–21 million cases of typhoid fever and 148,000–161,000 associated deaths around the world in year 2015.[16]

Asia has the highest burden of disease. With 93% of all cases across the world, Southeast Asia has the third-highest prevalence of enteric fever, with about 110 cases per 100,000 people. In Pakistan, the estimated rate of enteric fever in children ages 2-4 was 413/100,000, and in children ages 5–15, it was 573/100,000.[17] Typhoid fever is a periodic illness 45 percent of all cases recorded each year occur during the monsoon season. Because of the intense rains that occur during that time, the incidence of disease is highest in South Asia from July to October.[7]

In Pakistan and other countries with low and medium incomes, typhoid fever is a serious community health concern. Worldwide, typhoid cases were guessed to lie among eleven and twenty-one million, with 128,000 to 161,000 deaths.[18] Out of the sixteen Asian nations in which typhoid fever is endemic, the people who live in the Pakistani provinces of Sindh and Punjab are most likely to get it. As per a 2018 study, out of all the Southeast Asian countries, Pakistani residents are thought to have the greatest risk of *S. typhi* infection. There were approximately 493.5 cases reported for every 100,000 people.[9] According to estimates, *Salmonella enterica serovar Typhi* (*S. typhi*) is responsible for roughly 76.3% of enteric fever cases worldwide, with mortality rates considerably higher in children and adults from low- and middle-income countries (LMICs).[19]

The expanded disease trouble in countries with low and medium incomes is primarily ascribed to lacking disinfection and unfortunate arrangement of clean water and sewerage frameworks.[20] The summer months (July through September) had the highest incidence of typhoid cases (82.46%), while the winter months (December through February) had the lowest incidence (14.92%). Since enteric fever or typhoid fever are seasonal illnesses, it is reasonable to assume that the summer months will see a rise in the number of infections.[21] Although current surveillance studies in Karachi suggest that there are roughly 5 cases per 1000 individuals annually, the precise number of cases across Pakistan is unknown. High-income nations are similarly affected by travel-associated typhoid fever, with over 150 cases reported annually in England and Wales since 2008, Travel to South Asia has been a past experience for most of these situations.[22]

Routes of Transmission

The most common mode of transmission for *Salmonella enterica serovar typhi (S. typhi)* is orofecal transmission.[23] *S. typhi* is mostly spread by the fecal-oral pathway, which typically contaminates food and water. It is uncommon to find *S. typhi* in environments with widespread access to WASH infrastructure.[24,25] Humans can contract *Salmonella enterica serovar Typhi (S. typhi)* by the orofecal route, which is frequently through contaminated food and beverages.[26] Usually cannot transmitted by direct contact e.g. (kissing and sexual contact).[26]

S. typhi usually spreads passively through the consumption of food and beverages. *S. typhi* rarely grows in food or water, despite the fact that the bacteria may tolerate extended exposure to vehicles. People have understood the role that food and water

play in the transmission of typhoid fever since the late 1800s.[27]

In a survey conducted in 2020, the transmission patterns were established for 43 cases of typhoid fever outbreaks. Among these, 56% were attributed to waterborne transmission, 40% to food borne transmission, and 5% to direct contact. Waterborne outbreaks tend to have a higher average number of incidents per outbreak compared to those transmitted through food or direct contact. Factors contributing to water contamination included low-pressure water distribution systems, burst pipes, absence of water chlorination, and the proximity of drinking water sources to bathrooms.[28]

Mechanisms of Drug Resistance

The illness known as typhoid fever is typically treated with antibiotics, yet Salmonella is always challenging, and one way they may become resistant to drugs is by acquiring mutations in chromosomal, prophage, plasmid, or transposon genes. Numerous investigations have revealed that *S. typhi* is proliferating globally and resisting all first-line medications, like co-trimoxazole, ampicillin, and chloramphenicol. The bacterial strain is known as multi-drug-resistant (MDR).[11]

Genetic studies investigated to understand how antibiotic resistance spreads in *S. typhi*. Since late 1980s and early 1990s, a particular genetic variant, has rapidly spread worldwide due to its resistance to multiple antibiotics. Initially, this resistance was carried on IncHI1 plasmids, which are small DNA molecules. These plasmids are associated with multi-drug resistance and have been found in *S. typhi* globally. A key aspect of a specific subtype, 4.3.1.1, is its ability to integrate multi-drug resistance genes directly into its chromosome at various specific locations called IS1 sites. This integration mechanism contributes to the bacteria's ability to resist multiple antibiotics.[11]

Multi drug resistant *S. typhi* has genes linked to antibiotic resistance, including blaTEM-1 (which imparts ampicillin resistance), dhfR7 and sul1, (which provide co-trimoxazole resistance), and catA1 (which confers resistance to chloramphenicol). The plasmid or chromosome containing the IncHI1 region is present in all identified MDR *S. typhi*[11].[11]

The alteration of target sites by methylases, the antibiotic's deactivation by enzymes like esterases and phosphotransferases, and efflux facilitated by pumps involving those encoded by the mef and msr genes are some of the causes of resistance to macrolides.[29] Pakistan has witnessed the emergence of a novel mutant sublineage of *S. typhi* (4.3.1.1.P1), which produces XDR extended spectrum beta-lactamase (ESBL).[30] In conjunction with the gyrA-S83F single chromosomal point change that this sublineage possesses, yidA and an IncY plasmid (p60006) were incorporated by the MDR composite transposon carrying the ESBL blaCTX-M-15 and the quinolone opposition to the qnrS1 gene, which confer opposition to extended-spectrum beta-lactamases (ESBLs) and ciprofloxacin, respectively, making them extensively drug-resistant.[26,30,31]

Resistance to the quinolone is caused by alterations in the plasmid-mediated qnr, qepA, and aacs (6)-Ib-cr genes, as well as in the quinolone resistance determining areas of chromosomal genes, such as gyrA, gyrB, parC, and parE. In addition, work has revealed that ciprofloxacin susceptibility was decreased in plasmid-mediated opposition.[15]

Molecular analysis indicates that the isolates resistant to ceftriaxone express the blaCTX-M-15 gene within the resistant IncY plasmid (p60006), identified during the Sindh outbreak. Sulphonamides, chloramphenicol, and trimethoprim were positioned in a combination of mutationscoordinated into the chromosome yidA site in the extra-resistance factors that code for aminoglycoside resistant.Although the gyrA mutation Ser83Phe and the acquisition of qnrS prevented these strains from being as susceptible to fluoroquinolones.[32,33] In correlation with an ISEcp1 (tnpA), bandage screening (Figure 1) and the National Centre for Biotechnology Information (NCBI) BLAST verified the existence of ESBL CTX-M-15.[32]

Bandage analysis of the resistant strain of *S. typhi*. A Spades assemblage of sequenced regions (nodes) related with drug opposition from the *S. typhi* strain. Bandage permits visualization of how nodes (in gray) are conceivably linked (in black) to each other. The genes of interest, CTXM-15 and qnrS, are blasted opposite the assembled nodes, and their locations are determined. CTXM-15 is present in node 57, and a qnrS is present in node 49. Genes found on node 57 include CTXM-15, which is linked with the insertion element ISEc9. Additionally, there is an abbreviation noted: hp, indicating a hypothetical protein.[32]

S. typhi and paratyphiA, the bacteria that causes paratyphoid fever, can develop azithromycin tolerance due to a small change in the efflux pump AcrB at position R717Q/L. Arranged strains of Salmonella with typhoidal (typhi and *paratyphi* A) and primary non-typhoidal serovars (Typhimurium and Enteritidis) variations of AcrB R717Q/L were examined in order to figure out the worldwide distribution of this particular alteration. The two most frequently identified reason of non-typhoidal salmonellosis that may result in sudden deaths are the two last serovars (*Salmonella enteritidis and S. typhimurium*).[33,34]

Usage of Typhoid Conjugate Vaccine

The administration of typhoid immunizations had been suggested by the World Health Organization (WHO) in 2008. Vaccination-based management strategies, however, have not been adopted significantly. Both the orally administered Vi polysaccharide vaccine and the live-attenuated Ty21a vaccine were available, hence the Ty21a vaccine's capsules were indigestible for small kids and the Vi polysaccharide vaccine's immunogenicity was

impoverished in young children, both of these vaccines were considered inappropriate for widespread use.[35]

The two previous vaccinations, the three-dose oral Ty21a vaccine and the single intramuscular dosage of Vi-polysaccharide, have inadequate effectiveness, have brief protective duration it is not advised to use it within kids younger than two. Typhoid conjugate vaccine (Typbar-TCV), an innovative vaccine, was shown to have encouraging survivability in phase 2b and phase 3 trials. Furthermore, it is prequalified by the WHO and can be administered to infants as early as six months.[36-38]

World Health Organization (WHO) recommends that nations where typhoid fever is widespread set up laboratory-confirmed health facility-based monitoring in order to track antimicrobial resistance trends, evaluate vaccine efficacy, and estimate the burden of the disease [16].

In the past, immunization campaigns to control typhoid have not always been successful. There had earlier been three typhoid vaccines used: Ty21a, the polysaccharide vaccine (Vi-PS), and the inactivated whole-cell vaccine. Although Ty21a and the polysaccharide vaccine (Vi-PS) are currently available, their regular usage in typhoid-endemic nations has not materialized because of age limitations or practical difficulties.[39] By ensuring that people receive the immunization, clean water is accessible, and there is proper hygiene, the chance of contracting typhoid can be decreased. There have been multiple reports indicating that Pakistani travelers have introduced XDR typhoid to the United States, the Australia, United Kingdom, Taiwan, Denmark, and Canada.[21]

REVIEW LITERATURE

Evolution of Extensively Drug-Resistance Strains

In Pakistan, extensively drug-resistant *S. typhi* (XDR *S. typhi*) has emerged as a pressing medical problem. When *S. typhi* bacteria develop challenges to the third-line drugs cephalosporins, ampicillin, trimethoprim-sulfamethoxazole and fluoroquinolones, they are classified as XDR *S. typhi*.[40] In the 1940s, the first antibacterial used for the cure of typhoid was chloramphenicol.[41] The progressive evolution of antibiotic opposition in *S. typhi* has led to the emergence of MDR strains in bacteria. It has been demonstrated that certain first-generation medications, such as ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol, are resistant to these infections. But still, they are vulnerable to third-line drugs like cephalosporins. Since the 1980s, Bangladesh, India, Nepal, and Pakistan have been common of this type of intestinal fever.[17]

In the past, the first-generation antibiotic treatments for typhoid fever were ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. However, beginning in the 1990s and ending in the 1980s, reports came from nations throughout southern and Southeast Asia, including Pakistan, India, and others, indicating that *S. typhi* was resistant to these three first-line treatments, a condition known as MDR.[42] The subsequent development of resistance to it led to the 1970s prescription of co-trimoxazole and other antibiotics. However, studies from the 1980s claimed that *S. typhi* strains were resistant to every antibiotic that had been used before.[27]

Antibiotics that included ampicillin and trimetho prim-sulfamethoxazole became the preferred treatment for typhoid because of the resistance to chloramphenicol, despite the fact that they had less chance of effectiveness than other drugs prescribed earlier. However, it didn't take long for news of drug resistance to circulate around the world, and an increasing number of fatal cases were associated with resistant strains of these two drugs. Consequently, ciprofloxacin and other fluoroquinolones were used to treat enteric fever and typhoid.[41-43]

The concern about antibiotic treatment failure has been heightened by the increase in cases of typhoid in Pakistan, which is caused by MDR and XDR *S. typhi*.[44] Between 2010 and 2018, approximately 35% of *S. typhi* isolates that were reported from Asia and 75% from Africa have been identified as MDR to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxa- zole.[42]

MDR Salmonella infections are caused by strains of the bacteria that are resistant to first-line antibiotics. Second-generation medicines, such as ceftriaxone, and MDR Salmonella infections are cured using macrolides and third-line cephalosporins. Salmonella with XDR could arise from resisting at least one class II antibiotic.[3] Since 2015, there has been a noticeable increase of around 5·01% in XDR Salmonella cases in Pakistan.[45]

In 2016 a study also showed the appearance of the initial indication of XDR *S. typhi* in Karachi. Since the beginning of the epidemic in Sindh, over 17,000 cases of XDR typhoid have been reported. During the period of 2016–2017, Hyderabad alone recorded over 800 cases of XDR typhoid, making the entire region endemic for the disease. Thus, the numerous studies conducted in Pakistan have recently been limited to Sindh; nevertheless, due to frequent and international travel, there have been reports of cases appearing or arising throughout the nation and elsewhere.[41]

The first widespread epidemic was recorded in the province in southeast Pakistan in 2016. The frequency of XDR typhoid fever has arisen from 7 out of 100,000 to 15 out of 100,000 in the specific regions of Pakistan where the situation are favorable.[9]

Sindh province, Pakistan, has spread a significant XDR *S. typhi* outbreak since November 2016, primarily in the region of Karachi and Hyderabad.[14] There have been many other reports of cases similar to the XDR *S. typhi*-caused typhoid fever spread in Hyderabad. 5274 cases of XDR *S. typhi* were documented to the WHO in Karachi from November

2016 and December 2019. Infections resulting from XDR *S. typhi* are typically treated in Pakistan with meropenem and azithromycin, either administered individually or in a mixture.[17]

In many areas where enteric fever is widespread, the prevalence of antibacterial resistance is rising, which raises the need for action in addition to the high disease prevalence. In the 2016 typhoid outbreak in Hyderabad, Pakistan, the country was the first to report on MDR strains that were furthermore resistant to fluoroquinolones and Class III cephalosporins, a condition known as XDR. Pakistan still documents a significant percentage of XDR-*S. typhi* cases. There has been evidence of antimicrobial resistance in Asia to a growing range of drugs, such as azithromycin, a macrolide, and third-generation cephalosporins.[16] The possibility of antibiotic treatment failure has been heightened by the rising incidence of typhoid in Pakistan, which is caused by MDR and XDR *S. typhi*.[44]

The first case of XDR *S. typhi* occurred in Hyderabad, Pakistan, in 2016. These XDR *S. typhi* strains were resistant to the first-line medications like, fluoro-quinolone, and the class-III medication cephalosporin. The WHO statistics state that Pakistan has been the source of more than 10,365 cases of XDR *S. typhi* infections. The number of recorded cases gradually increased over the course of the two-year period from 2017 to 2018, in spite of attempts at infection control programs to contain the illness.[45,46]

Based on statistics from the WHO, 5274 XDR *S. typhi* cases were reported to the Provincial Disease Surveillance and Response Unit (PDSRU) in Sindh's 14 districts between 2016 and December 2018. Among them, 27% came from the Hyderabad district, 4% from different areas in Sindh, and 76% from Karachi city.[45] Numerous comparable cases have been documented after the extensively drug-resistance *S. typhi*-caused typhoid fever spread in Hyderabad. Over 5,000 cases of XDR *S. typhi* were noted by the WHO between November 2016 and December 2019 in Karachi.[47]

The 29 cases of typhoid fever having a travel background from Pakistan were noted in the USA in reaction to the XDR cases from Pakistan.[13] Despite the local government's implementation of control measures, there was anobservable rise in the number of reported cases between 2017 and 2018. Five cases from the USA and one from Canada were found during the global monitoring for XDR *S. typhi*. One case was found in the UK. All of these patients had traveled to Pakistan previously.[13,14,45] Furthermore, during the pandemic, there has been an upsurge in typhoid cases that resemble the coronavirus (COVID-19) in terms of clinical presentation in Pakistan, over 20 thousand cases were examined that existed between June 2020.[21] Around 10,365 cases of XDR *S. typhi* were recorded in 2019, according to estimates from the WHO. In September 2020, there were 2883 instances documented in Pakistan all cases were isolated with strong resistance.[9]

Between January 2016 and August 2019, 96 cases of Typhi infections towards US visitors to or from Pakistan were recorded by the CDC. 31% of these isolates had antimicrobial susceptibility testing results that indicated them to be XDR. Trimethoprimsulfametho xazole, ciprofloxacin, ampicillin, chloramphenicol, nalidixic acid, streptomycin, and sulfisoxazole were among the drugs that these isolates were resistant to. Out of the 20 patients who reported information regarding their travels within Pakistan, 12 (or 60%) visited Sindh province's Karachi or other regions, among the places where the XDR pandemic is known to have occurred. The remaining eight (or 40%) patients weren't reported as visiting Sindh but instead visited Punjab province.[48]

Since the strains of *S. typhi* demonstrated resistance to every antibiotic recommended for treating typhoid fever, including third-generation cephalosporins, they were dubbed *S. typhi* that is extensively resistant to drugs (XDR *S. typhi*). Additionally, the strains of XDR *S. typhi* have shown susceptibility to azithromycin or carbapenems (meropenem and ertapenem).[49]

Although official occurrence data analysis for XDR *S. typhi* is still unavailable beyond the province of Sindh, case reports from other parts of the country and the area are being passed on by travelers. Thus, whole genome sequencing (WGS) was further used to track the spread of viral and bacterial infections and to understand how *S. typhi* evolved mechanisms to resist drugs. Individual instances of XDR *S. typhi* from Denmark, Canada, and Taiwan that underwent whole genome sequencing revealed a nearest relationship (complete nucleotide match) with Pakistan's nearest sequenced XDR strain.[22]

Because of the unique characteristics of XDR *S. typhi*, there are currently no global treatment guidelines available. There are limited options for treatment, and little information is available regarding the results of XDR typhoid treatment. The present knowledge of the disease is crucial for creating treatment plans for typhoid patients with XDR. Additionally, observing information from this investigation could be helpful in the future in developing a more potent clinical experiment to cure XDR typhoid fever.[1]

Reports of *S. typhi* resistance to ampicillin, chloramphenicol and co-trimoxazole—also known as the multidrug-resistant *S. typhi* date back to the 1980s in nations with widespread infection, such as Pakistan, India, Bangladesh, and Nepal. The most effective treatments for *S. typhi* were fluoro-quinolones and third-generation cephalosporins until 2000. But during the course of the following 20 years, quinolone resistance emerged; over 90% of South Asian isolates of *S. typhi* showed decreased susceptibility to ciprofloxacin. Rare reports of *S.*

typhi isolates from Bangladesh, India, and Pakistan that are resistant to cephalosporins-class III drugs have been made.[49]

Reports have emerged from Bangladesh, India, and Pakistan regarding sporadic occurrences of *S. typhi* isolates that are opposing the cephalosporin-class III antibiotic. From 2016 to 2017, researchers observed a rise in *S. typhi* resistant to ceftriaxone infections originated in Pakistan's Sindh province.[49] A significant *S. typhi* outbreak that was resistant to ceftriaxone was documented in November 2016 among children who lived in Hyderabad, Pakistan. There were approximately 486 cases recorded and the infection was shown to be associated with contamination of water used for drinking.[17]

Carbapenems are a class of β-lactam antibiotic components that work against gram-negative bacteria in a wide range of situations. Prior to this, it was thought that carbapenems were the final choice when it came to treating gram-negative bacterial infections. Three main categories can be used to categorize genes that encode ESBL. On the other hand, Salmonella's resistance to ciprofloxacin is mostly caused by one variation in the parC gene and two doubled alterations in the gyrA gene.[3]

However, antimicrobial therapy is required for extra intestinal *Salmonella* infections, even though they typically resolve on their own. Serious cases of salmonellosis can occasionally be treated with antibiotics such as ceftriaxone, a third-generation cephalosporin, azithromycin, fluoroquinolone and ciprofloxacin, a fluoroquinolone prescription. When genes for resistance are found on plasmids containing extra-severe mechanisms, resistant *Salmonella* infections can be more serious, leading to a greater rate of hospitalization and even fatalities.[50,51]

The antibiotics named quinolones primarily target DNA topoisomerase IV and DNA gyrase, as well as the topoisomerases of bacterial type II. Both proteins influence DNA supercoiling and are coded by the genes gyrA, gyrB, parC, and parE. Quinolones quickly kill bacteria by inhibiting the functioning of enzymes, which causes chromosomal replication to be interrupted. It has been observed that bacteria have multiple resistance mechanisms to quinolones. Topoisomerase enzymes have a decreased affinity for binding quinolones when they have chromosomal gyr and par gene alterations in the quinolone resistance-determining regions (QRDRs).[5] The broad-spectrum antibiotics known as fluoro-quinolones directly prevent bacteria from synthesizing DNA. At the moment, the drugs that are prescribed for the majority of typhoid fever cases are ofloxacin and ciprofloxacin. Because of its high plasma and intracellular penetrations and greater bactericidal activity, ofloxacin was a great antibiotic choice for treating typhoidal Salmonella.[15] When *S. typhi* developed multidrug resistance in the 1990s, fluoroquinolones became the go-to medication for successful therapy. Up until the 2010s, nearly all strains was alteration in the quinolone resistance-determining regions (QRDR) of *S. typhi* in South Asia.[52]

An investigation into the antimicrobial resistance of *S. typhi* and *paratyphi* A in Pakistan over a three-year period (2009–11) revealed two cases of cephalosporin (cefixime and ceftriaxone) resistance and an increase in fluoroquinolone resistance, from 84.7% to 91.7%.[49] The WHO specifically designated Salmonella resistant to fluoroquinolones (FQ) as a high-priority infectious agent for investigation and development of new antibiotics in 2017.53 Fluoroquinolone FQ-resistant Salmonellae were ranked according to ten criteria, the most important of which were the following: the number of infections in the community, the susceptibility to spread and the potential for zoonotic disease, the duration of hospital stay following infection, and the likelihood of developing newly antibacterial promptly.[5]

Around the world, organisms with reduced susceptibility and ultimately increased resistance evolved owing to the extensive Fluoroquinolone usage. The two medications used to treat *S. typhi* that are resistant to fluoroquinolones are ceftriaxone and azithromycin. The macrolide azithromycin is well-liked because of its oral form and single daily dosing. The treatment of typhoid fever using macrolides has faced challenges due to the emergence of resistance, likely stemming from their prolonged use in treating various infections. Third-generation cephalosporins remain the primary choice due to their efficacy, yet there's growing apprehension about the potential widespread resistance development, leaving them as the last line of defense against typhoid fever.[54]

Azithromycin is the only oral antibiotic that is still readily available and has been shown to be effective throughout almost all of the healthcare system of Pakistan. There have already been reports of azithromycin-resistant strains in South Asia and that broad utilization of this medication may hasten the emergence of a defense against this medication.[55] Quinolone-resistant strains of *S. typhi* are still common, notwithstanding the decline of MDR strains. An analysis carried out from 2001 to 2006 at Pakistan's Aga Khan University indicates that during this time, theresistance to quinolones surged significantly from 1.6% to 64.1%, and there was a notable increase in the prevalence of multidrug-resistant *S. typhi* strains, rising from 34.2% to 48.5%.[2]

A single azithromycin-resistant variant in the AcrB efflux pump was discovered to have freely arisen in several *S. typhi* lineages in the 2021s. This poses a danger to the effectiveness of all oral antibiotics used to treat typhoid. Since the revelation from Bangladesh in 2019, at least six cases of AzmR *S. typhi* have been reported from Nepal, India, and Pakistan due to this mutation, which is the epicenter

of the XDR typhoid crisis.[52,56] Ceftriaxone and azithromycin are the preferred antibiotics in the event of fluoroquinolone resistance. Nonetheless, there have been cases of *S. typhi* that are defense against azithromycin and ceftriaxone.[57]

Class-III antibacterial cephalosporins have been the first line of treatment for infections at the end of the era as a result, attention to typhoid disease has progressively decreased in progressing nations. This distinct case has been attributed to the persistence, and many *S. typhi* are sensitive to these medicines.[8,58] Two of the relatively few effective treatments for typhoid fever include azithromycin and class III medicine cephalosporins. The first instances of cephalosporin resistance were documented in Bangladesh in 1998 and 2001; nevertheless, these instances appeared to be singular occurrences.[59]

Until November 2016, medications for *S. typhi* included ceftriaxone, a macrolide, azithromycin, and a third-generation cephalosporin. The province of Sindh in Pakistan has reported a significant epidemic of ceftriaxone-resistant infections, primarily from Hyderabad and Karachi. First-line medications, fluoroquinolones, and third-line cephalosporins are unsuccessful in opposing these XDR *S. typhi* strains. From the month of November 2016 to September 2017, 339 XDR *S. typhi* were isolated from the Sindh province of Pakistan.[60]

Azithromycin and Ceftriaxone and cefixime are two extended-spectrum cephalosporins, are suitable substitutes for *S. typhi*, which is vulnerable to decreased fluoroquinolones. Patients who do not respond promptly are often treated with cephalosporin and azithromycin combinations.[15] Azithromycin and meropenem combined are now the recommended course of treatment for confirmed XDR *S. typhi* cases in the UK.[32]

According to the majority of research, ceftriaxone resistance is spreading around the world. Typhoid fever morbidity, mortality and treatment burden have increased due to the rise in antibiotic resistance. The cornerstone of treatment for individuals with these XDR strains of Salmonella is the expensive carbapenems, which are sensitive to azithromycin.[61] One study from Bangladesh revealed numerous cases of *S. typhi* strains resistant to azithromycin, despite the fact that no major resistance to azithromycin or carbapenems has been documented in Pakistan or other endemic countries.[61,62]

An epidemic of XDR *S. typhi* was pointed out in Pakistan in February 2018. In the region, especially in Karachi and Hyderabad, XDR typhi cases are being recorded in over five thousand. These cases have shown resistance to ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, ampicillin, and third-line cephalosporins.[63] In addition, several *S. typhi* strains have shown multidrug resistance to trimethoprim-sulfamethoxa zole, ampicillin, and chloramphenicol. Azithromycin reduced susceptibility has also been documented, yet no multidrug-resistant or azithromycin-resistant XDR strains have surfaced.[43,63]

Five instances of the XDR *S. typhi* have been recorded in the US as of January 2019, and they were all cultured from kids who had recently traveled to Pakistan and were between the ages of 4 and 12.[63] There was a noticeable rise in ceftriaxone-opposing *S. typhi* infections in the Sindh region of Pakistan in the years 2016–17. A unique variety of *S. typhi* that possesses H58 ancestry was validated by completely sequencing the isolate genomes. It carried a plasmid containing additional resistance components, including the extended- spectrum β lactamase blaCTX-M-15, along with the qnrS fluoroquinolone resistance gene.[26,49]

Azithromycin and third-generation cephalosporins are recommended in more recent guidelines nevertheless, their effectiveness regarding the Fever Clearance Time (FCT) has been less substantial, with defects in some short-term strategies above 20% of treated cases. Cefotaxime and ciprofloxacin have together shown a synergistic effect against nalidixic acid (NA)-resistant isolates of *S. typhi* and Salmonella *paratyphi*.[64]

Third-generation drugs such as carbapenems (imipenem, meropenem) and macrolides (azithromycin) are still used as "next-option"oral and intravenous medications for *S. typhi* illnesses. Resistance to the macrolide azithromycin has emerged in the XDR strains of *S. typhi*. Additionally, there are claims of *S. typhi* strains resistant to the antibiotic carbapenem, as well as numerous instances of resistance to invasive nontyphoidal Salmonella (NTS) to carbapenem.[11,29]

As the use of azithromycin rises, there is a greater chance that isolates resistant to the antibiotic will arise and spread, which could lead to illnesses that are incurable or result in higher death rates. There is a dearth of information regarding the molecular mechanism underlying azithromycin opposing in typhoidal Salmonella, despite the occasional occurrence of treatment failures.[62] Because of its excellent accessibility, less harmfulness, and wide antibacterial action, azithromycin is among the most commonly given antibacterials for a variety of Gram-negative infections in humans, including traveler's diarrhea, salmonellosis, campylobacteriosis, and shigellosis.[51]

CONCLUSION

This review or exploratory investigation set out to ascertain the frequency of extensively drug-resistant (XDR) *S. typhi* and provide an overview of the current state of XDR *S. typhi* in Pakistan. In the outbreak of the extensively drug-resistant (XDR) *S. typhi* strain in Pakistan, there is widespread resistance observed across practically all treatments, including third-generation medications such as azithromycin and cephalosporins. This article examines the pathogenicity of *S. typhi*, its historical background and the progress of defense against

antibiotic. The purpose of this paper is to offer insightful information to researchers, policymakers, and healthcare professionals who are developing ways to effectively battle XDR *S. typhi*.

The worldwide spread of XDR and MDR-*S. typhi* presents a severe risk to public health. The first outbreaks of the XDR *S. typhi* strain are connected with the IncY plasmid and affected a wide range of Pakistan. Fluoroquinolones, third-generation cephalosporins, and first-line medications did not work on these XDR *S. typhi* strains. After all, these Salmonella XDR strains are sensitive to azithromycin plus expensive carbapenems, that is, the mainstays of cure for these individuals; however, there have also been reports of resistance to these antibiotics in certain endemic locations. A large portion of Pakistan is affected by the XDR strains of *S. typhi*, which are immune to antibiotic therapy. However, more effective antimicrobial use, vaccination, and improved sanitation are important aspects of treating typhoid infection. However, sadly, using antibiotics infrequently causes defense against first- and second-class medicines to increase, while rare resistance to third-line antibiotics is recognized.

The health authorities work on the XDR *S. typhi* in worldwide development to substantially enhance immunity to typhoid disease. *S. typhi* proceeded to disseminate and affect numerous regions. The poor management of the *S. typhi*-carrying population contributes to this situation. Alongside efforts to control the spread of XDR *S. typhi* strains, understanding the pathogenic mechanisms by which they develop antimicrobial resistance and induce chronic infections is crucial. The increasing incidence of XDR strains poses a significant threat to public health globally, necessitating a comprehensive understanding of the mechanisms behind resistance, current epidemiological trends, and potential strategies for improvement. Most areas where the XDR *S. typhi* strains emerged, including Sindh, Hyderabad, Karachi, and southern Punjab, were also included. The burden of XDR *S. typhi* in such areas is due to the misuse of drugs, self-medication, contamination of foods, and impure water.

REFERENCES

1. Qureshi, S., et al., Response of extensively drug resistant *S. typhi* to treatment with meropenem and azithromycin, in Pakistan. PLoS neglected tropical diseases, 2020. 14(10): p. e0008682.
2. 2.Akram, J., et al., Extensively drug-resistant (XDR) typhoid: evolution, prevention, and its management. BioMed Research International, 2020. 2020.
3. Zahid, I., et al., Antibiotyping and genotyping of extensively drug-resistant (XDR) Salmonella sp. isolated from clinical samples of Lahore, Pakistan. Journal of applied microbiology, 2022. 132(1): p. 633-641.
4. Rasheed, F., et al., Trend of antimicrobial resistance among *S. typhi* isolated from the pediatric population presenting at a tertiary care hospital in Lahore. Biomedica, 2023. 39(2): p. 61-66.
5. Cuypers, W.L., et al., Fluoroquinolone resistance in Salmonella: insights by whole-genome sequencing. Microbial genomics, 2018. 4(7).
6. Yang, Y.-A., A. Chong, and J. Song, Why is eradicating typhoid fever so challenging: implications for vaccine and therapeutic design. Vaccines, 2018. 6(3): p. 45.
7. Chandankhede, S.A., et al., A review on typhoid infectious bacterial disease. World Journal of Pharmaceutical Research, 2022. 11(17): p. 669-680.
8. Procaccianti, M., et al., First case of typhoid fever due to extensively drug-resistant Salmonella enterica serovar Typhi in Italy. Pathogens, 2020. 9(2): p. 151.
9. Jabeen, K., et al., Molecular Characterization of Extensively Drug Resistant Salmonella Enterica Serovar Typhi Clinical Isolates from Lahore, Pakistan. Infection and Drug Resistance, 2023: p. 2987-3001.
10. Fatima, M., et al., Morbidity and mortality associated with typhoid fever among hospitalized patients in Hyderabad district, Pakistan, 2017-2018: retrospective record review. JMIR Public Health and Surveillance, 2021. 7(5): p. e27268.
11. Kim, C., et al., The molecular basis of extensively drug-resistant *S. typhi* isolates from pediatric septicemia patients. PLoS One, 2021. 16(9): p. e0257744.
12. Ashurst, J.V., J. Truong, and B. Woodbury, *S. typhi*. 2018.
13. Chatham-Stephens, K., et al., Emergence of extensively drug-resistant *S. typhi* infections among travelers to or from Pakistan—United States, 2016–2018. Morbidity and Mortality Weekly Report, 2019. 68(1): p. 11.
14. Wong, W., et al., The first Canadian pediatric case of extensively drug-resistant *S. typhi* originating from an outbreak in Pakistan and its implication for empiric antimicrobial choices. IDCases, 2019. 15: p. e00492.
15. Veeraraghavan, B., et al., Typhoid fever: issues in laboratory detection, treatment options & concerns in management in developing countries. Future science OA, 2018. 4(6): p. FSO312.
16. Hancuh, M., et al., Typhoid fever surveillance, incidence estimates, and progress toward typhoid conjugate vaccine introduction—worldwide, 2018–2022. Morbidity and Mortality Weekly Report, 2023. 72(7): p. 171.

17. Shahid, S., et al., A review of clinical profile, complications and antibiotic susceptibility pattern of extensively drug-resistant (XDR) *S. typhi* isolates in children in Karachi. BMC Infectious Diseases, 2021. 21: p. 1-9.
18. Batool, R., et al., Coverage survey of typhoid conjugate vaccine among children aged 6 months to 15 years in an urban slum settlement of Lyari Town Karachi, Pakistan. Plos one, 2023. 18(8): p. e0289582.
19. Stanaway, J.D., et al., The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet Infectious Diseases, 2019. 19(4): p. 369-381.
20. Shaikh, O.A., et al., Antimicrobial resistant strains of *S. typhi*: The role of illicit antibiotics sales, misuse, and self-medication practices in Pakistan. Journal of Infection and Public Health, 2023.
21. Haqqi, A., et al., COVID-19 and *S. typhi* co-epidemics in Pakistan: a real problem. Journal of medical virology, 2021. 93(1): p. 184.
22. Rasheed, F., et al., Emergence of resistance to fluoroquinolones and third-generation cephalosporins in *S. typhi* in Lahore, Pakistan. Microorganisms, 2020. 8(9): p. 1336.
23. Masuet-Aumatell, C. and J. Atouguia, Typhoid fever infection–Antibiotic resistance and vaccination strategies: A narrative review. Travel Medicine and Infectious Disease, 2021. 40: p. 101946.
24. Walker, J., et al., Assessing the global risk of typhoid outbreaks caused by extensively drug resistant *S. typhi*. Nature communications, 2023. 14(1): p. 6502.
25. Cao, Y., et al., Geographic pattern of typhoid fever in India: a model-based estimate of cohort and surveillance data. The Journal of infectious diseases, 2021. 224(Supplement_5): p. S475-S483.
26. Klemm, E.J., et al., Emergence of an extensively drug-resistant Salmonella enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. MBio, 2018. 9(1): p. 10.1128/mbio. 00105-18.
27. Crump, J.A., Progress in typhoid fever epidemiology. Clinical Infectious Diseases, 2019. 68(Supplement_1): p. S4-S9.
28. Appiah, G.D., et al., Typhoid outbreaks, 1989–2018: implications for prevention and control. The American journal of tropical medicine and hygiene, 2020. 102(6): p. 1296.
29. Ahsan, S. and S. Rahman, Azithromycin resistance in clinical isolates of Salmonella enterica serovars Typhi and *paratyphi* in Bangladesh. Microbial Drug Resistance, 2019. 25(1): p. 8-13.
30. Kamal, R., et al., Identification of multiple variant extensively drug-resistant typhoid infections across Pakistan. The American Journal of Tropical Medicine and Hygiene, 2023. 108(2): p. 278-284.
31. Ingle, D.J., et al., Genomic epidemiology and antimicrobial resistance mechanisms of imported typhoid in Australia. Antimicrobial agents and chemotherapy, 2021. 65(12): p. 10.1128/aac. 01200-21.
32. Godbole, G.S., et al., First report of CTX-M-15 *S. typhi* from England. Clinical infectious diseases, 2018. 66(12): p. 1976-1977.
33. Nair, S., et al., ESBL-producing strains isolated from imported cases of enteric fever in England and Wales reveal multiple chromosomal integrations of bla CTX-M-15 in XDR *S. typhi*. Journal of Antimicrobial Chemotherapy, 2021. 76(6): p. 1459-1466.
34. Hooda, Y., et al., Mass azithromycin administration: considerations in an increasingly resistant world. BMJ Global Health, 2020. 5(6).
35. Shakya, M., et al., Phase 3 efficacy analysis of a typhoid conjugate vaccine trial in Nepal. New England Journal of Medicine, 2019. 381(23): p. 2209-2218.
36. Yousafzai, M.T., et al., Effectiveness of typhoid conjugate vaccine against culture-confirmed Salmonella enterica serotype Typhi in an extensively drug-resistant outbreak setting of Hyderabad, Pakistan: a cohort study. The Lancet Global Health, 2021. 9(8): p. e1154-e1162.
37. Milligan, R., et al., Vaccines for preventing typhoid fever. Cochrane Database of Systematic Reviews, 2018(5).
38. Organization, W.H., Typhoid vaccines: WHO position paper, March 2018–Recommendations. Vaccine, 2019. 37(2): p. 214-216.
39. Theiss-Nyland, K., et al., Assessing the impact of a Vi-polysaccharide conjugate vaccine in preventing typhoid infection among Bangladeshi children: a protocol for a phase IIIb trial. Clinical Infectious Diseases, 2019. 68(Supplement_2): p. S74-S82.
40. Organization, W.H., Typhoid fever–Islamic Republic of Pakistan. 2018. 2019.
41. Zakir, M., et al., Emerging trends of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *S. typhi* in a tertiary care Hospital of Lahore, Pakistan. Microorganisms, 2021. 9(12): p. 2484.

42. Marchello, C.S., S.D. Carr, and J.A. Crump, A systematic review on antimicrobial resistance among *S. typhi* worldwide. The American journal of tropical medicine and hygiene, 2020. 103(6): p. 2518.
43. Andrews, J.R., et al., Extensively drug-resistant typhoid—are conjugate vaccines arriving just in time? New England Journal of Medicine, 2018. 379(16): p. 1493-1495.
44. Rasheed, M.K., S.S. Hasan, and S.I. Ahmed, Extensively drug-resistant typhoid fever in Pakistan. The Lancet Infectious Diseases, 2019. 19(3): p. 242-243.
45. Saeed, N., M. Usman, and E.A. Khan, An overview of extensively drug-resistant *S. typhi* from a tertiary care hospital in Pakistan. Cureus, 2019. 11(9).
46. Majeed, M.M. and A. Munir, Pakistan: country report on children's environmental health. Reviews on Environmental Health, 2020. 35(1): p. 57-63.
47. Organization, W.H., Typhoid fever—Islamic Republic of Pakistan. Disease outbreak news. Geneva: WHO, 2018.
48. Watkins, L.K.F., et al., Update on extensively drug-resistant Salmonella serotype Typhi infections among travelers to or from Pakistan and report of ceftriaxone-resistant Salmonella serotype Typhi infections among travelers to Iraq—United States, 2018–2019. Morbidity and Mortality Weekly Report, 2020. 69(20): p. 618.
49. Qamar, F.N., et al., Outbreak investigation of ceftriaxone-resistant Salmonella enterica serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study. The Lancet Infectious Diseases, 2018. 18(12): p. 1368-1376.
50. Control, C.f.D. and Prevention, Antibiotic resistance threats in the United States, 2019 Available at: https://www. cdc. gov/drugresistance/biggest-threats. html. 2020, Accessed.
51. Ge, B., et al., Genomic analysis of azithromycin-resistant Salmonella from food animals at slaughter and processing, and retail meats, 2011–2021, United States. Microbiology Spectrum, 2024. 12(1): p. e03485-23.
52. Da Silva, K.E., et al., The international and intercontinental spread and expansion of antimicrobial-resistant *S. typhi*: a genomic epidemiology study. The Lancet Microbe, 2022. 3(8): p. e567-e577.
53. Tacconelli, E., et al., Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet infectious diseases, 2018. 18(3): p. 318-327.
54. Djeghout, B., et al., Ceftriaxone-resistant *S. typhi* carries an IncI1-ST31 plasmid encoding CTX-M-15. Journal of medical microbiology, 2018. 67(5): p. 620-627.
55. Levine, M.M. and R. Simon, The gathering storm: is untreatable typhoid fever on the way? MBio, 2018. 9(2): p. 10.1128/mbio. 00482-18.
56. Sajib, M.S., et al., Tracking the emergence of azithromycin resistance in multiple genotypes of typhoidal salmonella. MBio, 2021. 12(1): p. 10.1128/mbio. 03481-20.
57. Jacob, J.J., et al., *S. typhi* acquires diverse plasmids from other Enterobacteriaceae to develop cephalosporin resistance. Genomics, 2021. 113(4): p. 2171-2176.
58. Britto, C.D., et al., A systematic review of antimicrobial resistance in Salmonella enterica serovar Typhi, the etiological agent of typhoid. PLoS neglected tropical diseases, 2018. 12(10): p. e0006779.
59. Saha, S., et al., Antimicrobial resistance in typhoidal Salmonella: around the world in 3 days. Clinical Infectious Diseases, 2020. 71(Supplement_2): p. S91-S95.
60. Hussain, A., et al., Typhoidal Salmonella strains in Pakistan: an impending threat of extensively drug-resistant *S. typhi*. European Journal of Clinical Microbiology & Infectious Diseases, 2019. 38: p. 2145-2149.
61. Shah, S.A.A., et al., Antimicrobial sensitivity pattern of *S. typhi*: emergence of resistant strains. Cureus, 2020. 12(11).
62. Hooda, Y., et al., Molecular mechanism of azithromycin resistance among typhoidal Salmonella strains in Bangladesh identified through passive pediatric surveillance. PLoS neglected tropical diseases, 2019. 13(11): p. e0007868.
63. Petrin, C.E., et al., Drug-resistant *S. typhi* in Pakistan. Clinical pediatrics, 2020. 59(1): p. 31-33.
64. Zmora, N., et al., Open label comparative trial of mono versus dual antibiotic therapy for Typhoid Fever in adults. PLoS Neglected Tropical Diseases, 2018. 12(4): p. e0006380.