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REVIEW

Antibiotic resistome of *Salmonella typhi*: molecular determinants for the emergence of drug resistance

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Abstract **Resistome is a cluster of microbial genes encoding proteins with necessary functions to resist the action of antibiotics. Resistome governs essential and separate biological functions to develop resistance against antibiotics. The widespread clinical and nonclinical uses of antibiotics over the years have combined to select antibiotic-resistant determinants and develop resistome in bacteria. At present, the emergence of drug resistance because of resistome is a significant problem faced by clinicians for the treatment of *Salmonella* infection. Antibiotic resistome is a dynamic and ever-expanding component in *Salmonella*. The foundation of resistome in *Salmonella* is laid long before; therefore, the antibiotic resistome of *Salmonella* is reviewed, discussed, and summarized. We have searched the literature using PubMed, MEDLINE, and Google Scholar with related key terms (resistome, *Salmonella*, antibiotics, drug resistance) and prepared this review. In this review, we summarize the status of resistance against antibiotics in *S. typhi*, highlight the seminal work in the resistome of *S. typhi* and the genes involved in the antibiotic resistance, and discuss the various methods to identify *S. typhi* resistome for the proactive identification of this infection and quick diagnosis of the disease.**

Keywords *S. typhi*; antibiotic resistance; mechanism; resistome; identification methods

Introduction

Salmonella enterica serovar *typhi* is a causative agent of typhoid fever, which is transmitted to humans by the uptake of contaminated food and water. *Salmonella* spp. are the intracellular, Gram-negative, flagellated, non-spore-forming, facultative anaerobic bacteria that belong to the family Enterobacteriaceae. *Salmonella* comprises two species, namely, *S. bongori* and *S. enterica*, in which *S. enterica* causes a severe disease called salmonellosis. *S. enterica* spp. are recognized as potential pathogens of humans and further subdivided into > 2500 serovars on the basis of their antigenic differences (in the lipopolysaccharide-O antigen) and two flagellin structures [1]. Salmonellosis is divided into two subtypes: (1) typhoidal salmonellosis caused by *S. enterica* serotype *typhi*-A, B, C, C2, D, E, and paratyphi-A, B, C and (2) non-typhoidal salmonellosis caused by *S. enterica* serotype *enteritidis* and *S. enterica* serotype *typhimurium*. A wide range of vertebrates is the host of *Salmonella* and display various symptoms during infection. Typhoidal *Salmonella* causes a severe enteric fever that culminates into life-threatening diseases, whereas the non-typhoidal *Salmonella* causes gastroenteritis, which is associated with self-limiting diseases in immunocompromised individuals.

Typhoidal *Salmonella* is limited to humans and spreads by oral/fecal route or by direct contact with the infected person [2]. It is characterized by lengthy sustained fever, hepatosplenomegaly, leukopenia, neutropenia, rose spots, and relative bradycardia. The illness basically resolves by the end of the fourth week in an untreated patient [3]. Chills, high fever, abdominal pain, and headache are the major characteristics of this infection. The mortality rate of typhoid is 12%–30%; thus, antimicrobial intervention is essential for its treatment. Based on the literature, *S. typhi* shows resistance against most antibiotics (Fig. 1), and multidrug resistance (MDR) emerges in *Salmonella* because of the presence of resistome. Resistome is a group of bacterial genes that resist the action of antibiotics. Few treatment options are available for the treatment of salmonellosis, but they are not satisfactory. The parenteral

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Vi vaccine and live-attenuated Ty21a vaccine are commercially available but are partially effective in preventing this disease [4]. These vaccines also have some limitations, such as short-lived immunity in adults and low efficiency in children younger than 2 years. According to the World Health Organization (WHO), the protective efficacy of the injectable ViCPS (Vi capsular polysaccharides) vaccine is satisfactory, and revaccination is recommended after every 3 years to maintain the protection from *S. typhi* infection [5]. The Ty21a vaccine is an oral vaccine and based on the live-attenuated mutant strain Ty21a of *S. typhi*. This vaccine is supplied in enteric-coated capsules to protect it from the acidic environment of the stomach. However, revaccination is strictly advised every 3 years for persons living in poor sanitation areas or areas susceptible to infection [6]. Given the current treatment option of typhoidal *Salmonella*, the search for nontoxic inhibitors against resistome can be an appealing strategy for the resuscitation of the effective antimicrobials/novel drugs in surpassing this problem. Given these backgrounds, this review discusses the status of antibiotic resistance in *S. typhi* and its resistome and the important methods to detect *Salmonella* resistome on a single platform, which will be a good inventory for researchers who are working in this area.

Status of antibiotic resistance in *S. typhi*

The journey of *S. typhi* infection treatment starts with antibiotic chloramphenicol in the 1950s. However, in the 1960s and 1970s, classical first-line antimicrobials, such as ampicillin and co-trimoxazole, were the mainstay of treatment of *Salmonella* infection (Fig. 1). Afterward, WHO suggested the use of third-generation antibiotics, namely, fluoroquinolones (FQs) and cephalosporins, for the treatment of *Salmonella* infection in the year 2003. However, during the last decades, the insensible use of FQs has directed to the fast increase in ciprofloxacin-resistant isolates in South and South-east Asia [7]. Consequently, the global spread of *S. typhi* MDR strains ascends. The *S. typhi* MDR is found in many Asian countries during the last decade, and its increasing trend (26%–80%) is reported in Bangladesh, India, Pakistan, and Vietnam [8]. Cephalosporin-resistant (sporadic cases of third-generation antibiotics) *S. typhi* (producing β-lactamase and extended-spectrum β-lactamases) strains have also been reported in Bangladesh, Germany, India, and Philippines. The increasing occurrence of azithromycin resistance among *S. typhi* isolates has also been reported in India (33%) and Netherlands (14%) [9]. The sporadic reports on azithromycin resistance created a problem in selecting appropriate antimicrobials for the treatment of typhoid fever [10]. Therefore, a study was carried out with special reference to antimicrobial resistance (AMR) mechanisms, molecular subtypes, and associated haplotypes of *S. typhi* for the molecular characterization of *S. typhi* strains in Kolkata City, India, for 15 years (1998–2012). All clinical isolates of *S. typhi* in this study were found susceptible to azithromycin and ceftriaxone. Decreased ciprofloxacin-susceptibility (DCS) strains of *S. typhi* displayed mutation in *gyrA* that showed resistance to nalidixic acid, whereas strains having mutation in *gyrB* showed susceptibility to nalidixic acid. They have also found non-conjugative non-IncHI1 (180 kb) plasmids and conjugative IncHI1 (230 kb) plasmids in 14 and 23 MDR strains, respectively. MDR *S. typhi* strains with or without plasmid shared the same set of resistance genes (bla_{TEM-1}, *catA1*, *strA*, *strB*, *sul1*, and *sul2*) and class 1 integron-possessing *dfrA7* gene cassette. Two *S. typhi* strains harbored 50 kb transferrable plasmids carrying *aadA1* and *dfrA15* gene cassettes in class 1 integron [11].

AMR mechanism, prevalence, and serotyping of *S. enterica* were also studied in Saudi Arabi, in which 100 non-repetitive bacterial strains were isolated from clinical and environmental samples [12]. Among the strains, 33 were identified as *S. enterica*. The researchers have tested the susceptibility of these strains against 26 commonly

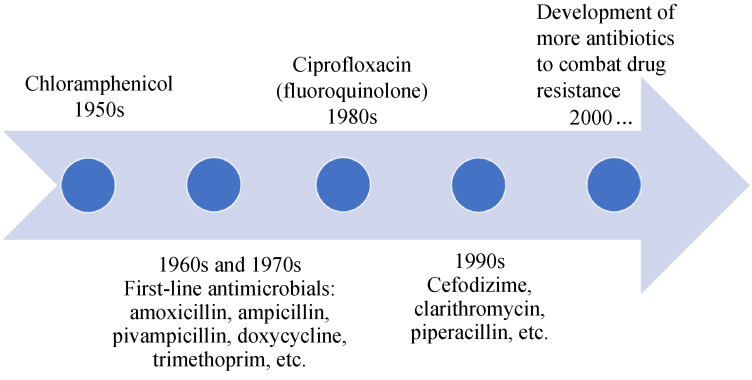

Fig. 1 Timeline of antibiotic discovery and their use of *S. typhi* infection treatment.

used antimicrobial agents. *S. enterica* serovar *enteritidis* showed the highest percentage of occurrence (39.40%), followed by *S. paratyphi* (21.20%), *S. typhimurium* (15.20%), *S. typhi*, and *S. arizona* (12.10%). Most isolates of *S. enterica* were susceptible to chloramphenicol antibiotics and few to β-lactam antibiotics (ampicillin, ampicillin-sulbactam, and piperacillin (PIP)). *S. paratyphi* C serotype showed a high level of resistance to erythromycin, kanamycin, neomycin, streptomycin, trimethoprim-sulfamethoxazole, and tetracycline. *S. paratyphi* B also exhibited resistance against most antibiotics, such as ampicillin-sulbactam, chloramphenicol, erythromycin, tetracycline, and streptomycin [12].

The emergence of MDR *S. enterica* serotype *typhi* was found in the Indian subcontinent because of the failure of conventional antibiotics in the late 1980s. Ciprofloxacin was developed as a first-line drug to treat enteric fever, but the reduced susceptibility to ciprofloxacin was reported extensively since 1994, which had created a therapeutic difficulty. Moreover, a report [7] that reviewed the situation of drug resistance among *S. enterica* serotype *typhi* in central India from 1988 to 2005 was published in 2006. The concurrent MDR to ampicillin, chloramphenicol, and cotrimoxazole had reached more than 90% in the year 1990–1991 but started to decline over the years and reached to a lower level of 5.6% in the year 2004–2005. On the basis of these observations, the older antibiotics (ampicillin, chloramphenicol, and cotrimoxazole) could be recalled to treat enteric fever [7].

S. enterica serotype *paratyphi* A (SPA) causes a mild form of the disease and has been reported less frequently (3.17%) in India. However, since 1996, the frequency of isolation of *S. paratyphi* has been augmented in contrast to *S. typhi* in the Indian population. A 5-year retrospective study in New Delhi, India, had documented the upsurge in the proportion of SPA from 6.5% in 1994 to 44.9% in 1998 [13]. In 2007, a similar increase in *S. paratyphi* isolation has been reported in Rourkela, Odisha, India [14]. A variation in the antimicrobial susceptibility pattern of SPA has also been reported from different parts of India. A study was also carried out on the SPA in 2004 at Kasturba Hospital, Sevagram, Maharastra Province, India, and reported 45.45% enteric fever because of SPA. Nearly 46.15% of SPA had also been reported in Nagpur, India, from 2000 to 2002. This dramatic switching of biotype SPA from *S. typhi* could be due to the widespread use of quinolones against *S. typhi* in the past decades. A report from Calicut, India, also documented 78.60% resistance to nalidixic acid in 2002. Nalidixic acid is a first-generation quinolone, and its resistance is a marker for forecasting low-level resistance to ciprofloxacin. In 2016, Mahapatra *et al*. isolated 167 *Salmonella* spp., including 83.8% *S. paratyphi* A and 16.6% *S. typhi* [3]. In this study, a dramatic increase in *Salmonella* MDR cases is reported. Various types of typhoid vaccines (a parenteral whole-cell vaccine and TAB (a combination of typhoid and paratyphoid A and B) vaccine) are used. However, paratyphoid is a less common cause of enteric fever, and the vaccines have poor protection. Afterward, TA (typhoid and paratyphoid A) and T (typhoid) vaccine were used. This finding may be another cause of the emergence of *S. paratyphi* A. The widely used live-attenuated vaccine is Ty21a (the mutant strain of *S. typhi* at *galE*), which results in the lack of enzyme uridine diphosphate galactose-4-epimerase (a major virulence factor of *S. typhi*) and is necessary for capsular polysaccharide formation. Capsular polysaccharide vaccine is a new generation subunit vaccine that stimulates B cells directly but not T cells; thus, memory cells are not produced, and antibody response is not boosted by additional doses [9].

Molecular characterization of typhoidal *Salmonella* for intermediate susceptibility to ciprofloxacin was conducted in 2016 by Veeraraghavan *et al*. [15]. They compared the criteria of the Clinical and Laboratory Standards Institute (CLSI), USA, and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), Switzerland, to verify ciprofloxacin susceptibility for typhoidal *Salmonella*. As per MIC testing using the CLSI guideline, 65.4% and 23.5% of typhoidal *Salmonella* cases were found to be intermediate and resistant to ciprofloxacin, respectively. According to the EUCAST-defined procedure, the resistance rate was high (93.9%). 96% of isolates assigned in the intermediate category had mutations in *gyrA* and *parC*, which might cause clinical failure in patients if treated with ciprofloxacin. Most ciprofloxacin resistance was facilitated by chromosomal mutations [15]. In USA, resistance mechanisms were recognized in animal isolates of *S. enterica* that showed resistance to aminoglycosides (alleles of *aadA*, *aadB*, *aacC*, *aphA*, *ant*, and *StrAB*), β-lactams (alleles of *blaCMY*-2, *PSE-1*, and *TEM-1*), chloramphenicol (alleles of *cat1*, *cat2*, *cmlA*, and *floR*), inhibitors of the folate pathway (alleles of *dfr* and *sul*), and tetracycline (alleles of *tetA*, *tetB*, *tetC*, *tetD*, *tetG*, and *tetR*). In the US, the MDR mechanisms in animal isolates of *Salmonella* were linked with mobile genetic elements and integrons such as IncA/C plasmids, which can be transported among bacteria. *Salmonella* antibiotic-resistant strains were originated from food animals and transmitted to humans from foodstuff. However, in the US, some antibiotic-resistant *Salmonella* strains were isolated from humans, and they had different elements for antibiotic resistance compared with those isolated from food, indicating a different etiology for some antibiotic-resistant human infections [16].

The combined effect of few drugs was studied for the treatment of *Salmonella* infection; thus, a combination of different drugs could be used for combinatorial therapy to surmount MDR in *S. enterica* [17]. The suppression of antibiotic resistance was reported in this study after using quinolones in combination with trimethoprim. This

combination might suppress quinolone resistance emergence [17]. A combination of trimethoprim–sulfamethoxazole (cotrimoxazole) was also suggested as an alternative to chloramphenicol [18]. After the study conducted on combinatorial therapy, ciprofloxacin became the drug of choice for MDR (ampicillin, chloramphenicol, and cotrimoxazole) against enteric fever. However, ciprofloxacin therapy was stopped because of an increase of MIC values in *S. enterica* serovar *typhi* isolates. Afterward, an *in vitro* evaluation of the two known antityphoid antibiotics was conducted and used in combination with ciprofloxacin and trimethoprim against 16 clinical isolates of *S. enterica* serovar *typhi* [19]. Drug combination exhibited a synergistic effect on all the tested isolates. This finding might help clinicians to make a clear decision in introducing a combinatorial chemotherapeutic approach against *S. enterica* serovar *typhi* MDR infection [19].

S. typhi resistome

Antibiotics are an advancement in the treatment of salmonellosis, but the emergence of antibiotic resistance because of the resistome of these bacteria is a great challenge for its therapy. Misapplication of antibiotics in humans and domestic animals leads to the emergence of MDR *S. enterica* strains that are significant public health problems worldwide. *S. typhi* resistome or resistome of any bacteria is a collection of proto-resistance genes (that have the potential to evolve a resistance function) and cryptic resistance genes (a resistance gene distributed in the chromosome of bacteria, but that is not associated with antibiotic resistance). These genes are either not expressed or expressed at a low level. Several shreds of evidence show that the MDR condition in *Salmonella* strains is attributed to the exhaustive use of antibiotics in animal feeding as a feed supplement or growth promoters. The exhaustive use of antibiotics to treat the infections of humans and animals both lead to flourishing the horizontal transfer of antibiotic-resistant genes among bacterial communities and now complementary/alternative therapy is advocated to overcome antibiotics resistance problem [20,21]. The resistome of *S. enterica* attributes to antibiotic resistance (Fig. 2) in host with the following mechanisms.

Enzymatic inactivation and degradation of some antimicrobial agents

S. enterica serovar *typhimurium* uses PmrA–PmrB and PhoP–PhoQ (two-component regulatory system) to identify the presence of cAMPs (cyclic adenosine 3′,5′-cyclic monophosphates), which is an environmental factor of the host tissues. It marks the enzymatic activation responsible for cAMP resistance and lipopolysaccharide modification [22]. In general, cephalosporin antibiotic is used against *Salmonella* infection. It is also known as cefalotin, cefpodoxime, cefuroxime, cefotaxime, cefoxitin, cefuroximeaxetil, or ceftriaxone. *S. typhi* is resistant to these antibiotics because it has a carb-like (carbenicillinase) gene, which encodes the β-lactamase enzyme. Ampicillin, ampicillin-sulbactam, PIP, and tazobactam are β-lactam antibiotics, and *Salmonella* shows resistance against β-lactam antibiotics because of the presence of carb-like (carbenicillinase), *tem*, and *oxa-1* genes in *Salmonella*. The ampicillin-resistant gene is $bla_{TEM\text{-}1}$ (β-lactamases), and many $bla_{TEM\text{-}1}$ genes are present in various bacteria. The β-lactam ring of penicillin is hydrolyzed by the penicillinase enzyme, which is one of the examples of enzymatic inactivation/degradation of antimicrobial agents by *S. typhi*. They can disrupt the β-lactam ring of penicillin and some cephalosporin. The initial strains of *S. typhi* antibiotic-resistant strain carry chloramphenicol acetyltransferase type I gene. This gene encodes an enzyme and inactivates the antibiotic chloramphenicol via acetylation [23]. Chloramphenicol contains two-OH groups that can be hydrolyzed in a reaction catalyzed by the chloramphenicol acetyltransferase enzyme with donor acetyl CoA. Aminoglycosides (amikacin, gentamicin, and tobramycin) can be inactivated or modified in numerous ways. Acetyltransferase catalyzes the acetylation of the amino groups, whereas aminoglycoside catalyzes the addition of either the adenyl groups (adenyltransferases) or phosphates (phosphotransferases) to the hydroxyl groups [24]. The widely used live oral vaccine is Ty21a using a mutant strain of *S. typhi* at *galE*, resulting in the lack of enzyme uridine diphosphate galactose-4-epimerase. This enzyme is a major virulence factor of *S. typhi* and necessary for capsular polysaccharide formation.

Activation of antimicrobial efflux pumps

Some genetic alterations conferring AMR are associated with the downregulation of genes encoding outer membrane proteins (OMPs) and upregulation of genes encoding efflux pumps of the plasma membrane. In bacteria, gene expression is primarily initiated by sigma factors RpoE, which plays a vital role in various responses to environmental stress. MDR efflux pumps constitute around 10% of the total number of transporters expressed by bacteria. This finding indicates that these proteins perform perilous roles. MDR efflux pumps are categorized into five major families on the basis of gene sequence similarities: (1) adenosine triphosphate-binding cassette (ABC) family, (2) small multidrug resistance family, (3) multidrug and toxic compound exporters, (4) resistance-nodulation-division proteins, and (5) the major facilitator superfamily (MFS) [25]. The MFS transporters represent the largest group of secondary active transporters because it consists of 25% of all known membrane transport proteins in prokaryotes. The putative members of *S. typhi* genes

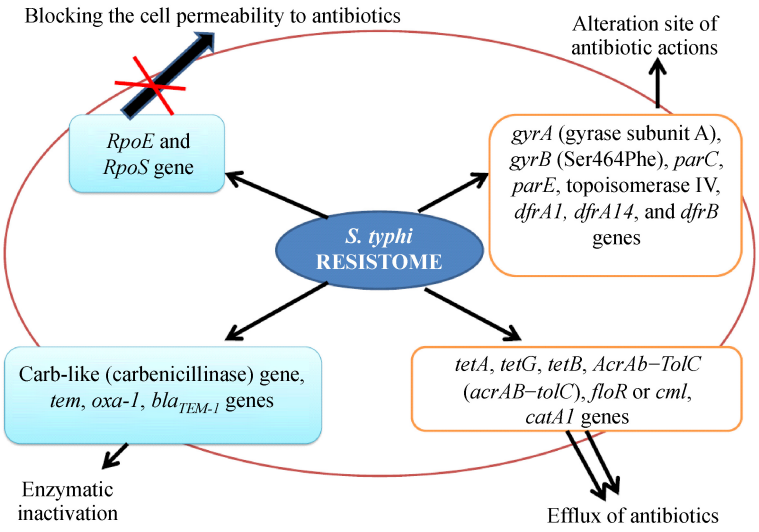

Fig. 2 Functioning of genes of *S. typhi* resistome in the drug-resistant mechanism.

encoding the MFS are cloned and expressed in the drug-hypersensitive *E. coli* and tested for the transport of 25 antibacterial compounds. This study also includes representative antibiotics of various classes (antiseptics, detergents, and dyes) and shows their function as an H^+-dependent exporter [26]. The empty drug efflux pump has two binding sites: (1) a substrate binding site facing inward and (2) the proton binding site facing outward of the membrane. The mechanisms of the expected drug efflux transport are as follows: (1) the H^+ binds at the outward-facing binding site of the empty efflux pump; (2) the drug binds at the inward-facing side of the pump; (3) the drug-binding affinity inside the pump increases; (4) a conformational change occurs to switch the orientation of drug- and proton-binding sites (so that the bound H^+ faces inside and bound drug faces outside); (5) the H^+ is released inward; (6) the drug is released outward; and (7) after this release, the efflux pump reorients the drug-binding site back to the inwards, and the H^+-binding site reverts to outwards. The empty drug efflux pump is prepared to start another cycle of the expulsion of drugs [27].

The AcrAB – TolC efflux pump of *Salmonella* was studied by creating mutations in AcrR (a local repressor) because it controls the efflux pump expression. If we see the structure of AcrR, then the arginine amino acid at the 45th position lies within the middle of a putative helix–turn–helix DNA-binding motif (highly conserved). The overexpression of the efflux pump occurs because of this mutation that ultimately leads to the loss of repressive function. The efflux pump is responsible for the resistance to FQ in many bacteria. Carbonyl cyanide m-chlorophenylhydrazone (an efflux pump inhibitor) is used to determine the role of the efflux pump in the antimicrobial activity of *S. typhi* [28]. Nalidixic acid (quinolone) resistance is also associated with the overexpression of efflux pumps. In nalidixic acid-resistant *S. enterica* strains, the role of AcrAB–TolC pump genes is to confer quinolone resistance. Tatavarthy *et al*. demonstrated that the regulator *RamA* increased the expression of the AcrAB efflux pump by causing ciprofloxacin resistance in *S. typhi* strains [5]. *RamA* binds in the gene promoter region and ultimately upregulates the expression of *AcrAb–TolC*. Active efflux involving the AcrAB–TolC system is a set of *S. typhi* definitive phage type 104 (DT104) isolates. It will be the primary mechanism for nalidixic acid resistance and DCS. This study also supports the possibility of resistance to other classes of drugs because of the efflux system [5].

The resistance of chloramphenicol is due to the existence of the *floR* gene in *Salmonella*. The chloramphenicol resistance is highly related to the efflux pump acquisition and expression that diminish the concentration of the drug in the cell of bacteria. *catA1* is the chloramphenicol-resistant gene of *Salmonella*, and the chloramphenicol efflux pumps of *Salmonella* are encoded by *floR* or *cml* gene. The resistance to tetracycline in *Salmonella* is due to the presence of *tetA*, *tetB*, and *tetG*, and resistance is highly allied with the acquisition and expression of the efflux pumps encoded by a set of *tet*. The intestinal tract is a suitable abode for the horizontal gene transfer of *tetA* and *tetB*. These genes are highly prevalent among the members of the entire Enterobacteriaceae family.

Alteration of the site of antibiotic actions

Quinolone (ciprofloxacin, nalidixic acid) and FG resistance develop because of the presence of mutations in *Salmonella gyrA/gyrB* (gyrase subunit A/B), *parC*, and

parE (topoisomerase IV). A point mutation found in *gyrA* at the 13th and 24th nucleotide positions leads to single amino acid substitution, and phenylalanine and aspartate were replaced by serine and tyrosine, respectively. Some other point mutations are also reported in *gyrA* (Ser83Phe, Ser83Tyr, Asp87Gly, Asp87Tyr, and Asp87Asn), whereas point mutations are detected at the 13th, 19th, and 28th nucleotide positions (Thr54Ser, Ser80Ile, and Glu84Gly) in *parC*. These mutations lead to the blocking of the binding sites of gyrase or topoisomerase genes targeted by the antimicrobial agents. Given this mutation, the complete frameshift of amino acid sequences is led to gyrase and topoisomerase. Among the resistant bacteria, a high variation of frameshift is detected in the *parC* gene that causes a major change in their proteins. The presence of these mutations in *parC* and *gyrA* makes bacteria to be more resistant to FQ. FQ resistance in *S. typhi* can be due to the gaining of plasmid-mediated quinolone-resistant (PMQR) genes or attributed to the mutations in the quinolone (nalidixic acid) resistance-determining regions (QRDRs) of topoisomerase genes.

Trimethoprim–sulfamethoxazole resistance in *Salmonella* is due to the presence of *dfrA1*, *dfrA14*, and *dfrB*. Resistance to trimethoprim is primarily due to mutations in the chromosomal gene that encodes dihydrofolate reductase enzyme. This enzyme reduces dihydrofolate to tetrahydrofolate. Sulphonamide resistance has also been found to be mediated by a chromosomal mutation in the gene coding for the target enzyme dihydropteroate synthetase that reduces the binding affinity of the drug [24].

Blocking cell permeability to antibiotics

The outer membrane (OM) of a Gram-negative bacteria is an important barrier that protects bacteria against toxic compounds. The toxic compound includes antibiotics and cationic antimicrobial peptides (host innate immune molecules). The toxic oxygen radicals of the host tissues regulate the permeability of the outer membrane by altering porin channels (OMP) to control β-lactam antibiotics and the influx of oxygen radicals [29]. Antimicrobial resistance in bacteria is due to the overexpression/downregulation of certain genes encoding OMPs and efflux pumps. Sigma factors (σ) initiate the bacterial gene expression and are controlled by various regulators (activators and inhibitors). σ can specifically recognize the target gene promoter and support RNA polymerase to assemble and promote the expression. It is an important means and the most common phenomenon for bacteria to adapt to different conditions [30]. rpoE is an important sigma factor (σE) that augments the transcriptional specificity by blocking the binding of RNA polymerase at the weak site of the promoter. It stimulates the synthesis of RNA by accelerating the recycle of core enzymes [31]. A study has shown that the *rpoE* mutant (Δ*rpoE*) of *S. typhi* has preeminent resistance to several antimicrobial agents (aminoglycosides, β-lactams, and quinolones). The result has shown that the expressions of two OMP genes (*ompC* and *ompF*) are pointedly downregulated in DrpoE (6 and 7-fold lower in comparison with wild-type strain) and RamA (a member of the efflux pump *AraC/XylS* family). They control the upregulation of the efflux system and downregulation of the OMP genes [32]. Under hyperosmotic stress in *Salmonella*, *RpoE* gene (a strong antimicrobial regulator in *S. typhi*) promotes flagellar gene expression, and extensive cross-talk is observed between RpoS and RpoE (two σ factors) [33].

The immune response of a healthy person produces reactive oxygen species (ROS), which is antimicrobial and kills attacking bacteria during infection. ROS can pervade through the membrane of bacteria and causes damage to bacterial DNA/proteins and other intra-bacterial molecules [34]. However, the OM of pathogenic Gram-negative bacteria provides defense against ROS. When genetic information changes in bacteria, the nature of proteins in the membrane also change, and the alteration in membrane permeability occurs. Such alterations change a membrane transport phenomenon so that antimicrobial agents cannot cross the membrane barrier. Resistance to aminoglycosides, quinolones, and tetracycline is obtained by this mechanism in *S. typhi*. A reduction in membrane permeability also leads to sulphonamide resistance in *S. typhi* [24]. Quinolone resistance has also been reported for decreased membrane permeability to quinolones. Permeability of the molecules across the membrane can be regulated by a change in the composition of membrane lipid or channel proteins.

The measurement of real-time H_2O_2 influx is performed by utilizing roGFP2 in the HpxF$^-$ background of *S. typhimurium* to study the membrane permeability for H_2O_2 [35]. Rapid intra-bacterial H_2O_2 detoxification can be avoided, and biologically relevant H_2O_2 concentrations can be investigated. The detoxifying power of HpxF$^-$ *S. typhimurium* mutant is approximately 90-fold lower than that of wild-type *S. typhimurium*, and exposure to increasing amounts of H_2O_2 leads to a dose-dependent response. Several OMPs form a β-barrel of the OM that potentially facilitates the diffusion of hydrophilic molecules across the OM. A single deletion mutant of the four abundant OMPs (*OmpA*, *OmpC*, *OmpD*, and *OmpF*) is created in HpxF$^-$ *S. typhimurium* to identify the OMPs responsible for H_2O_2 diffusion [36]. This study reported a novel stress response mechanism by which *Salmonella* regulates OM permeability by opening/closing of specific membrane pores. Under reducing condition or under mildly oxidizing condition (when bacteria can grow without extensive damage to enterobacterial components),

rapid acquisition of hydrophilic nutrients is required from the environment, and it comes from the wide OmpC pore. Bacteria protect themselves against ROS by limiting influx through the OmpC closure [36].

Methods to detect or identify *Salmonella* resistome

Several methods have been developed to investigate and identify the resistome of an organism. We discuss a few important methods for the detection of *S. typhi* resistome.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by disk diffusion assay using the respective disks of antimicrobial agents. The microbial strains were sub-cultured on fresh agar plates, and various antibiotic discs were placed on the surface of the agar plate. The strains were then incubated at their respective/favorable conditions. The minimum inhibitory concentrations (MICs) of selected antimicrobials were determined by E-test (Epsilometer test). The result could be interpreted by the guidelines of the standards development organizations, such as the CLSI, USA, and EUCAST. The antimicrobial susceptibilities of *S.typhi* strain were also determined on the basis of their MIC in mg/L or in a mole that explains whether a bacterial species is resistant or susceptible to an antibiotic. Bacteria were considered susceptible to antibiotics if the MIC was less than or equal to the susceptibility breakpoint. If the MIC was greater than this value, then the bacteria was considered intermediate or resistant. An antimicrobial susceptibility testing of *Salmonella* strain provided MIC breakpoints for all the antibiotics, which were used clinically. It provides an indication of the resistome in bacteria.

Polymerase chain reaction (PCR) analysis

PCR was used for the recognition of numerous antibiotic-resistant genes (molecular determinants of drug resistance) present in the microbial genome. It was also used to determine the mutation in the microbial genome that causes resistance toward various antibiotics. Using specific primers, PCR amplification of antibiotic-resistant genes and mutated genes could be carried out. DNA amplification was carried out in thermocycler by denaturation, annealing, and extension at required conditions. The amplified genes were analyzed by agarose gel electrophoresis. The genes were sequenced by automated DNA sequencing services and aligned with the known genes available in the online database NCBI. *S. typhi* haplotype H58 was detected by PCR using specific primers, and the result was obtained on the basis of the amplification products of 107 bp (a deletion of 993 bp) and 1100 bp that represented the H58 and non-H58 haplotypes, respectively.

DNA sequencing

After the amplification of the resistance gene from bacterial resistome, the amplicons (amplified product) could be subjected to direct DNA sequencing to detect mutations in the resistant genes of both strands using a DNA sequencer. Then, mutations at the level of the amino acid could also be analyzed by aligning the translated sequence with the *S. typhi* strain.

Mutation detection

Resistance against a particular drug was emerged by the mutations that alter the binding sites of the drug on the targets. This resistance ultimately increased the expression of endogenous efflux pumps. In *S. typhi*, FQ resistance could be attributed to mutations in the QRDRs of topoisomerase genes. The detection of chromosomal mutations at QRDRs of topoisomerase IV (*parC* and *parE*) and DNA gyrase (*gyrA* and *gyrB*) was performed by PCR with respective primers [11,12]. The obtained results could hint the resistome of *Salmonella*.

Plasmid analysis

Recent whole-genome sequencing revealed that the Tn2670-like complex transposable element encoded seven AMR genes (*blaTEM-1*, *dfrA7*, *strA*, *strB*, *catA1*, *sul1*, and *sul2*). This sequencing was due to the integration of ancestral IncHI1 plasmid to the chromosome of *S. typhi* MDR isolates [8]. The integration of MDR locus into the chromosome of *S. typhi* occurred since the emergence of the H58 lineage, and it might facilitate large transmissible IncHI1 plasmid loss in *S. typhi* MDR isolates [37]. Given the global emergence of drug resistance, *S. typhi* isolates were facilitated by the dissemination of a specific lineage H58 across the African and Asian countries. *S. typhi* MDR was conferred by the occurrence of resistance genes on the IncHI1 plasmids, but chromosomally translocated MDR locus has also been described by Pai *et al*. [38]. IncHI1 plasmid incompatibility type was detected by PCR using a specific set of primers. The presence of integrons (represented the main vehicle of antibiotic resistance) in *S. typhi* indicated the continuous transfer of drug-resistant genes from one organism to another regardless of the species, which was difficult with regard to the spread of AMR. Class 1 integron carried six drug-resistant genes (*aadA1*, *aacA4b*, *aac-(6)-IIa*, *blaP2*, *catB8*, and *dfrA1*) on a 50 kb plasmid of *S. typhi* [38]. Genes were detected at the 3′ end of the conserved segment within the variable region

of class 1 integron and *qacEΔ1*. *S. typhi* FQ resistance could be attributed to the acquisition of PMQR genes (*aac (6)-Ib-cr* and *qnr (qnrA*, *qnrB*, *qnrD*, *qnrS*)) [39].

Conjugation experiment for AMR transfer

Conjugation experiments were performed to determine whether the AMR was transferrable by the plasmids or not. Clinical MDR isolates of *S. typhi* were used as a donor strain and susceptible to all antibiotics that were used as recipient strains. At the exponential phase, donor/recipient strains were grown and incubated after mixing at an equal ratio. Transconjugant antimicrobial susceptibilities were tested by the disk diffusion test, and the presence of drug-resistant genes could be confirmed with PCR and sequencing.

Pharmacokinetic/pharmacodynamic (PK/PD) parameters

Once a patient was administered with the antimicrobial agent, some processes occurred in the body that direct the presence of drugs and the removal of the drug in the systemic circulation and tissues. These processes were jointly known as absorption, distribution, metabolism, and excretion. PK states "what the body does to the drug." PK studies explained the kinetic behavior, processes, and concentration–time profile of a drug. PK determined various parameters, such as bioavailability, body clearance, protein binding, and volume of distribution. These parameters allowed us to predict and characterize the time course of drug kinetics under physiological and pathological conditions.

When the drug reached the site (with the suitable concentration) of action, it stayed at this site for a longer time duration than its interactions with the target, resulting in some pharmacological actions. These entire processes were studied under PD. PD was also defined as "what the drug does to the body." With regard to antimicrobial therapy, the site of action of a drug was with or within the bacterial pathogen. The PK–PD approach was a fundamental concept in pharmacology that described the time course of the pharmacological effect, behavior, and dose regimen of drugs [40].

The PK/PD index was a quantitative relationship between a PK parameter and microbiological parameter. PK/PD indices were the best descriptors of antibiotic efficacy that depended on the activity pattern of each antibiotic. Three main PK/PD indices were identified, which were associated with the effect of the antibiotics. PK/PD analysis also predicted the antimicrobial efficacy. Three major patterns of antimicrobial activity have been categorized as (1) the first pattern that exhibited antimicrobial concentration/activity, which killed microbes with prolonged persistent effects; (2) antimicrobials with time-dependent killing with either short persistent effects or no effect; and (3) concentration-independent killing and prolonged persistent effects [41].

Dr. Harry Eagle was considered as the father of the PK–PD field, and these concepts were initially branded in the 1940s. Antimicrobial agents were classified on the basis of a PK–PD measurement that was predictive regarding efficacy. The three most common PK–PD measures are as follows: (1) time duration of a drug concentration that remained above the MIC (T1-MIC), (2) the ratio of the maximal drug concentration to the MIC (Cmax:MIC), and (3) the ratio of the area under the concentration–time curve at 24 h to the MIC (AUC0 24:MIC). The PK–PD profile of gatifloxacin against *S. enterica* serotype *typhi* was performed in 2018 using an *in vitro* PK–PD infection model [42]. Two isolates were used (1 susceptible strain and 1 resistant strain) in this study. Such isolates showed a strong correlation between free drug (*f*) and bacterial death. A relatively poor correlation was found in the proportion of the dosing interval, in which the concentration of free drug remained above the MIC. The gatifloxacin-resistant and -susceptible strains performed identically, that is, killing of bacteria was associated with similar drug exposure magnitudes irrespective of MIC value [42].

In chicken, a PK–PD model of enrofloxacin (ENR) was established to optimize the dosage schedule and compare the impacts of ENR on *Salmonella* infection at different dosage schedules in 2017 [43]. The best dosage regimen was explored, which could improve clinical efficacy but reduce the selection of decreased resistance/susceptibility in *Salmonella*. The dosage effects of ENR on clinical efficacy and selection of resistance were evaluated in three treatment groups and a non-treatment group of *Salmonella*. Consequently, a high dosage of ENR was found at short-time treatment, which could be effective for the treatment of *Salmonella* infection [43].

An *in vitro* dynamic model-based study was performed to apply the mutant prevention concentration and determine the PK/PD indices of FQs to minimize the development of resistance in *Salmonella*. Therefore, three FQs (marbofloxacin (MAR), ENR, and difloxacin (DIF)) at five dose levels and 3 days of treatment were studied [44]. The extreme loss in the susceptibility of FQ-treated *Salmonella* has been reported, which occurred at a simulated AUC/MIC ratio (area under the concentration–time curve over 24 h in the steady state divided by the MIC) of 47–71. The data indicated the AUC/MIC (AUC/mutant prevention concentration (MPC)) dependent selection of *Salmonella*-resistant mutants, with AUC/MPC ratios of 39 for MAR, 62 for ENR, and 69 for DIF, which was protective against resistant mutant selection [44]. The C_{max}/MIC ratio of $>$ 8–10 and AUC/MIC ratio of 125–250 were recommended to be the target PK/PD indices and associated with FQ treatment efficacy [45].

Salmonella used various stratagems to fight with oxidative stress resulting from the activity of NADPH phagocyte oxidase. ABC-type efflux pump MacAB, periplasmic glutathione, and Cu-Zn superoxide dismutase protected *Salmonella* against cytotoxicity resulting from NADPH phagocyte oxidase [46]. *Salmonella* was protected by the thioredoxin system from the bacteriostatic activity of H_2O_2 and thioredoxin-1 from the antimicrobial activity of the NADPH phagocyte oxidase. Virulence of *Salmonella* was co-dependent on the SPI2 type III secretion system and thioredoxin-1. Thioredoxin-1 aggravated a variety of oxygen-dependent and independent host defense by regulating the SPI2 type III secretion system expression. The results indicated that the horizontally acquired virulence determinant SsrB of *Salmonella* was post-translationally regulated by the ancestral protein thioredoxin-1. In the bacterial kingdom, thioredoxins were ubiquitous; therefore, the interactions established between SsrB and thioredoxin in ancestral bacteria might have been conserved after lateral gene transfer of the SPI2 pathogenicity island into the *Salmonella* lineage [47].

Acquisition of drug resistance and the development of resistome provided microbes a good tactic of survival. Therefore, we have discussed the common and popular methods that detect or identify the resistome of *Salmonella*. Knowledge about the resistome of *S. typhi*, such as pathogenic microbes, would be a good opportunity for the development of novel antimicrobials by targeting the resistome (Table 1).

Conclusions

The idea of an in-depth study of the resistome is an important background to understand the origins, evolution, and genetic complexity of antibiotic resistance in *Salmonella*. The widespread study on the antibiotic resistome in the past few decades has allowed us to address and understand the existing threats of antibiotic resistance in many microbes. Most of the past research on antibiotic resistance of *S. typhi* focuses narrowly on the emergence of antibiotic resistance in the clinical study. The evolution and spread of antibiotic resistance will continue and is evident from the pathogenesis and epidemiology study of *S. typhi*. The resistome of bacterial pathogens is formed by several determinants, that is, MDR efflux pumps chromosomally encoded antibiotic-inactivating enzymes and plasmid-borne antibiotic-resistant elements. The enlargement of activity and the spread of these elements in recent years are a serious reminder to us. The genes coding these determinants are highly conserved in all isolates of a bacterial species. Therefore, observing the trends and prevalence of genes within the antibiotic resistome is important to maintain the potential clinical use of existing antibiotics. Research on antibiotic resistance must be expanded, and a multi-targeting approach for novel drug development must be explored and accelerated for *Salmonella* infection. Regular antibiotic resistance identification and surveillance are necessary, and our efforts must lead to the development of new antibacterial agents

Table 1 Resistome of *S. typhi*

S.N.	Gene	Resistance against antibiotics	Product/function	Reference
1	Carb-like (carbenicillinase) gene, *blaCMY-2*, *TEM-1*, *PSE-1*	Cephalosporin (cefalotin, cefuroxime, cefuroximeaxetil) Penicillins, β-lactams (ampicillin, ampicillin sulbactam, and piperacillin/tazobactam)	β-lactamase enzyme, extended spectrum β-lactamases (ESBLs)	Das *et al*., 2017 [11]; El-Tayeb *et al*., 2017 [12]; Frye *et al*., 2013 [16]
2	*gyrB*, *gyrA*, *parC*	Ciprofloxacin (fluoroquinolone)	Mutation in QRDR	Das *et al*., 2017 [11]; El-Tayeb *et al*., 2017 [12]
3	*gyrA*	Nalidixic acid	Mutation	Das *et al*., 2017 [11]; El-Tayeb *et al*., 2017 [12]
4	*aadA*, *strA*, and *strB*	Streptomycin	Mutation	Das *et al*., 2017 [11]
5	*catA1*, *floR*, *cmlA*, *cat1*, *cat2*	Chloramphenicol	Mutation	El-Tayeb *et al*., 2017 [12]; Frye *et al*., 2013 [16]
6	*sul1*, *sul2*, *sul3* and *dfrVII*, *dfrA7/dfrA15*	Co-trimoxazole	Act on the folic acid pathway in bacteria by interfering with the production of dihydrofolic acid	Das *et al*., 2017 [11]
7	Alleles of *aacC*, *aadA*, *aadB*, *ant*, *aphA*, and *StrAB*	Aminoglycosides (gentamicin, amikacin, and tobramycin)	Binding to the 30S ribosomal subunit inhibiting protein translation	El-Tayeb *et al*., 2017 [12]; Frye *et al*. 2013 [16]
8	Alleles of *tetA*, *tetB*, *tetC*, *tetD*, *tetG*, *tetR*, *tetK*, *tetL*, *tetM*, *tetO*, *tetS*	Tetracycline	Targets the 30S subunit of the bacterial ribosome binding to the ribosome and inhibiting protein synthesis	Das *et al*., 2017 [11]; Frye *et al*., 2013 [16]

and antibiotic therapy. Significant challenges are found in developing new antibacterial agents because of the regulatory hurdles and clinical trials; however, using a high throughput approach, we must develop next-generation therapies that target the resistome of pathogenic bacteria, such as *Salmonella*. Recent advances in the field assures characterizing and countering emerging threats of antibiotic resistance and resistome studies in *S. typhi*.

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Compliance with ethics guidelines

Awanish Kumar and Anil Kumar declare that they have no conflict of interest related to this study. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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