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Review Article

Molecular Insights into Antimicrobial Resistance in Salmonella Species

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Abstract

Salmonella and its serovars have been known to cause typhoid, food poisoning and gastrointestinal infections via fecal-oral routes, unhygienic practices and contaminated food and water sources. Their host preference ranges from birds to mammals, with some serovars crossing the host barrier. With over 2500 serovars, they differ in their modes of dissemination and pathogenesis, as some are exclusive in their host adaptation. Some infections are self-limiting, however, infections like typhoid and acute salmonellosis require antimicrobial therapy. Typhoidal strains, non-typhoidal strains (NTS) and invasive non-typhoidal strains (iNTS) vary in their responses towards antimicrobial therapy. But now many available line of drugs is rendered futile due to the extensive drug resistance mechanisms in *Salmonella* serovars. The acquisition of class 1 integrons, *Salmonella* pathogenicity islands (SPI) and its variants, specific antibiotic resistance genes, transposable elements, point mutations and plasmid acquired resistance mechanisms have been studied extensively and attributed to pathogenesis and antimicrobial resistance. With accelerated resistance thwarting antibiotics, from first generation Penicillin to the current generation of Carbapenems, it is difficult to control this pandemic as *Salmonella* seems to acquire resistance with agility. Chemically modified antibiotics too are failing in their attempt to control *Salmonella* infections. As the world stumbles upon the hurdle of discovering new antibiotics, the global rampant scenario of multi drug resistance within *Salmonella* species, to the available line of antibiotics, does not seem to relent. It is now time to delve further into the molecular aspects of multi drug resistance within *Salmonella* serovars to fully grasp the mechanisms behind such incidences. The main aim of this review is to discuss multiple molecular mechanisms of *Salmonella* serovars that have been discovered or investigated in the recent times. This might give a detailed insight into the antibiotic resistant nature of the serovars which can be used effectively as therapeutic targets.

Keywords: Multi Drug Resistant Salmonella; Salmonella Pathogenicity Islands; Class 1 Integrons; PMQR Elements; Point Mutations; Global Patterns

Introduction

Salmonellae are Gram-negative motile bacteria. They cause enteric diseases in many animals [1]. They are members of the genus *Enterobacteriaceae*. They were originally identified and characterized by citrate and lysine metabolism and hydrogen sulfide production. However, classical biochemical testing alone is unreliable as it cannot differentiate between important pathogenic members. Thus, modern techniques based on serology and molecular methods are used. The most recent classification scheme relies on recognition of two principle *Salmonella* spp: *S. enterica* and *S. bongori*. In this scheme, *S. enterica* is classified into six subspecies: Subspecies I, or *S. enterica* subsp. *enterica*; Subspecies II, or *S. enterica* subsp. salamae; Subspecies IIIa, or *S. enterica* subsp. arizonae; Subspecies III b, or *S. enterica* subsp. diarizonae; Subspecies IV, or *S. enterica* subsp. *houtenae*; and Subspecies VI, or *S. enterica* subsp. Indica [2].

Serologic methods, based on three antigens: O, H, and Vi help in subtyping of the seven principle members of the *Salmonella* genus. This identifies >2500 serovars [3]. Majority of the isolates that have been cultured from humans and other warm-blooded animals include *S. enterica* subsp. *enterica* strains. *S. bongori* and the other members of *S. enterica* are more commonly isolated from cold-blooded animals and environmental sources. Rarely salmonellosis is caused by other serovars [3].

Salmonella enterica subspecies *enterica* causes salmonellosis in humans. Infection manifest as two forms of diseases: typhoid fever and non- typhoidal salmonellosis. Typhoid fever or Enteric fever is a febrile illness caused by a few serovars such as *Salmonella enterica* subspecies *enterica* serovar Typhi (S. Typhi) and S. Paratyphi A [4] and remains a major health problem [5]. Non- typhoidal salmonellosis is a self-limiting gastoentritis caused by other serovars [6]. It may cause invasive salmonellosis in immunocompromised, young and elderly patients. Non-typhoidal Salmonella (NTS) serovars cause foodborne illnesses world- wide. NTS infection occurs due to consumption of contaminated food such as poultry products, beef and pork as well as contact with infected animals [4].

Epidemiology

Typhoid and paratyphoid fevers affect millions worldwide. Factors usually include lack of clean potable water, poor sanitation, inadequate hygiene practices and low socio-economic status. Outbreaks may arise due to food or water that has been contaminated with the bacterium. In some cases the cause may be a chronic carrier, persistently shedding the bacterium due to infected gall bladder. Chronic carriage occurs following primary infection in the absence of antibiotic treatment and is strongly dependent on factors like age and sex. Enteric fever in Asia has been attributed to S. Paratyphi A. In developed countries, it can be attributed to travellers or migrant workers [5]. Geographic distribution indicates south-central and south-east Asia having the highest incidence. However, the contribution of chronic carriers to transmission in endemic regions is unknown [7].

Mode of transmission and host selectivity

Infectious agents within poultry are major concerns for both the industry and consumers. *Salmonella* infection has been associated with poultry products and some isolates have been found to be multi drug resistant. *Salmonella* species, can reside in healthy chickens causing asymptomatic illness. *Salmonella* colonizes primarily in the cecum in chickens. They may carry the bacteria at the time of slaughter and without being detected they may pose a food-safety risk for consumers. They also have the potential to contaminate farm workers, processing plants, food and the natural environment [6].

Infection depends on the strain and serovar, the host and various gastrointestinal barriers. Gastrointestinal acidity strongly influences the infectious dose. The stomach of healthy adults has a pH as low as 2. *Salmonella* must overcome this to initiate pathogenesis [3]. Infection has different stages: attachment and adhesion to host surfaces, production of factors responsible for invasion, initial multiplication, and ability to bypass host defence mechanisms [8].

Pathogenesis

Salmonella can target the specialized microfold cell (M cell) population. The M cells are found to be overlying lymphoid structures called Peyer's patches (PPs). They can also be found associated with smaller lymphoid aggregates (known as solitary intestinal lymphoid tissues) and rarely found when such structures are absent. They can penetrate the intestinal epithelium at M cell and non-M cell locations [9]. Various virulence factors enable *Salmonella* uptake by host cells. Contact with intestinal epithelial cells are mediated by SPI-4 genes, encoding Type 1 secretion system (T1SS) and SiiE (adhesion protein). Along with SiiE, SPI-3 encoded misL and protein ShdA (not on genomic island) aids in prolonged colonization by binding to fibronectin [10].

Salmonella Pathogenicity Island 1 (SPI-1), a set of viulence factor genes also helps in invasion. A needle-like Type III secretion system (T3SS) encoded by SPI-1 proteins allows the transport of many bacterial proteins into the host cell cytosol [9,10]. SPI-1 proteins, SopB, SopE, and SopE2, promote an inflammatory environment by release of IL-1b and IL-23 after interaction with epithelial cell Rho GTPases and NF-kB activation. Few SPI-1 proteins also promote colonization. It has been demonstrated by iNOS production in macrophages by SPI-1 proteins SipC, SipD, and SopE [10].

Upon crossing the epithelial barrier, the osmolarity of the surrounding tissue drops. This provides a signal for *Salmonella* to down-regulate SPI-1 and induce SPI-2 type III secretion system to synthesize survival proteins that are injected into infected cells. At this stage of infection, S. Typhimurium and S. Typhi follow different courses of infection that are genetically controlled. Both serovars possess fully functional SPI-2 and other virulence proteins. S. Typhi is equipped with genomic modifications, that helps it to avoid the host natural inflammatory response. As opposed to this, S. Typhimurium causes acute gut inflammation. S. Typhi has been an excellent example of "reductive genomic evolution". In contrast to S. Typhimurium, S. Typhi has evolved to become an exclusive human pathogen. It cannot cause productive infection in any other mammalian species and relies on the host's essential factors for survival and growth. Due to this, genetic disruptions and inactivations can be observed within some parts of its genome. Metabolic capacity loss and reduction in Toll-like Receptors (TLRs) mediated signaling expressed by innate immune cells, are the outcome of this disruption [3]. A capsular polysaccharide in combination with the inability to produce very long O-antigen chains as part of LPS and the differential regulation of the SPI-1 T3SS, results in a reduced inflammatory response to S. Typhi compared with S. Typhimurium [1].

Role of typhoid toxin in pathogenesis

The S. Typhi typhoid toxin is a tripartite exotoxin that is delivered to the extracellular milieu, affecting neighboring cells. The tripartite complex and the active subunit of CdtB (cytolethal distending toxin) in combination with PltA and PltB (homologues of pertussis toxin) causes DNA damage and subsequent cell-cycle arrest. The toxin secretion is dependent on TtsA, a muramidase, which is predicted to bind to peptidoglycan in the cell wall and may resemble a secretion mechanism that is poorly understood and thought to have evolved from phage endolysins. GtgE is an effector which cleaves and inactivates Rab29. In addition, the absence of the effector GtgE, enables Rab29-dependent vesicular export of typhoid toxin, which is transported to its targets [1]. Adhesion and invasion genes, plasmid encoded fimbriae (pefA) and hyper invasive locus (hilA) are also necessary for pathogenesis. *Salmonella* outer proteins (sop A-E) encoded by sop gene and (stn), codes for enterotoxin productions that are also associated with the actual manifestation of pathogenesis [11].

Clinical signs

Enteric fever caused by typhoidal serovars and NTS differs significantly. The average incubation period for typhoidal serovars is 14 days. Symptoms persist for up to 3 weeks. There is a gradual onset of fever. Other symptoms include chills, abdominal pain, hepatosplenomegaly, rash (rose spots), nausea, anorexia, diarrhea or constipation, headache, and a dry cough. In contrast to enteric fever, NTS infected patients present with nausea, vomiting, abdominal pain, fever, acute gastroenteritis and watery diarrhea that is self limiting. With NTS infection, symptoms appear 6–12 h after oral ingestion. The symptoms last less than 10 days. The case of iNTS (invasive nontyphoidal *salmonella*) infections is associated with immunodeficient patients. Patients often suffer from high fever, hepatosplenomegaly and have respiratory distress without intestinal symptoms and resembles enteric fever [12].

Treatments prescribed for *Salmonella* infections

Nowadays, electrolyte and fluid replacement is the best treatment due to self-limiting nature of the infections [13]. There is a risk of invasive life threatening infections for the elderly and immunocompromised and may require antimicrobial drug therapy [14]. The chemotherapeutics most often prescribed are: the fluoroquinolones, trimethoprim sulfamethoxazole (TMP-SMZ), ampicillin, or extended-spectrum cephalosporins (e.g., ceftriaxone or cefixime). Ampicillin (A), chloramphenicol (C), and cotrimoxazole are the first-line drugs for the typhoid fever [15]. Azithromycin and ceftriaxone have been recommended as treatment alternatives for typhoid fever [16]. There has been little to no benefit in the usage of fluoroquinolones as a chemotherapy. There has been a benefit in taking norfloxacin within 48 h of the onset of symptoms. Unfortunately, multidrug resistance has already been reported in many S. Typhimurium isolates [13].

Antimicrobial resistance mechanisms: a brief overview

Indiscriminate and thoughtless use of antibiotics, inadequate dosing and poor dedication to treatment regimen lead to the increase of antibiotic resistance [17]. Enzymatic inactivation of antimicrobial substrates has been one of the earliest known resistance mechanisms. They are hydrolytically metabolised into inactive metabolic end-products by various groups of enzymes. The enzymes groups involved include penicillinases called β-lactamases and extended spectrum β-lactamases (ESBLs) (inactivate penicillins and other β-lactam antimicrobials), Esterases (inactivate certain macrolide antimicrobials) and Acetyltransferases (attaches acyl groups to aminoglycoside antibiotics thereby inactivating it) [18].

The second mechanism is target alteration or modification which is exemplified by alterations in DNA gyrase by point mutations so that quinolones and fluoroquinolones cannot bind to these targets. The third mechanism is target protection. An example of this is the protection of tetracycline binding site on the ribosome by small peptides that prevent the tetracycline from binding to its target, that is the 30S ribsosome. A newer synthetic derivative of the tetracyclines, a glycylcycline called tigecycline, overcomes the effects of this protection mechanism but unfortunately that has also been reversed by active drug efflux transporters [18].

The fourth mechanism is called drug permeability reduction. This prevents access of antimicrobial agents to their intracellular drug targets, thus conferring antimicrobial resistance. The porin channel is a well known example found in the outer membrane of Gram-negative bacteria. They may be either downregulated or rendered defective to reduce their permeation activities. It confers resistance to the aminoglycosides, the β-lactams, the fluoroquinolones and the chloramphenicols [18].

The last mechanism is the active efflux of antimicrobial substrates. Several major of transporter protein superfamilies help in this mechanism. A multipartite complex consisting of the outer membrane protein, a periplasmic membrane fusion protein, and an inner membrane transporter in the cell walls may function in synergy to mediate the active efflux of multiple antimicrobials. The ATP-binding cassette (ABC) transporters is another family that uses ATP hydrolysis for removal of antimicrobial agents from the bacterial cell. Other super families use either passive or secondary active transport modes. Secondary active transport systems like the resistance-nodulation-cell division (RND) superfamily, the small multidrug resistance (SMR) superfamily multidrug and toxic compound extrusion (MATE) superfamily and the major facilitator su-

perfamily (MFS) of solute transporters such as those seen in the Enterobacteriaceae bacterial family like *S. enterica*, use ion gradients generated by respiration. Mobile genetic transfer elements (e.g., bacteriophages, plasmids, gene cassettes, integrons, transposons) and various modes of infection transmission (e.g., direct food animal to human and food-borne) have also facilitated the spread of newly emerged resistance determinants [18].

Factors responsible for resistance in Salmonella: Contributing factors may be located on chromosomes, plasmids, transposons and integrons [11]. These contain gene cassettes that confer resistance to aminoglycosides, b-lactams, chloramphenicol and trimethoprim as well as resistance to antiseptics and disinfectants [11]. Biofilms also contribute to increased resistance to multiple antibiotics, as they may contain mobile genetic elements that can be further dissemminated to other serovars or strains. In a study by Eguale., *et al*. *Salmonella* biofilms have been shown to harbour class 1 integrons and SGI-1 with multi drug resistance phenotype [19]. Resistance mechanisms to other antibiotic classes have been reported as follows:

Resistance to quinolones and fluoroquinolones

By PMQR elements

PMQR genes include qnrA, qnrB, qnrC, qnrD, qnrS and qnrVC, belonging to the Qnr families of pentapeptide repeat proteins, the modified aminoglycoside acetyltransferase gene aac(6)-1b-cr, as well as qepA and oqxAB, which encode the efflux pumps QepA and OqxAB, respectively [20]. Presence of PMQR elements and their variants confer resistance to ciprofloxacin but may or may not confer resistance against nalidixic acid [21]. The qnrA gene encodes a 218-amino acid protein that protects DNA-gyrase and topoisomerase IV from the quinolones' activity [22]. N08-2312, an S. Hadar isolate, was shown to harbor the PMQR qnrD2, a novel variant of qnrD1, differing by two amino acid exchanges (Ile189 to Thr and Leu202 to Phe) with no effect on resistance levels to quinolones [20]. In a novel finding with ciprofloxacin resistant Salmonella isolates of porcine origin, Salmonella Senftenberg isolate from cecum was found to have both aac(6=)Ib-cr and qnrB6. Few isolates were found to harbour qnrS2 genes or qnrB19 genes [23]. In a study by Campbell., *et al*. 75% of S. newport isolates harboured qnrB genes with plasmid alleles ColE, IncP, IncI1, and IncA/C [21].

By Mutations

Chromosomal mutations in DNA gyrase and topoisomerase IV genes also confer quinolone resistance. DNA gyrase is encoded by gyrA and gyrB genes, while topoisomerase IV is encoded by parC and parE genes. Both DNA gyrase and topoisomerase IV are tetrameric enzymes [24]. Resistance to quinolones in Salmonella spp. is mostly attributed to point mutations in the quinolone resistance determining regions (QRDRs) of the target genes gyrA, gyrB, parC, and parE [5]. In Salmonella spp., mutations in gyrA and parC are related to nalidixic acid (NAL) resistance and reduced susceptibility to FQs, such as ciprofloxacin [22] In recent years, the rate of resistance to ciprofloxacin has increased considerably in both clinical and food isolates of Salmonella [4].

For the gyrA gene, coding the A subunit of DNA gyrase, a single mutation resulting in an amino acid substitution at the position 83 (Serine to Phenylalanine or to Tyrosine) or at the position 87 (Aspartic acid to Asparagine or Glycine) has been the most frequently described. A second mutation leading to the amino acid change at the position 80 (Serine to Isoleucine or to Arginine) of the ParC subunit of topoisomerase IV in S.Typhi and S. Paratyphi A human isolates from India whereas three mutations, i.e., a double mutation in gyrA at both codons 83 and 87 and one mutation in parC, were shown to confer ciprofloxacin resistance in S.Typhi and S. Paratyphi A human isolates from India or from Taiwan [5].

By drug efflux systems: Other mechanisms of FQ resistance include overexpression of efflux pumps. The cr variant of the aminoqlycoside acetyltransferase aac(6′)-Ib, the QepA determinant (an efflux pump) and the multi-resistance efflux pump OqxAB also confer resistance to FQs [25]. A study by Wong., *et al* revealed that plasmid encoded oqxAB and aac(6')Ib-cr together with a single gyrA mutation in S. typhimurium were sufficient to confer ciprofloxacin resistance [26]. Genome sequencing from a patient revealed a mutation in the efflux pump gene, acrB, that failed ciprofloxacin therapy. The G288D substitution which changed the binding of drugs to the distal binding pocket of AcrB was shown by computational modelling [27].

Resistance to beta lactams

The β-lactams have a unique four membered "β-lactam" ring that when acted upon by cell wall building enzymes, forms an irreversible bond with the enzyme. This inactivates the enzyme and stops the enzyme from completing cell wall synthesis. Penicillin was one of the first β-lactams to be used and also one of the first antibiotics to which bacteria gained resistance [28]. Because of this, new β-lactams, which were chemically modified forms of the older ones, were used so that they become resistant to the β-lactamases. These include modified penicillins such as methicillin and oxacillin, the cephalosporins like cephalothin, cefoxitin, ceftriaxone, and cefipime, which are 1st through 4th generation

cephalosporins, respectively and the carbapenems such as imipenem and meropenem [29]. Most resistance to β-lactams is conferred by β-lactamases. β-lactamases are a class of enzymes that enzymatically cleave and inactivate the β-lactamring [29]. Penicillin-binding proteins (PBPs) are another family of enzymes that bind to β-lactam antibiotics and are responsible for the polymerization of the glycan strand and the cross-linking between glycan chains. PBPs can be classified into two groups: low molecular weight PBPs and high molecular weight PBPs [28].

The penicillin-binding domains of PBPs function as DD-transpeptidases, which catalyze the final step of cell wall biosynthesis by cross-linking two strands of peptidoglycan, or DD-peptidases, which remove the C-terminal D-alanine from the peptidoglycan. Both PBPs and beta-lactamases, interact with beta lactam antibiotics in two steps. In the first acylation step, the active-site serine attacks the b-lactam ring present in these antibiotics forming a covalent acyl-enzyme complex. The second deacylation step is very fast with b-lactamases but extremely slow with PBPs. Resistance to beta-Lactam antibiotics has been found to be conferred by point mutations in Penicillin-Binding Proteins PBP3, PBP4 and PBP6 in Salmonella enterica [28].

Periplasmic factors like the pre-GOB-18 MBL, whose gene is encoded by plasmids and that aid in MBL biogenesis, have been found in dacD mutants of Salmonella enterica. Such mutants showed an altered ability to develop biofilm growth, sensitivity to cefotaxime and concomitant lower accumulation of GOB-18 in the periplasm, suggesting that the lack of DacD negatively affects the stability of secreted apoMBLforms. DacD is a widely distributed low-molecular-mass (LMM) penicillin binding protein (PBP6b) with low DD-carboxypeptidase activity whose functions are still not clearly understood [30].

Resistance to cephalosporins

In Salmonella enterica serovar Typhimurium (S.Typhimurium), a chromosomally integrated multidrug resistance genomic island, GI-VII-6, containing a gene encoding CMY-2 β-lactamase (blaCMY 2) has been found to have extended-spectrum cephalosporin resistance [31]. Infections caused by ESBL and AmpC- producing organisms are on the rise [32]. In general, ESBL-producers are resistant to all penicillin, cephalosporin, and monolactam antibiotics. ESBLs several families of enzymes encoded by plasmids (TEM, SHV, cefotaxime (CTXM), and oxacillin (OXA)). They can also be encoded on the chromosome or be transposon-mediated. TEM1 hydrolyzes penicillins and first generation cephalosporins [33]. The metallo-beta-lactamases (MBLs) are especially worrisome. This group is constituted entirely of metalloenzymes employing Zn2+ for catalysis of a broad spectrum of substrates and can be disseminated over a wide range of serovars. Design of general MBL inhibitors have been undertaken however face challenges due to the huge diversity of active-site structures among these metalloenzymes [5].

In ceftriaxone-resistant S. Enteritidis strains, obtained from China, a 87,255-bp IncI1 plasmid, pSE115, was found to harbor a blaCTX-M-14 gene in a novel multidrug resistance region (MRR) within the tra locus. These strains were genetically unrelated and originated from Henan Province. They harbored a variety of blaCTX-M group 1 and group 9 elements. The novel MRRsite at the tra locus in pSE115 was not detectable in the other IncI1 plasmids. Thus, through this study, it has been shown that cephalosporin resistance in S. Enteritidis strains, collected in China, was mainly due to the dissemination of IncI1 plasmids carrying blaCTX-M [34]. Cefotaxime resistance has been found to be due to an extended-spectrum cephalosporin (ESC), conferred by TEM-20,TEM-52 and CTX-M-25 extended-spectrum β-lactamases (ESBLs) [35].

Cefoxitin resistance has been shown to be mediated by CMY-2 AmpC β-lactamase. In S. Infantis, the blaTEM−20 and blaCMY 2 genes has been associated with IncP plasmids, blaTEM has been linked with a non-typable plasmid and blaCTX−M−25 has been found to be carried by an IncA/C plasmid. ESC-resistant S. Infantis carrying blaTEM−52 has remarkably increased and S. Infantis strains harboring blaCMY−2, blaTEM, or bla− CTX−M−25 genes have emerged from broilers in Japan for the first time [35] 80% of the Salmonella isolates tested in one study in Nigeria were cefoxitin resistant. Plasmid-mediated AmpC β-lactamase and ESBL enzymes were recorded. Salmonella isolates possessed 380 bp AmpC fox gene, with the highest occurrence found in S. typhi strains followed by S. typhimurium. There was no AmpC fox gene detected in S. paratyphi strains [32] Salmonella enterica serotype Enteritidis resistant to oxyimino cephalosporins has also been studied which confirmed the presence of blaCTX-M-14 linked to IS903 in a 95-kb IncI1 conjugative plasmid. This is the first report of blaCTX-M-14 in Salmonella Enteritidis of human origin in South America [36]. In a study by Gelinski., *et al*, all isolates of S. Minnesota serotype had ESBL phenotype. Aztreonam resistance was the least common amongst the Salmonella isolates, followed by ceftazidime. These results are very indicatives of the presence of ESBL genes in Salmonella isolates from a broiler supply chain, reaffirming the growing global problem of ESBL resistance [33].

Resistance to tetracyclines

Tetracycline targets the 30S subunit of the bacterial ribosome and inhibits protein synthesis [29]. In Salmonella, resistance to tetracyclines in conferred by tet genes belonging to classes A, B, C, D, and G. These genes can also be found on SGI-1. In individual and respective studies, tet A from porcine isolates [37] and tet B and tet G from equine isolates [38] displaying high tetracycline resistance have been demonstrated.

Resistance to aminoglycosides

The aminoglycosides bind to the 30S ribosomal subunit inhibiting protein translation. Salmonella enzymatically modifies aminoglycosides as a resistance mechanism. Enzymes used for this purpose include of acetyltransferases, phosphotransferases, and nucleotidyltransferases. Phosphotransferases confer resistance to kanamycin and neomycin and are usually named aph. Their nomenclature is based on the location they modify on the antibiotic [e.g., aph(3')]. The phosphotransferase aph(6)-Ia gene (also named strA) and the aph(6)-Id gene (also named strB) confer streptomycin resistance. These genes have been described as being part of transposon Tn5393 and are frequently located on plasmids. Nucleotidyl- transferases can confer resistance to gentamicin, tobramycin, or streptomycin and include aad and ant groups of genes. Alleles of aminoglycoside resistance genes include aac(3'), aac(6'), aadA, aadA1, aadA2, aadA12, aphAI, aph(3')-Ii-iv, strA,and strB [29]. In a study by Arguello., *et al*. kanamycin resistant isolates were found to harbour gene aphAI. Approximately 94% Gentamicin resistant isolates were found to harbour aac3-IVa and remaining isolates were observed with aac3-IIa. Isolates resistant to spectinomycin and streptomycin were found to harbour aadA1-like and aadA2 genes [37].

Resistance to phenicols

Chloramphenicol and related compounds such as florphenicol bind to the 50S ribosomal and inhibit protein synthesis. It is primarily used for treatment of systemic salmonellosis. The resistance mechanisms include efflux pumps floR and cmlA and inactivating enzymes such as chloramphenicol acetyltransferase, cat1. In addition, the chloramphenicol resistance gene floR is often found in the class I integron located in Salmonella Genomic Island 1(SGI-1) [29].

Resistance to folate pathway inhibitors

Folate pathway inhibitors are compounds that compete for substrates of the essential folic acid pathway in bacteria. Both sulfonamides and trimethoprim act on the folic acid pathway in bacteria by interfering with the production of dihydrofolic acid (DHF). This is done at two different steps: inhibition of DHPS (dihydropteroate synthase) by sulphonamides and inhibition of DHFR (dihydrofolate reductase) by trimethoprim. Acquisition of genes encoding enzymes, that do not bind these compounds, confers resistance to both. These include the sul genes, sul1, sul2 and sul3 that encode an insensitive DHPS enzyme and are found in Salmonella globally. Resistance to trimethoprim is by DHFR encoding genes, either dhfr or dfr, both of which have been found in Salmonella as dfr1, dfrA, dfrAI, dhf, and dhfrI [29].

Resistance to carbapenems

Carbapenem-hydrolyzing enzymes such as New Delhi metallo-β-lactamase-1 (NDM-1) confer carbapenem resistance in Enterobacteriaceae. NDM-1 has been found in 2 strains of Salmonella spp., isolated from feces and urine specimens in patients from India. blaNDM-1 gene was detected in one Salmonella strain isolated from the feces of an 11-month- old girl at Lishui Central Hospital, Zhejiang Province, China [39] A carbapenem resistant Salmonella enterica serovar Senftenberg isolate BCH 2406 with blaNDM−1 was isolated from a diarrheal child in Kolkata, India. The isolate was resistant to all the tested antimicrobials except tetracycline. The blaNDM 1 was found to be located between IS26 and IS4321, on a 146.13-kb mega plasmid pNDM-SAL, which could be conjugally transferred. Downstream of the blaNDM−1, other genes, such as bleMBL, trpF, tat and an ISCR1 element with class 1 integron containing aac(6')-Ib were detected. Another β-lactamase gene, blaCMY−4 was found to be inserted in IS1 element within the type IV conjugative transfer loci of the plasmid [40].

Global patterns of multi drug resistance

The global patterns of multi drug resistance of multiple Salmonella serovars isolated from various parts of the world have been enlisted in table 1 below [51,52].

Concluding Remarks

Multiple factors contribute to multi drug resistance in Salmonella serovars. With over 2500 serovars discovered till date, it is not possible to deduce a single overall reason for their multi drug resistance, since each serovar behaves differently in different hosts under different physiological conditions. However few mechanisms like mutations in basic genetic regulatory genes like gyrA and parC, acquisition of plasmid encoded resistance genes specific for the antibiotics and gain of pathogenicity islands and its variants and dissemination of all these by conjugative mega or mini plasmids remain the plausible explanation for almost every serovar yet encountered. The biggest concern, that still remains as a hindrance, are the ways to stop wide spread dissemination of resistance de-

terminants between inter and intra species. Multiple reports have suggested the dissemination of Salmonella resistance genes among Vibrio and Escherichia species. Such incidents are worrisome as it gives rise to new formidable variants of the once susceptible organisms. Since the serovars share similar genetic regulation, understanding the molecular mechanisms of these resistance patterns, can thus prove to be fruitful in designing molecular drugs, specific for the genetic target, as designing drugs for the individual serovars cannot be possible.

Isolate	Resistance profile	Mechanisms of resistance	Reference
Salmonella enterica serovar Brancaster strain PS01	aminoglycosides, fluoroquinolones, fosfomycin, macrolides, phenicols, sulphonamides, tetracyclines, trimethoprim, beta lactams	aph(4)-Ia,aac(3)-IVa, aadA1 and aph(3)-Ic, qnrS1 , fosA, mph(A) , mef(B), floR, sul3, tet(A) dfrA14, blaTEM-176	[41]
Salmonella enterica serovar Typhimurium	Ciprofloxacin, Cephalosporin and Azithromycin	Mutation in gyrA and parC, PMQR genes qnrB, qnrS, and aac(6')-Ib-cr.ESBL gene blaCTX-M, blaTEM 1,blaOXA-1, blaSHV-12, blaCTX-M,blaCTX-M-14 blaCTX-M-55, blaCTX-M-123 and blaCTX-M-125. mphA gene	[42]
NTS enterica Strain SALH-394-2 of serovar Typhimurium	Florfenicol and sulphonamide	floR gene, sul2 gene, AcrABC and MdtC systems efflux pumps, EmrABC operon, marR (DNA-binding transcriptional repressor), marABC system, β-lactamase, streptomycin 3''-O- adenyltransferase genes.	[6]
NTS enterica Salmonella hadar strain ABBSB1020-2	tetracycline sulphonamides aminoglycoside	tetA gene, sul1 with Class 1 integron, aac3-VI and aadA	[6]
S. Kentucky ABB1087	Macrolide, aminoglycoside and tetracycline	IncF plasmid with RND efflux system macA, the aminoglycoside 3'-phosphotransferase and tetA.	[6]
NTS	Erythromycin, tetracycline, amoxicillin-clavulanic acid, trimethoprim sulfamethoxazole, streptomycin, nalidixic acid, ampicillin-sulbactam, gentamycin, ampicillin, chloramphenicol, ciprofloxacin and ceftriaxone.	Class 1 integrons, aac(3')-Id, aadA2, aadA4, aadA7, sat, dfrA15, lnuF and estX. point mutations in the aac (3')-Id of S. Derby, aadA2, estX-sat genes of S. Typhimurium, frame shift mutation in aadA7 genes of S. Typhimurium, virulence genes sopB, pefA, hilA, stn.	[11]
Salmonella enterica serovars from US and Canadian slaughters	Aminoglycosides, Beta lactams, Chloramphenicol, Sulfamethoxazole, Tetracycline, Trimethoprim	aac, aad, aph, strA/B, blaTEM, blaCMY, blaPSE-cat, flo, cmlA, sulI, tet(A, B, C, D) tetR, dfrA	[43]
Salmonella enterica serovars Ohio ST329 and Senftenberg ST210	Kanamycin, neomycin, tetracycline, erythromycin, apramycin, netilmicin, tobramycin, hygromycin, sulphonamides, spectinomycin and streptomycin	A/C2 plasmids with sul2, aphA1, tetA(D) and erm gene, resistance island RI-119 with aacC4, hph, sul1 and aadA2 genes.	[44]
food-borne Salmonella strains	Ciprofloxacin, ampicillin, nalidixic acid, kanamycin, gentamicin, azithomycin, streptomycin, chloramphenicol, tetracycline and sulfamethoxazole ceftriaxone	oqxAB and aac (6')-Ib-cr, qnrS, qnrB and qnrD, aac (6')-Ib-cr-oqxAB-qnrS2. blaCTX-M-65(in S. Indiana), blaCTX-M-55(in S. enteritidis and S. derby) blaCMY-2(in S. Indiana and S. heidelberg) and blaCMY-72(in S. Heidelberg)	[45]
Salmonella enterica serovar Corvallis	carbapenems, fosfomycin, aminoglycosides, co-trimoxazole, tetracyclines, and macrolides	IncA/C2 pRH- 1238 plasmid, blaNDM-1, blaCMY-16, fosA3, sul1, sul2, strA, strB, aac(6')-Ib, aadA5, aphA6, tetA(A), mphA, floR, dfrA7, and merA genes	[46]

Salmonella spp (from food samples in India and human samples in Nigeria)	Tetracycline, cotrimoxazole, nalidixic acid Nitrofurantion, piperacillin/tazobactin	tetA, tetB, tetC, and tetG, sul1, sul2, and sul3 cmlA and cmlB, aph(3)11a, aac(3)lla,	[47]
Salmonella enterica subsp. enterica serovar Derby	streptomycin/ spectinomycin, tetracycline	sul1 and tetA genes and class 1 integrons carrying aadA26	[48]
S. Enteritidis strain SE402	ampicillin, nalidixic acid, streptomycin, sulfamethoxazole and tetracycline	blaTEM, strAB, sul2 and tet(A) in IncN conjugative plasmid	[49]
S.enteritidis STYMXB.0061	ampicillin chloramphenicol streptomycin sulfamethoxazole and tetracycline	SGI1	[49]
S.enteritidis STYMXB.0110	streptomycin sulfamethoxazole and tetracycline	sul1 and sul2, aadA1 and tet(C)-flanked by an IS26 element	[49]
Salmonella enterica SARA33 (Heidelberg)	ampicillin, chloramphenicol, tetracycline, streptomycin, sulfisoxazole, and kanamycin, gentamicin	aac(6')-ly, aadA5, aadB, aa(6')-33, and aadA1, sul1 and sul2, blaOXA-2 and blaTEM, tetD.	[50]

Table 1: List of isolates with their respective multi drug resistance profiles and mechanisms of resistance.

Conflicts of Interest

Authors declare no conflict of interest.

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