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Impact of Antibiotic Resistance on Public Health and Food Safety: A Review of *Shigella* Infections

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Abstract: Foodborne infections, particularly those caused by *Shigella spp*., pose a significant threat to global public health, with a disproportionate impact on underdeveloped regions. *Shigella*, responsible for acute enteric infections, particularly affects children under 5 years old in developing countries, leading to high morbidity and mortality rates. The emergence of antibiotic-resistant strains, fueled by indiscriminate antibiotic use in food production and clinical settings, further complicates the management of *Shigella* infections. Antibiotics play a pivotal role in animal husbandry and aquaculture, contributing to the development of antibiotic-resistant bacteria in food-producing animals and subsequent transmission to humans through the food chain. The emergence of plasmid-mediated resistance genes, such as mcr-1 conferring resistance to colistin, further exacerbates the problem of antibiotic resistance in *Shigella* infections. Addressing antibiotic resistance in *Shigella* infections requires a multifaceted approach, encompassing prudent antibiotic use, enhanced surveillance systems, and the development of alternative treatment modalities. In this review comprehensive analysis of the epidemiology, pathogenesis, clinical features, treatment options, and mechanisms of antibiotic resistance in *Shigella* infections was carried out. Various mechanisms underpin antibiotic resistance in *Shigella*, including enzymatic inactivation, target site modifications, and efflux pump systems, which diminish the efficacy of commonly used antibiotics such as fluoroquinolones, β-lactams, and sulfonamides. Moreover, the challenges associated with current treatment strategies are discussed, emphasizing the urgent need for novel therapeutic approaches to combat multidrug-resistant *Shigella* infections. We have underscored the importance of continuous research and global collaboration to mitigate the impact of antibiotic resistance on public health and food safety, particularly in vulnerable populations.

Keywords: *Shigella*, Foodborne infections, Antibiotics, Antibiotic resistance, Multidrug-resistant

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Introduction

Foodborne infections present a significant hazard to food safety, especially in underdeveloped nations where sanitation and hygiene are typically lacking. *Shigella spp*. are Gram-negative, rod-

shaped, non-motile, and non-spore-forming bacteria that act as causative agents of foodborne shigellosis, an acute enteric infection (CDC Report, 2013). *Shigella* remains a major pathogen responsible for increased rates of morbidity and mortality due to dysentery globally, particularly affecting children under 5 years old in developing countries. The four types of *Shigella spp*. include subgroup A (*S. dysenteriae*), subgroup B (*S. flexneri*), subgroup C (*S. boydii*), and subgroup D (*S. sonnei*), each containing several serotypes. Shigellosis can manifest in pandemic, epidemic, and sporadic forms (Kumar and Varela, 2013).

Antibiotics have been extensively used in animal husbandry and aquaculture for various purposes such as feed efficiency, prophylaxis, and illness therapy. Indiscriminate antibiotic use for non-therapeutic purposes has led to the emergence of antibiotic-resistant bacteria in food production. This resistance can result from sub-lethal antibiotic exposure in the environment or through direct acquisition of resistance mechanisms from other bacteria via DNA transfer processes (ECDC Report, 2009). Antibiotic use in food and agriculture directly impacts the development of antibiotic resistance in bacteria associated with animals that can enter the food chain through meat and fish consumption.

According to the United States Centers for Disease Control and Prevention (CDC), over two million people in the United States are infected by antibiotic-resistant bacteria annually, leading to approximately 23,000 deaths. In Europe, an estimated 25,000 deaths are attributed to antibiotic-resistant bacteria (US – FDA Report, 2014). Nearly 80% of antibiotics produced in the United States are used in animal husbandry. The increase in global meat demand necessitates a corresponding rise in meat-producing livestock. *Campylobacter* quickly developed resistance to fluoroquinolone antimicrobials after their introduction in the US poultry sector. The use of antibiotics in livestock and aquaculture contributes to the development of resistance in human pathogens, as susceptible bacteria are eliminated, allowing resistant variants to thrive.

Key contributors to antibiotic resistance include the overuse of antibiotics in humans and animals, poor hygiene and sanitation practices, and inadequate infection prevention and control in healthcare settings. Continuous surveillance is essential to identify active antibiotics for appropriate treatment. The ongoing need for new antibiotics to combat resistance underscores the importance of research and development in the pharmaceutical industry (Jacoby and Bush, 2005). However, the race between antibiotic development and resistance in pathogens like *Shigella* poses challenges, as effective antibiotics for future Shigellosis epidemics are currently unavailable. Relying on the pharmaceutical industry to produce novel and cost-effective antibiotics frequently is not a sustainable long-term solution.

Epidemiology:

Bacillary dysentery poses a significant global health threat, often stemming from factors such as inadequate access to clean drinking water, poor sanitation, substandard hygiene practices, and socioeconomic disparities (Lee *et al.*, 1991). Contaminated food and water serve as common vectors for outbreaks of this condition (David *et al.*, 2001). The frequency and severity of these outbreaks vary due to factors like geographical location, climate, host-pathogen interactions, and prevailing management strategies.

Epidemic dysentery, particularly caused by multidrug-resistant (MDR) *S. dysenteriae* serotype 1, presents intermittent challenges. Epidemiological data indicate that *S. flexneri* predominantly causes epidemic diarrheal diseases in developing nations, while *S. sonnei* is more prevalent in industrialized countries. According to the World Health Organization (WHO) *Shigella* species are primarily responsible for community-acquired bacterial infections. Globally, an estimated 164.7 million cases of shigellosis occur annually, with children bearing a significant burden, accounting for 69% of cases and 61% of related deaths (Lee *et*

al., 1991; Naimi *et al.*, 2003). *S. sonnei* demonstrates remarkable survivability, persisting for up to seven weeks on contaminated surfaces, five days in brackish environments, and 12-30 h in stagnant water. Additionally, carriers can shed the bacteria for up to two weeks post-infection, or even longer in some cases (Nisar *et al.*, 2013). Seasonality plays a role, with *S. sonnei* infections peaking during summer and rainy seasons, while in tropical climates, the pathogen remains prevalent year-round. Bacterial virulence genes are typically activated and expressed within a temperature range of 30–37°C, at pH range of 7 to 8, and under mild osmotic pressure conditions (Dorman and Porter, 1998).

Pathogenesis:

Numerous characteristics of *Shigella* contribute to its invasiveness and pathogenicity. These bacteria can invade enterocytes in colonic and rectal epithelia, disrupt intracellular phagocytic vacuoles, escape into the cytoplasm, multiply, and invade neighboring cells. This invasive ability is linked to the presence of large plasmids (120–140 Mdal) encoding outer membrane proteins like invasion plasmid antigens (Ipa) or invasins. Strains lacking this plasmid are non-virulent. Ipa proteins play a crucial role in recognizing epithelial cells, initiating a signal transduction cascade that leads to actin polymerization and bacterial-directed endocytosis. By colonizing the epithelial layer, *Shigella* avoids exposure to the extracellular environment. Intracellular growth leads to epithelial cell death, ulceration, and mucosal inflammation (Jacoby and Bush, 2005).

Apart from the discussed virulence factors, endotoxins also contribute to the pathogenicity of *Shigella spp.,* potentially causing systemic symptoms like fever, malaise, and body aches. *S. dysenteriae* type 1 produces a potent protein cytotoxin known as Shiga toxin (Schnappinger and Hillen, 1996). While Shiga toxin is not essential for disease development, toxin-producing strains are more pathogenic. Shiga toxin consists of functional (A) and binding (B) subunits. Once inside the cytoplasm, the A subunit catalyzes the hydrolysis of 28S RNA in the 60S ribosomal subunit, leading to irreversible inhibition of protein synthesis and cell death. Shiga toxin is implicated in hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura (Delcour, 2009). Additionally, *Shigella* can inhibit pro-inflammatory cytokines like IL-8 through epigenetic mechanisms.

Treatments prescribed for Shigella infections:

The treatment of shigellosis involves both rehydration and antibiotics. Fluid losses in shigellosis are not as pronounced as in secretory diarrhea, and dehydration is generally not a significant concern. It can typically be managed with oral rehydration solutions. As per current WHO guidelines and systematic reviews, the recommended treatment for shigellosis includes fluoroquinolones (first-line, preferably ciprofloxacin), cephalosporins (second-line), and β-lactams (second-line) for a duration of 7 to 10 days. In regions with high ciprofloxacin resistance rates, azithromycin may be considered as an alternative treatment option. There is only one related study on an antimicrobial susceptibility case series that reports resistance in various regions and highlights treatment challenges for infections caused by *Shigella*. Overall, a decrease in susceptibility to first-line and alternative agents has been observed. Consequently, there is an urgent need for the development of new therapeutic strategies to address multidrug-resistant (MDR) *Shigella* infections.

Clinical features and complications:

The clinical presentation of *Shigella* infections varies from watery, loose stools to severe symptoms such as fever, abdominal pain, tenesmus, and bloody diarrhea. The severity of the condition depends on the infecting species; *S. dysenteriae* infections typically result in dysentery, which can also occur with *S. flexneri* infections, while *S. boydii* and *S. sonnei* infections commonly present with limited watery diarrhea. Severe complications like toxic megacolon, peritonitis, and septicemia are frequently observed in

severely affected children, especially in the absence of early antibiotic treatment. *Shigella dysenteriae* can lead to serious complications including persistent diarrhea, severe loss of appetite, weight loss, malnutrition, colonic dilation, seizures, neurological damage, and hemolytic-uremic syndrome. Bacteremia may occur in infants and immunocompromised adults. Additionally, pneumonia associated with *S. sonnei* has been documented in severely affected children, individuals with HIV, and those with underlying health conditions.

Antimicrobial resistance mechanisms: *A brief overview*:

Throughout their evolutionary history, bacteria have developed several key mechanisms to resist antimicrobial agents. These mechanisms include enzymatic inactivation, where bacterial enzymes hydrolytically convert antimicrobial agents into inactive compounds (Schnappinger and Hillen, 1996). An example is the production of β-lactamases, such as penicillinases, which deactivate β-lactam antimicrobials like penicillin. Another mechanism involves biochemical modification of antimicrobial drugs by microbial enzymes, rendering them ineffective (Nikaido, 1988). Target protection is another resistance mechanism, where bacterial peptides shield antimicrobial targets, as above in resistance to tetracyclines. Additionally, bacteria can reduce the entry of antimicrobial agents into the cytoplasm, known as drug permeability reduction, preventing access to intracellular targets. Finally, active efflux pumps expel antimicrobial agents from bacterial cells, reducing drug concentrations and conferring resistance (Delcour, 2009).

Resistance to beta lactams:

β-lactam antibiotics have emerged as the primary treatment choice due to their mechanism of action targeting penicillin-binding proteins (PBPs), pivotal for cell wall synthesis. Upon binding to essential PBPs, β-lactams exhibit bactericidal effects in susceptible species (Falagas *et al.*, 2010). Despite the potential for *Shigella* to develop β-lactam resistance through mutations reducing PBP affinity, the predominant and clinically significant resistance mechanism in Gram-negative bacteria involves the production of β-lactamases (Salam and Bennish, 1991). These enzymes degrade the β-lactam ring, rendering the antibiotics ineffective.

Class A β-Lactamases:

Tazobactam and clavulanic acid inhibit class A-lactamases, enzymes capable of hydrolyzing narrow-spectrum penicillin but not carbapenems or cephalosporins. Within Ambler class A, extended-spectrum β-lactamases (ESBLs) are included. *Shigella* strains have been identified with ESBLs, imparting resistance to third-generation cephalosporins (Kim *et al.*, 2014). The emergence of ESBLs in *Shigella* poses a significant public health threat. *Shigella* isolates harbor various β-lactamases from Ambler class A, including TEM, SHV, and CTX-M enzymes.

In 1995, France reported the first *S. flexneri* isolate producing an ESBL, carrying the blaSHV-2 gene on a plasmid. Over 40 variants of CTX-M-type β-lactamases have been identified, easily transferred across *Shigella* strains via conjugative plasmids like IncF, IncZ, and IncI groups. Lee *et al.* (1991) found ISEcp1 adjacent to all blaCTX-M genes in *Shigella*, suggesting its involvement in blaCTX-M gene mobilization. In Vietnam, the IncI1 plasmid pKHSB1 containing blaCTX-M-15 was found in a clonal population of *S. sonnei*. Another study revealed the complete sequencing of IncI1 plasmid pSH4469, carrying blaCTX-M-15 from a clinical *S. sonnei* sample during an epidemic in the South. In China, a novel hybrid β-lactamase, CTX-M-123, was discovered in *S. flexneri* isolates, carried by IncHI2 and IncF conjugative plasmids (Li *et al.*, 2015). Various CTX-M β-lactamase subtypes, including CTX-M-79, CTX-M-27, CTX-M-24, CTX-M-15, CTX-M-14, CTX-M-64, CTX-M-65, CTX-M-55, and CTX-M-3, have been identified in clinical *Shigella* strains across China. ESBL genes may have transferred from *E. coli* to *Shigella spp*., particularly *S. sonnei,* through conjugation in the human gut. The rise of multidrug resistance and ESBLs in *Shigella* strains can lead to treatment failures and limited therapeutic options.

Class B β-Lactamases:

The Class B β-lactamase enzyme can break down carbapenems and other β-lactams, excluding aztreonam, while traditional β-lactamase inhibitors like tazobactam and clavulanic acid are ineffective against them. Metallo-β-Lactamase was initially identified in a transferable plasmid from *Pseudomonas aeruginosa*, with IMP-1 being first recognized in various Gram-negative rods in Japan. O'Hara *et al.* (1998) described a new variant of metallo-β-lactamase called MET-1, which was linked to a plasmid in *S. flexneri* (Qu *et al.*, 2014). MET-1 was believed to be a modified form of IMP-1 β-lactamase and conferred resistance not only to β-lactams but also to sulfonamide and kanamycin. MET-1 had two amino acid alterations compared to IMP-1, leading to the designation of the gene as IMP-3, potentially preceding IMP-1 β-lactamase. This gene was located on a cassette within a class I integron, widely spread among *Shigella* species, providing resistance to almost all β-lactam antibiotics (O'Hara *et al*, 1998). Recently, carbapenem resistance mediated by blaVIM and blaIMP genes was detected in *S. sonnei* and *S. flexneri* isolates from pediatric diarrhea patients in India's Andaman and Nicobar Islands.

Class C β-Lactamases:

Cifrofloxacin-resistant *Shigella* isolates should be treated with ceftriaxone. However, certain strains of *Shigella spp*. now carry a gene for cephalosporin resistance. Amp C type enzymes, commonly known as Class C-lactamases, confer high-level cephalosporin resistance. Both plasmid and chromosomal genes encode Amp C-lactamase, and the first report of plasmid-encoded CMY-2-type Amp C-lactamase was found among ceftriaxone-resistant *S. sonnei* isolates from a bacillary dysentery outbreak in Taiwan. CMY-2 enzymes have been found in epidemic strains in China, Taiwan, Costa Rica, Iran, and India (Zhang *et al.*, 2014). In *Shigella* isolates collected from diarrhea patients in China, Zhang *et al.* (2014) discovered two Amp C lactamase producers bearing the genes blaCMY-2 and blaDHA.

Class D β-Lactamases:

Class D-lactamases, also referred to as OXA-type-lactamases, provide resistance to ampicillin and cephalothin and can break down oxacillin, cloxacillin, and benzyl penicillin, but they are not inhibited by tazobactam or other inhibitors. Initially, blaOXA-lactamases were exclusively identified in *P. aeruginosa* isolates, but subsequently, blaOXA genes have been detected in integrons and plasmids of various Gram-negative bacteria. Ampicillin resistance in *Shigella* species is primarily associated with OXA-type β-lactamase (Zhang *et al.*, 2014). In 2000, blaOXA-30 was identified in ampicillin-resistant *S. flexneri* strains in China. Certain studies suggest that *S. flexneri* isolates are a probable host specifically for blaOXA-type β-lactamase. Another study conducted in Iran revealed that all blaOXA-positive isolates carried blaOXA-1, with the majority found in *S. flexneri* isolates, indicating a host preference for these enzymes in *S. flexneri*.

Resistance to Fluoroquinolones and Quinolones due to Chromosomal Target–Site Mutations:

DNA gyrase and topoisomerase IV consist of subunits encoded by specific genes: gyrA, gyrB, parC, and parE. DNA gyrase comprises two gyrA and two gyrB subunits, while topoisomerase IV is composed of two parC and two parE subunits (Tamanna and Ramana, 2016). Most mutations are clustered in a region near the beginning of the gyrA gene known as the quinolone resistance–determining region (QRDR), spanning Ala67 to Gln107. Several studies have reported that mutations frequently occur at codons 83, 87, and 211 in gyrA, while mutations in gyrB are less common across different studies. Some researchers suggest that a single mutation in gyrA may confer resistance to quinolones, but additional mutations in parC and gyrA regions are necessary for decreased susceptibility to fluoroquinolones. Among *Shigella* isolates, mutations in the parC gene are most found at codon 80. gyrA mutations are more prevalent than gyrB mutations (Datta *et al.*, 2005). Variations in nucleotide and amino acid sequences within

QRDRs of gyrA, gyrB, parC, and parE have been observed among *Shigella spp*. Mutations in QRDRs have been identified worldwide, including two novel mutations at parC codons 86 and 129, as well as a mutation at gyrA codon, in *S. sonnei* strains from China in 2009. Although these mutations in chromosomal target sites (QRDRs) do not directly contribute to quinolone and fluoroquinolone resistance, other mechanisms may be at play in *Shigella* isolates, necessitating further research (Perea, 1978).

Resistance to aminoglycosides:

Aminoglycosides represent a class of antibiotics utilized in the treatment of various infections, acting by disrupting protein synthesis. Resistance mechanisms to aminoglycosides include enzymatic inactivation, ribosomal alterations, and active efflux pumps, with aminoglycoside-modifying enzymes being prevalent in clinical settings (Falagas *et al.*, 2010; Muthuirulandi *et al.*, 2019). These enzymes are activated through three primary processes: adenylation, acetylation, and phosphorylation. Aminoglycoside adenyl transferase (encoded by aadA gene cassettes) is particularly common in Enterobacteriaceae, notably among *Salmonella* and *Shigella* isolates, imparting resistance to streptomycin and spectinomycin (Parajuli *et al.*, 2019). While various types of aadA gene cassettes have been identified among Enterobacteriaceae, aadA1 and aadA2 are prevalent among *Shigella* isolates (Fourmy *et al.*, 1998). Additionally, amino-glycoside phosphotransferases encoded by strA and strB genes are widely dispersed among *Shigella* isolates via plasmids like IncFII and pNVY394 (Shaw *et al.*, 1996; Fourmy *et al.*, 1998). However, class 1 integrons containing the gene-cassette array of blaOXA-30 + aadA1, with complete 3′CS, have been observed on plasmids in *Shigella spp*. isolates, *Salmonella enterica serovar Typhimurium*, and *E. coli* strains. Transferable plasmids may facilitate the spread of resistance genes within integrons, underscoring the role of plasmids in horizontal resistance gene transfer.

Resistance to Tetracyclines:

Tetracyclines, bacteriostatic antimicrobial drugs, were introduced in the early 1950s, acclaimed for their broad-spectrum activity against both gram-negative and gram-positive bacterial infections. They interact with the 30S component of the bacterial ribosome, reversibly inhibiting protein synthesis by impeding aminoacyl-tRNA binding. Understanding tetracycline's mode of action necessitates consideration of uptake and ribosomal binding mechanisms. Explaining their antibacterial and antiprotozoal activities, as well as microbial selectivity, is pertinent. Tetracyclines traverse the outer membranes of gram-negative enteric bacteria as positively charged cation-tetracycline coordination complexes through OmpF and OmpC porin channels (Miranda *et al.*, 2016). The uptake of tetracycline across the cytoplasmic membrane relies on energy and is regulated by the pH component of the proton motive force.

Efflux proteins:

Tetracycline export lowers intracellular drug concentrations, protecting ribosomes within the cell. Both gram-positive and gram-negative bacteria have efflux genes. Tetracycline resistance is conferred by most of these efflux proteins, but not by minocycline or glycylcyclines. The gram-negative tet(B) gene, on the other hand, codes for an efflux protein that confers tetracycline and minocycline resistance but not glycylcycline resistance (Miranda *et al.*, 2016). Glycylcycline resistance has been linked to laboratory-derived mutations in tet(A) or tet(B), showing that bacterial resistance to this class of medications may emerge over time and with clinical use.

Ribosomal Protection Proteins:

These are cytoplasmic proteins that protect ribosomes from tetracycline and confer doxycycline and minocycline resistance. They confer a broader range of tetracycline resistance than bacteria that contain tetracycline efflux proteins., apart from Tet(B). Elongation factors EF-Tu and EF-G are related to the ribosomal

protection proteins (Taylor and Chau, 1996; DePaola *et al.*, 1998) The N-terminal region, which houses the GTP-binding domain, shows the most similarity. Tetracycline sensitivity of ribosomes is reduced by the Tet(M), Tet(O), and OtrA proteins.

Unknown Mechanism of Protein:

Tetracycline resistance is conferred via the tet(U) gene (Taylor and Chau, 1996). This gene produces an 11.8-kDa protein with 105 amino acids, which is lower than efflux proteins (45 kDa) and ribosomal proteins (72 kDa) but larger than the efflux proteins (45 kDa). Between the Tet(U) and Tet(M) proteins, there is a 21 per cent similarity in the first 105 amino acids, starting around the carboxy terminus of the latter. The consensus GTP-binding regions in Tet(M) and related proteins are hypothesized to play a role in resistance. The sequence, however, does not resemble either the efflux or ribosomal protection genes, hence, and the mechanism is thus listed as unknown.

Resistance to Phenicols:

Phenicols have been increasingly utilized worldwide for treating *Shigella* infections in recent years, leading to significant selective pressure for antibiotic resistance (Williams and Berkley, 2018). In *Shigella*, resistance is primarily associated with enzymatic inactivation of unfluorinated phenicols by chloramphenicol acetyltransferase genes, including catA (catA1, catA2, catA3) and catB (catB2, catB3, catB7, catB8) variants, as well as active efflux mediated by cmlA (cmlA1, cmlA4, cmlA9) and/or major facilitator–superfamily proteins for both fluorinated and unfluorinated phenicols (floR) (Schwarz *et al.*, 2004). Chloramphenicol (Cm), characterized by a novel structure comprising a p-nitrophenyl group at C-1 and an N-dichloroacetyl substituent at C-2 attached to a 1,3-propanediol with two chiral centers (C-1 and C-2), was the first naturally occurring chemical identified to contain a nitro group. Cm, being the first antibiotic commercially synthesized due to its relative simplicity, has been exclusively produced in this manner since 1950. Its antibiotic activity resides solely in one of the four potential diastereoisomers (D-threo), with the C-3 primary hydroxyl group, assumed crucial for suppressing protein synthesis via its affinity for the 50S ribosome's peptidyltransferase, replaceable with fluorine. Besides the fluoro substitution at C-3 (as in florfenicol), only minimal changes can be tolerated without compromising antibacterial efficacy. Thiamphenicol and florfenicol, substituting a sulfomethyl group ($-SO_2CH_3$) at the para position of the 1-phenyl moiety for the nitro group ($-NO_2$), previously linked to dose-unrelated aplastic anemia, have shown beneficial effects. Chloramphenicol is stable at room temperature and remains amphiphilic and unionized at physiological pH, allowing it to traverse biological membranes efficiently, including the blood-brain barrier. Florfenicol, a fluorinated derivative of chloramphenicol, exhibits antibiotic activity only in the D-threo stereoisomer, with poor solubility in aqueous solutions and lipophilicity facilitating excellent tissue penetration due to its amphiphilic nature.

Mode of action and spectrum of activity:

Chloramphenicol (Cm) is an exceptionally potent and selective inhibitor of protein synthesis in prokaryotes. Its primary mode of action involves the inhibition of peptide chain elongation, leading to the suppression of bacterial protein production. This bacteriostatic effect is achieved through reversible binding to the peptidyl transferase center of the 50S ribosomal subunit within the 70S ribosomes (Schlunzen *et al.*, 2001). Importantly, Chloramphenicol and its derivatives do not affect the function of eukaryotic cells' 80S ribosomes.

Bacterial resistance to Chloramphenicol:

Over time, bacteria have developed various strategies to evade the inhibitory effects of chloramphenicol. The enzymatic inactivation of Cm by distinct types of chloramphenicol acetyltransferases (CATs) represents the earliest and most prevalent mechanism of bacterial resistance to Cm. Additionally, other mechanisms

contributing to Cm resistance, including efflux systems, phosphotransferase inactivation, alterations in target sites, and the presence of permeability barriers, have been documented (Shaw, 1983). The presence of cat genes is strongly associated with chloramphenicol resistance in *Shigella* isolates.

Resistance to Colistin:

Colistin (polymyxin E) is a polypeptide anti-microbial agent that interacts with the outer membranes of various Gram-negative bacteria. The plasmid-mediated polymyxin resistance gene mcr-1 was first reported in an *E. coli* isolate in China in November 2016. Since then, mcr-1 has been identified in *Salmonella enterica* and *Klebsiella pneumoniae*, with sporadic reports in other Enterobacteriaceae members. It has also been detected in genera such as *Shigella, Cronobacter, Kluyvera*, and *Enterobacter* isolated from various sources including vegetables, the environment, food, animals, and humans (Pham *et al.*, 2016). In most cases, mcr-1 is the only resistance gene found on related plasmids, suggesting that selective pressure associated with polymyxin use drives its acquisition. The presence of mcr-1 in *Shigella* isolates leads to a four to eightfold increase in the minimum inhibitory concentration (MIC) of polymyxin B. mcr-1 is carried on plasmids of varying sizes, including IncI2, IncFI, IncHI2, IncFIB, IncP, IncY, and IncX4, and may coexist with ESBL-encoding genes or other resistance genes. Plasmid-mediated colistin-resistant *S. flexneri* isolates from animal feces on farms suggest dissemination via the fecal-oral route. In addition, mcr-1 has been found in *S. sonnei* isolates with polymyxin B resistance in Shanghai (Schwarz and Johnson, 2016). Furthermore, a phosphatidyl ethanolamine transferase modifies lipid A in Gram-negative bacterial cell membranes, resulting in reduced antimicrobial activity of polymyxins due to decreased affinity for colistin and related polymyxins.

Resistance to Sulfonamide:

Sulfonamide resistance in *Shigella* is primarily mediated by the presence of sul1, sul2, and sul3 genes (Table 1). Among these, sul1 is particularly prevalent in *Shigella* isolates, often found within the 3'-conserved sequence region of class 1 integrons. Additionally, sul3, which is linked to an unusual 3'-conserved segment, is commonly associated with class 1 integrons. Sul2, another sulfonamide-resistance gene, is typically situated on large transmissible plasmids or small non-conjugative plasmids, initially identified on a small non-conjugative plasmid of *E. coli* (Antunes *et al.*, 2005). Antibiotic-resistance gene clusters containing strA, strB, and sul2 are widespread among Gram-negative bacteria, with a significant presence observed in *Shigella* isolates. Studies have indicated that sul1, sul2, sul3, integron 1, and integron 2 genes are present in multidrug-resistant *S. flexneri* 2a strains, with sul2 notably absent in sulfamethoxazole-sensitive strains but consistently present in sulfamethoxazole-resistant strains. Expression of sul1, sul3, integron 1, and integron 2 genes did not differ between sulfamethoxazole-resistant and -sensitive *S. flexneri* 2a strains. Furthermore, the removal of a 4.3 MDa plasmid resulted in the loss of sul2 and increased susceptibility to sulfamethoxazole, suggesting the involvement of sul2 and this plasmid in sulfamethoxazole resistance (Schwarz *et al.*, 2004). Additionally, an unusual class 1 integron linked with sul3 was discovered in 24 *S. sonnei* isolates, encoding various resistance factors including esterase, lipase, and resistance to streptomycin, chloramphenicol, and quaternary amines.

Fosfomycin Resistance:

Fosfomycin (Fom) is a broad-spectrum antibiotic that works by inhibiting bacterial cell-wall formation through the inactivation of the MurA enzyme. Despite being used for microbial infections for over four decades, Fosfomycin has maintained its effectiveness against common pathogens, with resistance remaining rare worldwide. However, recent observations have

Table 1: Prevalence of antimicrobial resistance genes in *Shigella spp.* isolated from different regions of the world

Antimicrobial Class	Resistance Mechanism	Genes mediating Antimicrobial Resistance	Geographic Origin
Sulfonamides	Plasmid-borne Resistance	sul1	Australia, South Korea, Taiwan
		Sul2	Taiwan, India, Peru, Chile, Bangladesh, South Korea
		Sul3	Taiwan
Phenicol's	Chloramphenicol acetyltransferase genes	CatA-like	Taiwan, Mozambique, India, Peru, France, Brazil, Pakistan
	Chloramphenicol acetyltransferase genes	catP P	Pakistan
	Efflux pumps	cmlA1	Taiwan, Mozambique
Colistin	Plasmid-borne Resistance	mcr-1	China, Vietnam
Macrolide	Enzymatic inactivation	mphA	Palestine, Switzerland, Vietnam, China, Canada, UK, Peru
	rRNA methylase	ermB	Vietnam, Canada, UK
Quinolones	Plasmid-borne Resistance	qnrA	Iran
		qnrB	China, India, Iran
		QnrB4	Switzerland
		qnrB19	Switzerland
		qnrC	India
		qnrS	China, India, Iran, Pakistan
		qnrS1	Iran, China, Switzerland, US
		Aac-(60)-Ib-cr	US, India, Japan, China, Iran
	Efflux pumps	qepA	China, Switzerland
Fosfomycin	Fosfomycin Resistance Enzymes	FosA3	China
Aminoglycosides: Streptomycin	Adenyltransferase	strA	India, China, Pakistan, South Korea
		strB	India, Chile, Pakistan, South Korea
		aadA1	Senegal, Bhutan, India, Taiwan, Spain, China, Iran, France, Australia, Brazil
		aadA2	Taiwan, Spain, China, South Korea, Australia, Korea
		aadA5	Taiwan, China, Iran
Tetracycline	Efflux Pumps	tetA	Mozambique, Taiwan, Chile, Peru, Brazil, Spain, Pakistan, South Korea

		tetB	Mozambique, Taiwan, Peru, Brazil, Spain, Pakistan, France
		tetG	Mozambique, Spain
β-Lactams	Class A β-lactamases	blaSHV-2	Argentina, France
		blaSHV-11	India
		blaSHV-12	China, Turkey
		blaTEM-2	Argentina
		blaTEM-1	Lebanon, Chile, China, India, Iran, US,
			Djibouti, Denmark, France, Greece, Brazil, South Korea, UK, Romania
		blaTEM-1b	China, South Korea
		blaTEM-15	South Korea
		blaCTX-M-15	China, Spain, Iran, South Korea, India, US, Lebanon, Japan, Poland, New Zealand,France, Romania
		blaCTX-M-22	China
		blaCTX-M-24	China
		blaCTX-M-27	China
		blaCTX-M-28	China
		blaCTX-M-39	Israel
		blaCTX-M-55	China, South Korea
		blaCTX-M-57	China
		blaCTX-M-64	China
		blaCTX-M-65	China
		blaCTX-M-79	China
		blaCTX-M-123	China
β-Lactams	Class B β-lactamases	blaIMP-like	India, France
		blaKPC	Senegal, France
		blaIMP-3	Japan
		blaVIM-like	India
	Class C β-lactamases	blaCMY-2	China, Mexico, India, Iran, Taiwan,Costa Rica, Romania
		blaCMY-59	Iran
		blaDHA-1	China, India, Israel
	Class D β-lactamases	blaoxa-1-like	Mozambique, Chile, China, India, US, Egypt,Djibouti, Spain, Greece, Denmark, Peru, Iran
		blaoxa-2-like	Mozambique, Spain, Israel
		blaoxa-5-like	Mozambique, Spain
		blaoxa-30-like	Senegal, China, France, Japan, Spain, Brazil

identified Fom resistance in *E. coli* strains determinants known as FosC2 and FosA3. Two main mechanisms contribute to fosfomycin resistance: mutations in uhpA/T and glpT genes, which encode proteins involved in fosfomycin uptake, and the acquisition of fosfomycin-modifying enzymes like FomA, FomB, FosX, FosA, and FosB (Lui *et al.*, 2017).

The discovery of fosfomycin-modifying enzymes in *S. flexneri* strains from Chinese patients marked a significant finding. Studies indicate that the increasing prevalence of fosA3 may be linked to the dissemination of IncN and IncI plasmids, facilitating rapid spread. Notably, transconjugant plasmids carrying both ESBL (blaCTX-M-123, blaCTX-M-55, or blaCTX-M-15) and fosA3 genes were identified across various incompatibility groups, conferring resistance to cefotaxime, ceftriaxone, and fosfomycin (Phuc *et al.*, 2009). The presence of these resistance determinants on conjugative plasmids underscores their role in disseminating fosA3 and ESBL genes among diverse *Shigella* isolates with high genetic diversity, emphasizing the importance of vigilant monitoring.

Macrolide Resistance:

Macrolide resistance is primarily driven by four mechanisms: enzymatic inactivation through phosphotransferases encoded by mph genes or esterases encoded by ere determinants, target-site modification by an rRNA methylase encoded by erm genes, point mutations in rplV (encoding L22 ribosomal protein), rplD (encoding L4 ribosomal protein), and rrlH (23S rRNA), and drug resistance facilitated by efflux pumps like OmpA, OmpW, mefA, and msrA. Resistance to azithromycin and erythromycin can arise from any of these macrolide resistance mechanisms. The transmission of plasmids from *E. coli* has been identified as the primary source of macrolide mphA resistance mechanisms in *Shigella* spp. All mphA-associated plasmids reported so far have been detected in *E. coli* isolates, suggesting their potential role as a reservoir for antimicrobial resistance in *Shigella spp*. Instances of *E. coli* carrying novel transferable fosfomycin-resistance transferring mphA to *S. sonnei* have been documented (Phuc *et al.*, 2009).

Conclusion

The bacteria responsible for food poisoning, *Shigella*, poses a significant public health concern, with the evolution of antibiotic resistance necessitating the development of improved antimicrobial therapies. There is a possibility that they may develop resistance to newly developed medications, highlighting the importance of understanding resistance mechanisms and finding effective solutions. Efflux pump activity has been studied as a resistance mechanism, but no inhibitors have yet received clinical approval. Consequently, treating shigellosis has become a therapeutic challenge. Managing multidrug-resistant (MDR) bacteria has become the primary focus, emphasizing the critical need to address MDR bacteria as the ultimate mission.

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