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REVIEW

Shigella: Antibiotic-Resistance Mechanisms And New Horizons For Treatment

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Abstract: *Shigella* spp. are a common cause of diarrheal disease and have remained an important pathogen responsible for increased rates of morbidity and mortality caused by dysentery each year around the globe. Antibiotic treatment of *Shigella* infections plays an essential role in reducing prevalence and death rates of the disease. However, treatment of these infections remains a challenge, due to the global rise in broad-spectrum resistance to many antibiotics. Drug resistance in *Shigella* spp. can result from many mechanisms, such as decrease in cellular permeability, extrusion of drugs by active efflux pumps, and overexpression of drug-modifying and -inactivating enzymes or target modification by mutation. Therefore, there is an increasing need for identification and evolution of alternative therapeutic strategies presenting innovative avenues against *Shigella* infections, as well as paying further attention to this infection. The current review focuses on various antibiotic-resistance mechanisms of *Shigella* spp. with a particular emphasis on epidemiology and new mechanisms of resistance and their acquisition, and also discusses the status of novel strategies for treatment of *Shigella* infection and vaccine candidates currently under evaluation in preclinical or clinical phases.

Keywords: *Shigella*, antibiotics, resistance, drug resistance, mechanism, treatment, biofilm, efflux pumps, prevention, vaccine

Introduction

Shigella spp. are a Gram-negative, rod-shaped, immotile, and non-spore–forming bacteria and a causative agent of acute diarrhea that may progress to bloody mucoid diarrhea, also known as bacillary dysentery (or shigellosis).[1] *Shigella* is the most common cause of diarrheal disease and has remained a major pathogen responsible for increased rates of morbidity and mortality caused by dysentery each year around the globe, particularly affecting children aged <5 years in developing countries.[2] The four types of *Shigella* spp. comprise subgroup A (*S. dysenteriae*), subgroup B (*S. flexneri*), subgroup C (*S. boydii*), and subgroup D (*S. sonnei*). Each subgroup contains several serotypes. Shigellosis can occur in pandemic, epidemic, and sporadic forms. Epidemiological reports have shown that the epidemic subgroup of diarrhea typically occurs as a result of infection with *S. flexneri* in developing countries and *S. sonnei* in industrialized countries.[1,3] *Shigella* spp. are categorized by the World Health Organization (WHO) as bacteria mainly causing infections in the community.[4] Shigellosis is a great public health threat, because its infective dose is on the order of 10–100 organisms compared to other enteric pathogens (usually it is 105–108 for *Salmonella* and *Vibrio*, respectively).[5,6]

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Antibiotic treatment of common bacterial infections plays an important role in reducing prevalence and death rates of the disease. However, incorrect antibiotic use or overuse in treating diarrhea increases antibiotic resistance. *Shigella* spp. are resistant to most antibiotics, and drug treatment related to these bacteria is costly, time-consuming, and sometimes problematic, particularly in areas with limited medical care.[7,8] About half the strains of *Shigella* in many parts of the world are now resistant to multiple drugs. Recently, various antibiotic-resistance mechanisms have been described by researchers, and these antibiotic-resistance mechanisms limit therapeutic options for treatment of *Shigella* infections.[8,9]

Drug resistance in *Shigella* spp. can result from many mechanisms, such as extrusion of drugs by active efflux pumps, decrease in cellular permeability, and overexpression of drug-modifying and -inactivating enzymes or target modification by mutation.[6,10,11] The current study was done to review various antibiotic-resistance mechanisms of *Shigella* spp., with a particular focus on epidemiology and new mechanisms of resistance and their acquisition, and also to discuss treatment and prevention measures for diseases caused by these organisms.

Search Strategy

We searched the biomedical electronic databases Web of Science, Scopus, PubMed (Medline), Embase, Cochrane Library, and Google Scholar for articles on *Shigella* published in English between 1990 and May 2019, using the key terms (alone and in combination) "*Shigella*", "drug resistance", "mechanism", "biofilm", "efflux pump", "vaccine", and "treatment". We excluded case reports and some studies on the implementation of established techniques. Articles published before 1990 were also excluded, except when necessary. A total of 193 relevant articles were identified from the databases and included in this review.

Drug-Resistance Mechanisms In *Shigella* Spp

Role Of Outer-Membrane Permeability

Natural resistance to antimicrobial drugs by various mechanisms preventing the drug from being absorbed is capable of transforming the drug, its biotransformation into the cell, or reducing affinity with the drugs' target.[12] Cell walls of microorganisms are the first barrier against penetration of the drug. Some modifications of membrane permeability or changes in the membrane lead to porin loss, which can result in an increase in minimum inhibitory concentration(MIC) for antimicrobial agents.[13] Most antibiotics used in treatment of *Shigella* infection should be able to penetrate the cell membrane to reach intracellular accumulation and target sites. For example, quinolone antibacterial agents, such as nalidixic acid, ofloxacin, and ciprofloxacin, interfere with DNA replication by inhibiting DNA topoisomerase IV and gyrase. Aminoglycoside antibiotics, such as streptomycin and spectinomycin, mediate inhibition of protein synthesis by binding to ribosomal subunits and reaching intracellular targets. β-Lactam antibiotics, eg, penicillin and cephalosporin, are a class of antibiotics containing a β-lactam ring in their molecular structures and inhibit cell-wall biosynthesis by targeting penicillin-binding proteins. Mutation or absence of ~39 kDa porin in the membrane of such Gram-negative bacteria as *Shigella* spp. mainly influences susceptibility to slow penetration of β-lactams, such as aztreonam and dianionic moxalactam, and also low permeability of hydrophilic antibiotics, such as penicillin and piperacillin.[6,13] Indeed, resistance toward β-lactam antibiotics is associated with modification of the outer-membrane porins OmpF (~38 kDa) and OmpC (~42 kDa) and cytosolic proteins of ~26 kDa, OmpR as a transcriptional regulator.[6] In a study, three imipenem-resistant mutants of *S. dysenteriae* were obtained from India and showed lower levels of both major OMPs (~38 and ~43 kDa). Increasing imipenem resistance in mutants was associated with permeability of outer-membrane proteins.[14] Lipopolysaccharides (LPSs) have been recognized as an essential outer-membrane component needed for assembly of trimeric PhoE porin and confer colicin E_2 resistance in *S. flexneri* strains,[15] and have also been reported to be linked with the rise in resistance toward imipenem in *S. dysenteriae*.[14] Some outer-membrane components, such as *IcsA* molecules, are not only associated with bile salts resistance but are also related to promotion in development of biofilm by mediating bacterial cell–cell interactions. Consequently, they produce resistant phenotypes.[16]

Efflux Systems

Active efflux pumps play a significant role in antibiotic-resistance phenotypes of Gram-negative bacteria and

expelling toxic compounds from their cells. Efflux systems are grouped into five families: the major facilitator superfamily (MFS), resistance–nodulation–division family, small multidrug resistance (MDR) family, ATP-binding cassette superfamily, and multidrug and toxic compound extrusion family.[17] AcrAB–TolC pump is involved in antibiotic resistance phenotype of *Escherichia* spp., *Enterobacter* spp., *Salmonella* spp., and *Shigella* spp isolates. The AcrAB–TolC system is a tripartite complex comprising TolC (outer-membrane channel), AcrB (inner-membrane transporter protein), and periplasmic AcrA involved in assembly and maintenance of these two integral membrane proteins. AcrAB–TolC belongs to resistance–nodulation–division family of efflux pumps, associated with efflux of quinolones, and one of factors responsible for development of resistance among *Shigella* isolates. Indeed, overexpression of AcrAB–TolC results in overall decreased accumulation of quinolones inside bacterial cells, also resulting in reduced susceptibility to them.[18] AcrAB-associated to bile-salt resistance has been found in some strains of *S. flexneri*. Their expression has been shown to increase after exposure to bile salts, and enabled *Shigella* to resist bactericidal effects of bile. Researchers believe that this phenomenon may confer resistance to other antimicrobial agents. Furthermore, overexpression of AcrB has been found to be linked to multiple drug-resistance phenotypes in some Gram-negative bacteria.[19]

Synergistic action regarding activation of *acrAB–tolC* efflux pumps has been shown to decrease expression of outer-membrane porins, gyrase, and topoisomerase target–gene mutations toward fluoroquinolone resistance in *Shigella* isolates.[20,21] Drug-efflux pumps, such as *marA*, *tolC*, *ydhE*, and *mdfA*, confer quinolone resistance. Kim et al demonstrated that resistance to fluoroquinolone is due to increased expression level of MdfA efflux pump in *Shigella* spp. This efflux is a member of the MFS antibiotic-efflux system, and MdfA efflux pump–mediated fluoroquinolone resistance was first identified among MDR *Escherichia coli*.[22] Tetracycline efflux and resistance is associated with the MFS antibiotic–efflux system encoded by various *tet* genes in Gram-negative bacteria, such as *Shigella* spp. and *Klebsiella* spp. Among *tet* efflux systems, it seems that *tetA* and *tetB* are mediated by resistance to tetracycline in *S. sonnei* and *S. flexneri*, respectively (for more details, refer to "Tetracycline Resistance" section).[11]

Resistance To β-Lactam Antibiotics

Class A β-Lactamases

Class A β-lactamases can hydrolyze narrow-spectrum penicillin, but not carbapenems or cephalosporins, and are inhibited by tazobactam and clavulanic acid. Extended-spectrum β-lactamases (ESBLs) belong to Ambler class A. ESBLs conferring resistance to third-generation cephalosporins have been found in *Shigella* isolates. The first report documenting identification of ESBL-producing *Shigella* strains was from Bangladesh in 2004.[23] Emergence of ESBLs in *Shigella* spp. is a global major health threat affecting both developed and developing countries. Different β-lactamases belonging to Ambler class A have been reported among *Shigella* isolates, such as TEM, SHV, and CTX-M enzymes. The first isolate of *S. flexneri* producing an ESBL and harboring a plasmid encoding the $bla_{\text{SHV-2}}$ gene was reported from France in 1995.[24]

To date, several reports from Argentina, Israel, Canada, Turkey, Lebanon, Japan, Iran, South Korea, China, and other various regions in Asia have identified *Shigella* spp. harboring different types of ESBL genes (Table 1).[23,25–32] Although most ESBLs are derivatives of TEM and SHV β-lactamase families, which were first identified, *Shigella* spp. can also express the CTX-M family, among which CTX-M-15 is one of the most relevant findings associated with the current epidemiology of ESBLs, which has been predominantly identified in commensal and pathogenic ESBL-producing *Shigella* isolates around the world. Despite many surveillance studies and investigations, the reason for this epidemiological shift remains unknown.[24,33] These enzymes are responsible for selective hydrolysis of ceftriaxone and cefotaxime and even more distinctly for ceftazidime, although some types of CTX-M, such as CTX-M-15, may hydrolyze ceftazidime.[33] In general, CTX-M-15 has been found to have great catalytic efficiency (high $k_{\text{cat}}/K_{\text{m}}$) against piperacillin, benzylpenicillin, ceftriaxone, and cefotaxime, as reported for other of CTX-M-types, such as CTX-M-3, CTX-M-16, and CTX-M-18.

To date, CTX-M-15 has been detected in *Shigella* isolates from various countries across the world, including Canada, Russia, Poland, the UK, France, Bulgaria, Turkey, and Iran.[33,34] CTX-M-type β-lactamases contain at least 40 enzymes, and these can be readily transferred among *Shigella* isolates by conjugative plasmids belonging to IncF, IncZ, and Incl groups.[34,35] Li et al revealed that IS*Ecp1* was present adjacently to all $bla_{\text{CTX-M}}$ genes in *Shigella* strains, meaning that it plays an important role in mobilizing $bla_{\text{CTX-M}}$ genes (Figure 1).[36] A large study conducted in Vietnam analyzed IncI1 plasmid pKHSB1 carrying $bla_{\text{CTX-M-15}}$ collected from a clonal population of *S. sonnei*.[37] Another study reported full sequence of IncI1 plasmid pSH4469 carrying $bla_{\text{CTX-M-15}}$ in a clinical isolate of *S. sonnei* isolated from an outbreak in South

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	Origin	Geographic Origin	Reference
β-Lactams	Class A β-lactamases	bla_{SHV-2}	P	Argentina, France	32
		bla_{SHV-11}	C, P	India	191
		bla_{SHV-12}	P	China, Turkey	40
		bla_{PER-2}	—	Argentina	32
		bla_{TEM-1}	I, P	Lebanon, Chile, China, India, Iran, US, Djibouti, Denmark, France, Greece, Brazil, South Korea, UK, Romania	25,26,31,40,120,192
		bla_{TEM-1b}	I, P	China, South Korea	34,40
		bla_{TEM-15}	–	South Korea	28
		bla_{TEM-17}		South Korea	28
		bla_{TEM-19}	–	South Korea	28
		bla_{TEM-20}	–	South Korea	28
		bla_{TEM-52}	P	South Korea	28
		$bla_{CTX-M-1}$	P	China	40
		$bla_{CTX-M-2}$	P	Argentina, Turkey, South Korea, Israel	30,32,39
		$bla_{CTX-M-3}$	P	China, Turkey, Argentina, South Korea, India, Israel	30,39,40
		$bla_{CTX-M-14}$	P	China, Turkey, Argentina, South Korea, US, Japan	39,40,193
		$bla_{CTX-M-15}$	C, P	China, Spain, Iran, South Korea, India, US, Lebanon, Japan, Poland, New Zealand, France, Romania	26,31,33,34,40,194
		$bla_{CTX-M-22}$	P	China	51
		$bla_{CTX-M-24}$	P	China	40
		$bla_{CTX-M-27}$	P	China	40
		$bla_{CTX-M-28}$	–	China	36,51
		$bla_{CTX-M-39}$	–	Israel	30
		$bla_{CTX-M-55}$	P	China, South Korea	35,40
		$bla_{CTX-M-57}$	–	China	51
		$bla_{CTX-M-64}$	P	Japan	195
		$bla_{CTX-M-65}$	P	China	192
		$bla_{CTX-M-79}$	P	China	40
		$bla_{CTX-M-123}$	P	China	40

(*Continued*)

Table 1 (Continued).

Antimicrobial Class	Resistance Mechanism	Genes Mediating Antimicrobial Resistance	Origin	Geographic Origin	Reference
	Class B β-lactamases	*bla*$_{IMP-like}$	P	India, France	43,46
		bla$_{KPC}$	–	Senegal, France	46
		bla$_{IMP-3}$	P	Japan	44
		bla$_{VIM-like}$	–	India	43
	Class C β-lactamases	*bla*$_{CMY-2}$	C, P	China, Mexico, India, Iran, Taiwan, Costa Rica, Romania	48,51
		bla$_{CMY-59}$	C	Iran	31
		bla$_{DHA-1}$	C, I, P	China, India, Israel	30,51
	Class D β-lactamases	*bla*$_{oxa-1-like}$	I, P	Mozambique, Chile, China, India, US, Egypt, Djibouti, Spain, Greece, Denmark, Peru, Iran	105,193,196
		bla$_{oxa-2-like}$	I	Mozambique, Spain, Israel	30,105
		bla$_{oxa-5-like}$	–	Mozambique, Spain	105
		bla$_{oxa-30-like}$	I, P	Senegal, China, France, Japan, Spain, Brazil	51,91,120
Quinolones	Plasmid-borne resistance	*qnrA*	P	Iran	50,75
		qnrB	P	China, India, Iran	64,65,75
		qnrB4	P	Switzerland	61
		qnrB19	P	Switzerland	61
		qnrC	P	India	65
		qnrS	P	Iran, China, India, Pakistan	53,66,75,94
		qnrS1	P	Iran, China, Switzerland, US	48,61,64
		aac-(60)-Ib-cr	P	US, India, Japan, China, Iran	50,64–66
	Efflux pumps	*qepA*	P	China, Switzerland	61,64
Fosfomycin	Fosfomycin resistance enzymes	*fosA3*	P	China	38
Aminoglycosides: streptomycin		*strA*	MGE	India, Australia, Chile, Pakistan, South Korea	86,93,94,97,122
		strB	MGE	India, Chile, Pakistan, South Korea	86,94,97,122
	Adenyltransferase	*aadA1*	I, P	Senegal, Bhutan, India, Taiwan, Spain, China, Iran, France, Australia, Brazil, Pakistan, South Korea	83,86,88,91,94,97,98,120
		aadA2	I, P	Taiwan, Spain, China, South Korea, France, Australia, Korea	46,83,88,97,98
		aadA5	I, P	Taiwan, China, Iran	88,98

(*Continued*)

Table 1 (Continued).

Antimicrobial Class	Resistance Mechanism	Genes Mediating Antimicrobial Resistance	Origin	Geographic Origin	Reference
Tetracycline	Efflux pumps	*tetA*	C, P	Mozambique, Taiwan, Chile, Peru, Brazil, Iran, Spain, Pakistan, South Korea	11,88,94,97,104–106,120
		tetB	C, P	Mozambique, Taiwan, Peru, France, Brazil, Iran, Spain, Pakistan	11,46,88,94,104,106,120
		tetG	C, P	Mozambique, Spain	104,105
Trimethoprim	Dihydrofolate reductases	*dfrA1*	I, P	Spain, Taiwan, Senegal, Mozambique, India, Bhutan, China, Iran, South Korea, Peru, France, Chile, Australia	46,83,86,88,91,98,105,122
		dfrA5	I, P	Spain, Senegal, China	91,98
		dfrA7	I, P	Spain, Iran, France	46,106
		dfrA8	P	Mozambique, Chile	105,122
		dfrA12	I, P	Spain, Taiwan, Korea, Australia, South Korea	11,83,88,97
		dfrA13	P	South Korea	97
		dfrA14	P	Mozambique, Chile	105,122
		dfrA14-like	P	Mozambique	105
		dfrA15	I, P	Spain, Senegal	91
		dfrA16	P	Spain	91
		dfrA17	I, P	Taiwan, China, Iran, Brazil	88,98,106,120
		dfrV	I, P	China, India	98
Sulfonamides	Plasmid-borne resistance	*sul1*	I, P	Australia, South Korea, Taiwan	83,88,97
		Sul2	I, P	Taiwan, India, Peru, Chile, Bangladesh, South Korea	86,88,97,122,126
		Sul3	I, P	Taiwan	88
Phenicols	Chloramphenicol acetyltransferase genes	*CatA*-like	P	Taiwan, Mozambique, India, Peru, France, Brazil, Pakistan	46,86,88,94,120
		catP	P	Pakistan	94
	Efflux pumps	*cmlA1*	I, P	Taiwan, Mozambique	88,105
Colistin	Plasmid-borne resistance	*mcr-1*	P	China, Vietnam	112,116
Macrolide	Enzymatic inactivation	*mphA*	P	Palestine, Switzerland, Vietnam, China, Canada, UK, Peru	61,116,127,128,133
	rRNA methylase	*ermB*	P	Vietnam, Canada, UK	128,133

Abbreviations: P, plasmid; C, chromosome; I, integron; —, unknown; MGE, mobile genetic element.

Korea.[34] The overall structure of plasmid pSH4469 was nearly identical to pKHSB1, and it seemed that both were responsible for dissemination of $bla_{\text{CTX-M-15}}$ among *Shigella* isolates (Figure 1).

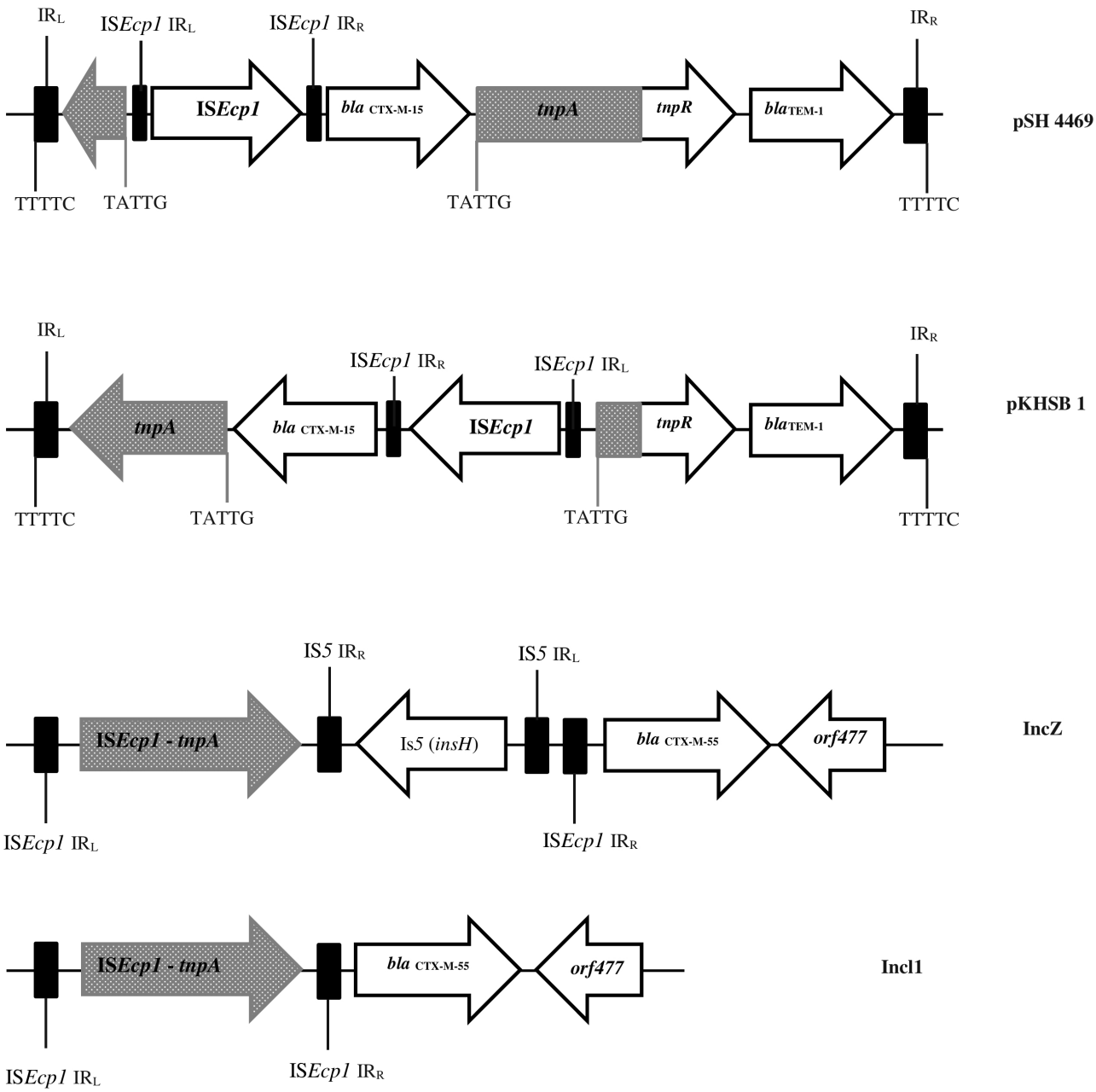

Figure 1 Schematic representation of $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}15}$, and $bla_{TEM\text{-}1}$ genes in different types of plasmid. Arrows indicate positions and directions of different genes and IRL, terminal inverted repeats at the left, IRR, terminal inverted repeats at the right.

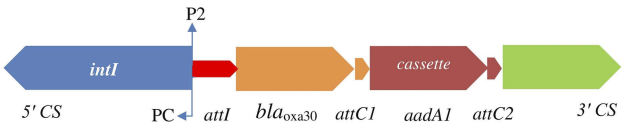

Figure 2 Physical map of *Shigella* atypical class 1 integron and locations of $bla_{OXA\text{-}30}$ and *aadA* genes.

Recently, a new hybrid of CTX-M-9 and CTX-M-1 β-lactamases named CTX-M-123 was identified among *S. flexneri* isolates from patients in China. In this study, $bla_{CTX\text{-}M\text{-}123}$ was carried by two conjugative plasmids named IncHI2 and IncF, and these conjugatable plasmids were responsible for dissemination of $bla_{CTX\text{-}M\text{-}123}$ in *S. flexneri* isolates.[38] Moreover, several new β-lactamase subtypes — 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 found in clinical *Shigella* strains isolated from different provinces in China.[36,39,40] ESBL genes among *Shigella* isolates might be transferred from *E. coli* isolates to *Shigella* spp., especially *S. sonnei* isolates, through conjugation in human

gut.[41,42] The increase in MDR and emergence of ESBL in *Shigella* spp. may be the cause of treatment failures and accordingly limitation in therapeutic options.[41]

Class B β-Lactamases

Class B β-lactamase enzyme can hydrolyze carbapenem and other β-lactams, except for aztreonam, and classical β-lactamase inhibitors, such as tazobactam and clavulanic acid, do not inhibit them. Metallo-β-Lactamase was first detected in a transferable plasmid from *Pseudomonas aeruginosa*, and also IMP-1 was first identified from many kinds of Gram-negative rods in Japan.[43,44] O'Hara et al reported a novel type of metallo-β-lactamase named MET-1 mediated by a *S. flexneri* plasmid. They believed that MET-1 was a derivative of IMP-1 β-lactamase.[44] This plasmid conferred resistance against sulfonamide and kanamycin, in addition to β-lactamase.[44] Lyobe et al found that MET-1 experienced two amino-acid changes from IMP-1. The gene was renamed IMP-3, and thus IMP-3 could be considered an ancestor of IMP-1 β-lactamase. They also showed that this gene was located on a cassette inserted in a class I integron, widely disseminated among other species of *Shigella*, and conferred resistance to almost all β-lactam antibiotics.[45]

Carbapenem resistance conferred by bla_{VIM} and bla_{IMP} genes has recently been detected in *S. sonnei* and *S. flexneri* isolates from pediatric patients with diarrhea in the Andaman and Nicobar islands in India.[43] In this study, after analysis of nucleotide sequencing of bla_{IMP} and bla_{VIM} genes by BLAST, 100% similarity with sequences of these genes isolated from *Acinetobacter baumannii* and *P. aeruginosa* available at the NCBI database was confirmed. After spread of carbapenem resistance to *Shigella* in another part of the world, an indication of a potential public health challenge, treatment options will be limited, and infection-control measures remain of high importance.[43] Finally, although class A β-lactamase KPC is one of the most commonly identified carbapenemases among other Gram-negative bacteria in some parts of the world, it has not yet been identified in *Shigella* isolates, except for a single bla_{KPC}-carrying *S. flexneri* strain isolated from the National Senegalese Enterobacteriaceae Center located at the Pasteur Institute in Dakar.[46]

Class C β-Lactamases

Ceftriaxone is recommended for treatment of ciprofloxacin-resistant *Shigella* isolates. However, today some strains of *Shigella* spp. have a resistance gene to cephalosporins. Class C β-lactamases, also known as AmpC-type enzymes, confer high-level resistance against cephalosporins. AmpC β-lactamase is encoded by both plasmid and chromosomal genes, and the first report of plasmid-encoded CMY-2-type AmpC β-lactamase was detected among ceftriaxone-resistant *S. sonnei* isolates obtained from an outbreak of bacillary dysentery in Taiwan.[47] CMY-2 enzymes have been reported in China, Taiwan, Costa Rica, Iran, and India from several epidemic strains.[47–50] Zhang et al found two AmpC β-lactamase producers with bla_{CMY-2} and bla_{DHA-1} in *Shigella* strains recovered from diarrhea patients in China.[51]

Tajbakhsh et al reported on the first AmpC β-lactamase (bla_{CMY-2}) producers in *S. sonnei* isolated from patients in Tehran, Iran.[48] In recent years, other studies conducted in Iran have indicated spread of resistance to extended-spectrum cephalosporins among *Shigella* isolates.[31,50] In a similar study carried out in Iran, studying cephalosporin-resistant *Shigella* isolates, the researchers identified gene *CMY-59* in one *S. sonnei* isolate from pediatric patients aged <12 years.[31] However, the majority of AmpC-positive isolates studied in other parts of the world belonging to the *CMY-2* genotype and other AmpC genes (bla_{MOX}, bla_{FOX}, $bla_{MIR(ACT-1)}$, bla_{CIT}, and bla_{ACC}) have been identified in *Shigella* isolates; however, so far there has not a study on them.[52,53] Indeed, few reports have described the presence of AmpC β-lactamases among *Shigella* isolates worldwide (see Table 1 for more details).[47,48]

Class D β-Lactamases

Class D β-lactamases or OXA-type β-lactamases confer resistance to ampicillin and cephalothin and can hydrolyze oxacillin and cloxacillin, as well as benzylpenicillin, but they are not inhibited by tazobactam or other inhibitors.[52,54] Initially, bla_{OXA} β-lactamases were reported among *P. aeruginosa* isolates, although now bla_{OXA} genes have been identified in integrons and plasmids in many Gram-negative bacteria.[55] In *Shigella* spp., resistance to ampicillin is mainly associated with an OXA-type β-lactamase.[54,56] bla_{OXA-30} was initially described in ampicillin-resistant *S. flexneri* strains from China in 2000.[57] Results of some studies have shown that *S. flexneri* isolates are a probable host specific for bla_{OXA}-type β-lactamase.[52,58] Another study from Iran showed that all bla_{OXA}-positive isolates carried bla_{OXA-1} and many of them were present in *S. flexneri*, suggesting an individual host preference of these enzymes in *S. flexneri* isolates.[50]

bla_{OXA-1} and bla_{OXA-30} genes, containing Tn2603 and Tn1409 transposons, respectively, differ from each other by having one mutation at codon 131. A gene encoding bla_{OXA}β-lactamases is carried on integron.[58] Furthermore,

other studies have shown that bla_{OXA-30} and *aadA1* are located in the gene cassettes of class 1 integrons (Figure 2). Therefore, class 1 integrons carry resistance traits for β-lactams (bla_{OXA}) and trimethoprim (*dfrA1*).[52]

Quinolone And Fluoroquinolone Resistance

Resistance To (Fluoro)quinolones Due To Chromosomal Target–Site Mutations

Corresponding subunits for DNA gyrase and topoisomerase IV are *gyrA*, *gyrB*, *parC*, and *parE*, encoded by genes *gyrA*, *gyrB*, *parC*, and *parE* genes, respectively. DNA gyrase consists of two *gyrA* subunits and two *gyrB* subunits, and topoisomerase IV contains two *parC* and two *parE* subunits. The most mutations have been found in a small region near the start of the *gyrA* gene termed a quinolone resistance–determining region (QRDR), between Ala67 and Gln107, and as reported in several studies (Table 2), most frequently mutations occur at codons 83, 87, and 211, while mutations in *gyrB* were detected with lower frequencies in different studies.[59,60] Some researchers believe that when a single mutation occurs in *gyrA*, it may confer resistance to quinolones, but for decreased susceptibility to fluoroquinolones, a number of further mutations in *parC* and *gyrA* regions are needed.[61,62] *parC* gene mutations most frequently occur at codons 80 among *Shigella* isolates. *gyrA* gene mutations have been confirmed to be much more prevalent than mutations in the *gyrB* gene.[63,64] Most nucleotide and amino-acid changes in QRDRs of *gyrA*, *gyrB*, *parC*, and *parE* among *Shigella* spp. are shown in Table 2. Novel mutations in QRDRs have also been identified from different regions of the world. Two novel mutations at codons 86 and 129 in *parC* and a mutation in codon 211 of *gyrA* were first reported in *S. sonnei* strains recovered from China in 2009.[65]

Finally, two novel mutations at codons 408 and 458 in *parE* have recently been discovered among *Shigella*spp., isolated in India in 2013 and Jiangsu Province in China in 2016.[10,63] Mutation in codon 458 is believed to result in resistance to ciprofloxacin and nalidixic acid, while a single isolate with a mutation at codon 408 in *parE* is related to resistance to nalidixic acid, but susceptible to ciprofloxacin. It seems that both the novel mutations in *parE* of *S. flexneri* isolates may be correlated with the increased MIC for ciprofloxacin and mediate fluoroquinolone resistance. Also, in neither study were *parE* mutations identified among quinolone-sensitive isolates.[10,63]

Data presented in Table 2 show that mutation patterns in *gyrA* and *parC* genes, particularly common mutations, are similar to those reported by other studies, reflecting a universal pattern among *Shigella* spp. A direct contribution to quinolone and fluoroquinolone resistance by each of these mutations in chromosomal target–site mutations (QRDRs) remains unknown. However, other mechanisms may be present in *Shigella* isolates, and further investigations are needed.

Resistance To (Fluoro)quinolones Due To Plasmid-Mediated Resistance Mechanisms

Distribution of plasmid genes called plasmid-mediated quinolone-resistance regions (PMQRs) namely *qnr* (*qnrA, qnrB, qnrC, qnrD, qnrS, qep, aac[6']-lb-cr*) genes is the main reason for resistance to quinolones among *Shigella* isolates, and they are usually associated with transposable or mobile elements on plasmids.[64,66] *qnr* genes, which are often incorporated into integrons, may allow for dissemination among *Shigella* and possibly other members of the Enterobacteriaceae family, and then quinolone-resistance isolates may spread across geographic regions and even across countries with population mobility.[65,67] The *aac(6')-Ib-cr* gene encodes an acetyltransferase associated with reduced quinolone activity, and is identified in many members of the Enterobacteriaceae family.[68] PMQRs have been identified widely among human and animal isolates, and have become a pressing issue worldwide. In a study conducted in China, fluoroquinolone-resistance rates in animal isolates of *S. flexneri* were reported to be higher than those in human strains.[68]

In the US, the *Shigella* resistance rate to fluoroquinolones reached 87% during 2014–2015.[69] Resistance of *Shigella* isolates to fluoroquinolone is mainly due to mutational alterations in QRDRs of DNA gyrase and topoisomerase IV genes, but PMQRs may facilitate in selection of isolates exhibiting higher levels of resistance through extrachromosomally encoded mechanisms and confer reduced susceptibility to quinolones (or fluoroquinolones).[68] *aac(6')-Ib-cr* and *qnrS* genes were first identified in isolates of *S. flexneri* 2a in 1998 and *S. flexneri* serotype 1a in 2002, respectively.[70,71] Furthermore, the *qnrS* gene was identified in isolates of *Shigella flexneri* 2b in 2005 in Japan.[70] Also, as reported in two previous studies, *aac(6')-Ib-cr* and *qnrS* were predominant PMQR determinants across two provinces in China, conferring high levels of fluoroquinolone resistance.[72,73] These studies indicated that *aac(6')-Ib-cr*–positive *Shigella* isolates have been present in China for many years.[70,72,73]

Table 2 Frequency Of Amino-Acid And Nucleotide Changes In The Quinolone Resistance–Determining Regions Of *Shigella*Isolates In Different Parts Of The World

Target Site Mutations	Codon	Amino-Acid Changes	Nucleotide Mutation	*Shigella* spp.	Country Of Detection	Reference(s)
gyrA	57	Asn→Lys	AAT→AAA	*S. flexneri*	China	197
	69	Gln→Trp	—	*S. sonnei*	India	65
	71	Phe→Ser	—	*S. sonnei*	India	65
	72	Ser→Pro	—	*S. sonnei*	India	65
	75	Met→Leu	—	*S. sonnei*	India	65
	80	His→Pro	CAT→CCT	*S. flexneri*	China	197
	80	His→ Gly	CAT→GGT	*S. dysenteriae*	Belgium	197
	83	Ser→Leu	TCG →TTG	*S. sonnei*, *S. flexneri*, *S. dysenteriae*, *S. boydii*	China, Bangladesh, Switzerland, Thailand, India	21,58,61,64
	87	Asp→Asn	GAC→AAC	*S. sonnei*, *S. flexneri*, *S. dysenteriae*	China, Bangladesh, Switzerland, India	21,52,64
	87	Asp→Gly	GAC→GGC	*S. sonnei*, *S. flexneri*, *S. dysenteriae*	China, Switzerland, Thailand, India	21,61,64
	87	Asp→Tyr	GAC→TAC	*S. sonnei*, *S. flexneri*	Switzerland	61
	90	Ser→Cys	—	*S. sonnei*	India	65
	94	Met→Leu	—	*S. sonnei*	India	65
	106	His→Pro	—	*S. sonnei*	India	65
	161	Asn→His	—	*S. sonnei*	India	65
	163	Thr→Ala	—	*S. sonnei*	India	65
	196	Val→Ala	—	*S. flexneri*, *S. dysenteriae*	India	21
	211	His→Tyr	CAC→TAC	*S. sonnei*, *S. flexneri*	China, Bangladesh	8,62,64
gyrB	517	Gln→Arg	CAG→CGA	*S. flexneri*	China	62,71
parC	64	Ala→Asp	GCC→GAC	*S. sonnei*, *S. flexneri,*	India, China	65
	64	Ala→Cys	GCC→TGC	*S. sonnei*	China	65
	80	Ser→Ile	AGC→ATC	*S. sonnei*, *S. flexneri*, *S. dysenteriae,*	China, Bangladesh, Switzerland, India	21,40,57,61,62,64
	81	Ala→Pho	—	*S. flexneri*	China	71
	83 85	Ser→Leu Ala→Thr	— GCG→ACG	*S. flexneri* *S. flexneri*	China China	30 71
	85	Ala→Ser	GCG→TCG	*S. boydii*	Switzerland	61
	86	Met→Trp	ATG→TGG	*S. sonnei*	China	64
	91	Gln→His	—	*S. flexneri*	China	71
	93	Phe→Val	—	*S. flexneri*, *S. dysenteriae*	India	21
	101	Asp→Glu	—	*S. flexneri*, *S. dysenteriae,*	India	21

(*Continued*)

Table 2 (Continued).

Target Site Mutations	Codon	Amino-Acid Changes	Nucleotide Mutation	*Shigella* spp.	Country Of Detection	Reference(s)
	110	Asp→Glu	—	*S. flexneri*, *S. dysenteriae*	India	21
	111	Asp→His	GAT→CAT	*S. flexneri*	China	62,64
	129	Ser→Pro	TCC→CCC	*S. sonnei*	China	62,64
parE	408	Gly→Asp	GGC→GAC	*S. flexneri*	China	62
	458	Ser→Leu	TCG→TTG	*S. sonnei*, *S. flexneri*, *S. dysenteriae*	China, India	62
	458	Ser→Ala	TCG→GCG	*S. sonnei*, *S. flexneri*, *S. dysenteriae*	India	62

Note: —, unknown.

S. flexneri serotypes 1a, 2a, 2b, 4c, and *S. sonnei* carrying the *qnrS* gene have been reported worldwide.[67]

Importantly, *qnrS*-positive isolates of *Shigella*, especially *S. flexneri* strains, show high-level resistance to fluoroquinolones, and many researchers from different part ofs the world suggest that the plasmid-mediated quinolone-resistance gene *qnrS* plays an essential role in reduced susceptibility of *Shigella* strains to fluoroquinolones.[67,72,73] Indeed, *qnrS* plasmid could change fluoroquinolone susceptibility of *S. flexneri* isolates containing both *gyrA*83 and *parC*80 mutations into ciprofloxacin-resistant isolates.[74] Overall, *aac(6')-Ib-cr* is the most prevalent gene, followed by *qnrS* detected in isolates of *S. flexneri* from the US, India, Japan, China, and Iran, and also most studies have highlighted an increased prevalence of PMQR determinants through the years.[50,70,75–77] A recent study conducted in China reported that *aac(6)-Ib-cr*–positive isolates and *qepA*-positive isolates expressed high levels of quinolone resistance. This finding indicates that other mechanisms, such as reduced outer-membrane permeability, active efflux pumps, and harboring of different resistance genes, may be responsible for resistance to quinolones.[18,77]

Fosfomycin Resistance

Fosfomycin (Fom) is a broad-spectrum antibiotic inhibiting bacterial cell-wall biogenesis by inactivating the MurA enzyme.[38,78] Despite the use of Fom in treatment of microbial infections for four decades, Fom has remained effective against common uropathogens, and Fom resistance has remained rare throughout the world.[79] However, Fom resistance was observed among *E. coli* strains to harbor novel transferable fosfomycin-resistance determinants named FosC2 and FosA3.[80] Two primary resistance mechanisms have been described for fosfomycin resistance: mutations in *uhp*A/T and *glpT* genes encoding proteins for two carrier-dependent systems responsible for fosfomycin uptake, and attainment of fosfomycin-modifying enzymes containing two kinases, FomA and FomB, and three types of metalloenzymes: FosX, FosA, and FosB.[81]

Fosfomycin-modifying enzymes were discovered for the first time among *S. flexneri* strains isolated from patients in China.[38] Some studies have suggested that increasing prevalence of *fosA3* was due to dissemination of IncN and IncI plasmids, facilitating its quick dispersal.[38,80] Indeed, ESBL ($bla_{CTX\text{-}M\text{-}123}$, $bla_{CTX\text{-}M\text{-}55}$, or $bla_{CTX\text{-}M\text{-}15}$) and *fosA3* genes were cocarried by transconjugant plasmids from diverse incompatibility groups, and all of them contained determinants encoding resistance to cefotaxime, ceftriaxone, and fosfomycin.[38,80] In this regard, conjugatable plasmids are likely to play an essential role in dissemination of *fosA3* and ESBL genes among *Shigella* isolates with high clonal diversity, and they should be closely monitored.[38]

Aminoglycoside Resistance

Aminoglycosides are used to treat a wide range of infections. Aminoglycosides mediate inhibition of protein synthesis.[82] Resistance to aminoglycosides is associated with enzymatic inactivation, ribosomal modification, and active efflux pumps. Among these mechanisms, aminoglycoside-modifying enzymes are the most common in the clinical setting.[83–85] These enzymes are activated through three general reactions, resulting in adenylation, acetylation, or phosphorylation. Aminoglycoside adenyltransferase (*aadA* gene cassettes) are very common in Enterobacteriaceae, especially among *Salmonella* and *Shigella* isolates, conferring resistance to streptomycin and spectinomycin.[84,86] Indeed, streptomycin resistance is

strongly associated with integrons because of the high prevalence of *aadA* gene cassettes within class 1 and 2 integrons. A typical class 2 integron has a gene cassette of 2.2 kb with a resistance-gene arrangement (*dfrA1–sat–*aadA1) conferring resistance to trimethoprim, streptothricin, and spectinomycin/streptomycin, respectively, while *aadA1* was absent in atypical class 2 integrons.[87,88] Class 2 integrons have been identified in transposon Tn7 and predominantly inserted into chromosomes with high frequency.[89] An atypical class 1 integron with an unusual 3′ conserved sequence carrying a *estX–psp–aadA2–cmlA-aadA1–qacH* cassette array has been detected among different Gram-negative species (*E. coli*, *Shigella*, and *Salmonella*) from different hosts (human, animal, and food), periods and geographical regions. Accordingly, horizontal transfer of these integrons by plasmids promotes spread of multiple-resistance genes in sporadic and outbreak isolates of *Shigella*.[89–91]

Many types of *aadA* gene cassettes have been identified among Enterobacteriaceae, but types *aadA1* and *aadA2* have high prevalence among *Shigella* isolates.[84,89,92] Aminoglycoside phosphotransferases encoded by *strA* and *strB* are the most common genes dispersed among *Shigella* isolates by plasmids, such as IncFII and pNV-Y394.[83,87,93,94] The gene encoding *strA* has been identified in 42.1% of *Shigella* isolates recovered from diarrheal patients in Pakistan.[95] In a study conducted in India, 100% and 88% of *S. dysenteriae* type 1 and *S. sonnei* strains harbored *strA* genes, encoding resistance to streptomycin.[96] The majority of *Shigella* isolates harbored *strA* and *strB*, along with unrelated resistance determinants, which are coded by bla_{TEM}, bla_{CTX-M}, *qnrS*, *aadA1*, *tet*(B), *catA*, and *catP*.[95,96] A 6.3 kb plasmid has been detected in the *S. flexneri* 3a strain and is involved in a streptomycin-resistance phenotype. This plasmid is likely to cause acquired resistance to streptomycin.[97] Also, a study conducted in South Korea showed that resistance to streptomycin was mediated by *strA* or *strB* among *S. sonnei* isolates obtained there and revealed that *tetA*, *strA–strB*, and *sul1* were encoded and present in 8.4 kb of untransferable R plasmid.[98]

Occurrence of aminoglycoside-resistance genes among *Shigella* isolates does not occur, because these drugs have long been excluded for treatment of shigellosis in different geographical areas.[85] However, there is still a concern, because class 1 integrons containing the gene-cassette array of bla_{OXA-30} + *aadA1* with complete 3′CS have been reported on plasmids in *Shigella* spp. isolates, *Salmonella enterica* serovar Typhimurium and *E. coli* strains, and transferable plasmids may enhance spread of resistance genes within integrin, establishing arole of plasmids in horizontal transfer of resistance genes.[99]

Tetracycline Resistance

Tetracyclines are used against a wide variety of diseases in humans and animals.[100] Tetracycline-resistant bacteria are found in opportunistic pathogens and normal flora species. According to the study by Roberts[101] and nomenclature for tetracycline resistance genes(https://faculty.washington.edu/marilynr), five tetracycline-efflux genes — *tet*(A), *tet*(B), *tet*(C), *tet*(D), and *tet*(G) — and one ribosomal protection protein encoded by *tet*(M) have been identified among *Shigella* isolates, most of which are encoded in transmittable elements, with extensive dissemination in different groups of bacteria. In a study, a 20.4 kb genomic island was identified encoding MDR genes, such as a wide variety of *tet* genes flanked by transposases.[102] This identical MDR cassette was first identified in *S. flexneri* serotype 2a strain YSH6000 and was referred to as *Shigella* resistance locus–pathogenicity island.[103] Interestingly, these MDR genes have recently been found in an *E. coli* plasmid, pRSB225, with a similar arrangement.[104] The IncB/O/K/Z-type plasmid, termed p866, carrying resistance genes *tet*(A) and tet(B) have been identified in *S. sonnei* strains.[105] These findings suggest that *tet* genes might be dispersed among other species by horizontal gene transfer.

Among 154 tetracycline-resistant isolates recovered as confirmed causes of traveler's diarrhea in Spain, 79.2% (n=122) harbored at least *tet*(A) or *tet*(B). Combinations of *tet*(A) + *tet*(B), *tet*(A) + *tet*(G), and *tet*(B) + *tet*(G) were found in five, one, and seven isolates, respectively.[106] Results of two studies revealed that *tet*(A) was more frequent among *S. sonnei* strains, whereas *tet*(B) was more frequent among *S. flexneri* strains.[106,107] Also, *S. sonnei* and *S. flexneri* differed from each other in terms of prevalence of plasmid Inc groups.[106] In 50 isolates of *Shigella* spp. identified from stool samples collected from children with diarrhea in Iran, 90% and 18% of isolates carried *tetA* and *tetB*, respectively, and no positive results were identified for *tet*(C) or *tet*(D) in this study.[11] Results of the same study conducted in Iran revealed that *tet*(A) and *tet*(B) were present in 75.7% and 21.42% of *Shigella* spp. and that *tet*(A) was more frequent in *S. flexneri* and *S. sonnei* populations.[108]

A study carried out in Mexico identified genes *tet*(A), *tet*(B), and *tet*(C) in 1, 6, and 18 *S. sonnei* isolates, and 2,

7, and 1 *S. flexneri* isolates, respectively, whereas *tet*(D) was observed only in *S. sonnei* isolates (8%).[109] Among 20 *S. dysenteriae* isolates from dysentery outbreaks obtained from different parts of India, *tet*(B) was more common (90%) than *tet*(A) (10%). In the same study, genes *tet*(A) and *tet*(B) were detected in 15% and 79% of *Shigella* isolates, respectively, samples of which were obtained from children with diarrhea in southern Mozambique.[107] Based on recent studies,[107,108,110] it seems that efflux-mediated tetracycline resistance to tetracycline in *S. sonnei* and *S. flexneri* strains may be related to expression of *tet*(A) and *tet*(B), respectively. According to tetracycline resistance–gene nomenclature (https://faculty.washington.edu/marilynr), *tet*(B) is able to confer resistance to minocycline, while other efflux pumps encoded in a transferable *tet* gene do not have such a property and may cause clonal dissemination of *tet*(B)-positive *Shigella* isolates worldwide. In general, *tet*(A) and *tet*(B) are the most prevalent tetracycline-resistance genes in *Shigella* spp. Other tetracycline-efflux genes, such as *tet*(C) or *tet*(D), are rarely detected alone.

Phenicol Resistance

Phenicols have used to treat *Shigella* infections during the past few years around the world, and application of them led to strong selective pressure for resistance to these antibiotics.[111] Resistance in *Shigella* is associated with enzymatic inactivation of unfluorinated phenicols by chloramphenicol acetyltransferase genes encoded by variants of *catA* (*catA1*, *catA2*, *catA3*) and *catB* (*catB2*, *catB3*, *catB7*, *catB8*), as well as active efflux by *cmlA* (*cmlA1*, *cmlA4*, *cmlA9*) and/or fluorinated and unfluorinated phenicols (*floR*) by major facilitator–superfamily proteins.[112]

Chloramphenicol resistance in *Shigella* isolates is mainly associated with the presence of *cat* genes. Among 95 *Shigella* isolates collected from diarrheal patients in Pakistan, at least 69 (72.6%) were resistant to chloramphenicol. *catA* and *catP* were detected in 32 (33.68%) and 24 (25.26%) isolates, respectively, from chloramphenicol-resistant *Shigella* isolates.[95] Among 103 *S. sonnei* isolates associated with several waterborne outbreaks in Taiwan, in 84% of sporadic isolates, *tetB* and *catA* genes were transferred along with 130 kb plasmids.[89] The presence of *catA* encoding chloramphenicol *O*-acetyltransferase was detected and confirmed in *S. flexneri* strains with *strA* or *aadA1* genes or both.[96]

Colistin Resistance

Colistin (polymyxin E) is a polypeptide antimicrobial agent interacting with outer membranes of Gram-negative bacteria. Since the first report of the plasmid-mediated polymyxin-resistance gene *mcr-1* was published in an *E. coli* isolate in November 2016 in China,[113] this gene has also been identified in *Salmonella enterica* and *Klebsiella pneumoniae*, but is rarely reported in other Enterobacteriaceae members here.[114] This gene has now been identified in other Enterobacteriaceae genera, such as *Shigella*, *Cronobacter*, *Kluyvera*, and *Enterobacter* isolated from vegetables, the environment, food, animals, and human beings.[115,116] The mechanism of resistance of *mcr-1* is produced by a phosphatidylethanolamine transferase, leading to modification of lipid A present as a result of addition of phosphoethanolamine to lipid A in cell membranes of Gram-negative bacteria, resulting in more cationic LPSs and lower affinity for colistin and related polymyxins and consequently reduced antimicrobial activity.[117] The presence of *mcr-1* in *Shigella* isolates leads to a four- to eightfold increase in the MIC of polymyxin B.[118] *mcr-1* is located on various plasmid backbones, including IncI2, IncFI, IncHI2, IncFIB, IncP, IncY, and IncX4, sized 58–252 kb (Figure 3).[114] ESBL-encoding genes or other resistance genes might coexist with it. Also, plasmid-mediated colistin-resistant *S. flexneri* isolates recovered from animal feces on a farm showed that it might be circulated via the fecal–oral route, at least between animals on that farm, and possibly distributed via the food-production network.[118] In most reports, *mcr-1* is known to be the only resistance gene for related plasmids, indicating that selective pressure associated with polymyxin is responsible for *mcr-1* acquisition.[114,118] It means that other plasmids conferring MDR phenotypes can be achieved from resistant *S. flexneri* strains. Mobile elements, such as IS and integrons, could also help isolates acquiring other resistance elements from the environment.[118] A novel transposon, Tn*6390* has been detected in *S. flexneri* C960, in which two copies of ISApl1 bracketed to the *mcr-1* gene play a pivotal role in transposition of *mcr-1*.[118] Recently, *mcr-1* was identified in *S. sonnei* isolates from Shanghai (2010–2012) with polymyxin B resistance (MIC 4–8 μg/mL).[118]

Sulfonamide And Trimethoprim Resistance

After the spread of trimethoprim–sulfonamide resistance in different parts of the world, these agents are currently considered ineffective for empirical therapy of shigellosis.[119] Acquired resistance mechanisms have frequently been

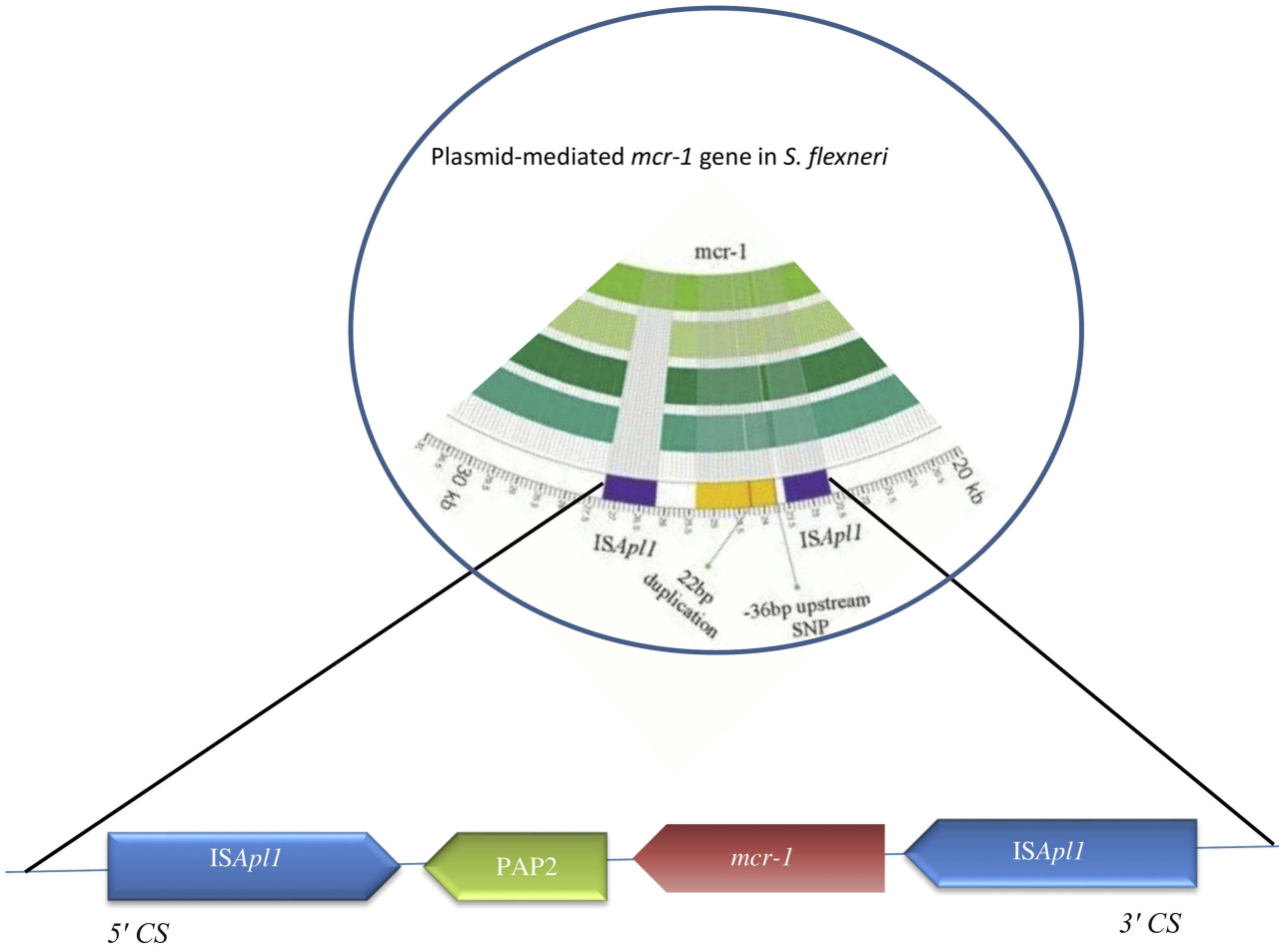

Figure 3 Structure of genes surrounding *mcr-1* in *S. flexneri.*

identified, mostly due to mutational or recombinational changes in target enzymes (dihydropteroate synthase and dihydrofolate reductase, respectively) or acquired resistance by drug-resistant target enzymes, such as acquired *sul* genes coding for drug-resistant dihydropteroate synthases or *dfr* genes coding for drug-resistant dihydrofolate reductases.[120,121]

Resistance To Trimethoprim

At least 42 *dfr* genes conferring trimethoprim resistance have been detected in different groups of bacteria worldwide, 12 of which have been identified in *Shigella* spp. Resistance to trimethoprim might be explained by the presence of integron-borne *dfr* genes (Figure 4). Gene cassettes within class 1 integrons detected on plasmids or chromosomes in *Shigella* isolates often encode resistance to trimethoprim (*dfrA*), streptomycin (*aadA*), and ampicillin (*oxa-1*).[58,85] Class 2 integrons borne on Tn7 have often been found in *Shigella* spp., and gene-cassette arrays of them usually contain *dfrA1*, *sat1*, and *aadA1*.[122]

The presence of *dfrA1* genes among *Shigella* isolates is the main mechanism of trimethoprim resistance, occurring in a cassette in both class 1 and class 2 integrons (Figure 4). Two types of class 2 integrons among *S. sonnei* strains have been identified in Japan. One of them was typical type of class 2 integrons (2,158 bp) with *dfrA1*, *sat1*, and *aadA1* cassette arrays, and the other was an atypical type of class 2 integrons (1,313 bp) carried only two gene-cassette arrays with *dfrA1* and *sat1*.[123] This integron-associated antibiotic resistance can be transferred to other species via plasmid conjugation. *dfr12–orfF–aadA2, dfr17–aadA5*, and *aadA1* cassette arrays carried by class 1 integrons have been recognized in *S. sonnei* isolates recovered from South Korea, China, Vietnam, and Australia.[84,85,99] Also, *dfrA1–sat1–aadA1–orfX*, free *aadA1*, or *free orfX* cassette arrays carried by class 2 integrons have been detected in Senegalese *Shigella* spp. isolates.[92] In contrast to *dfrA* genes, *dfrB* genes have not been identified among *Shigella* isolates. Recently, trimethoprim-resistance clones were sequenced

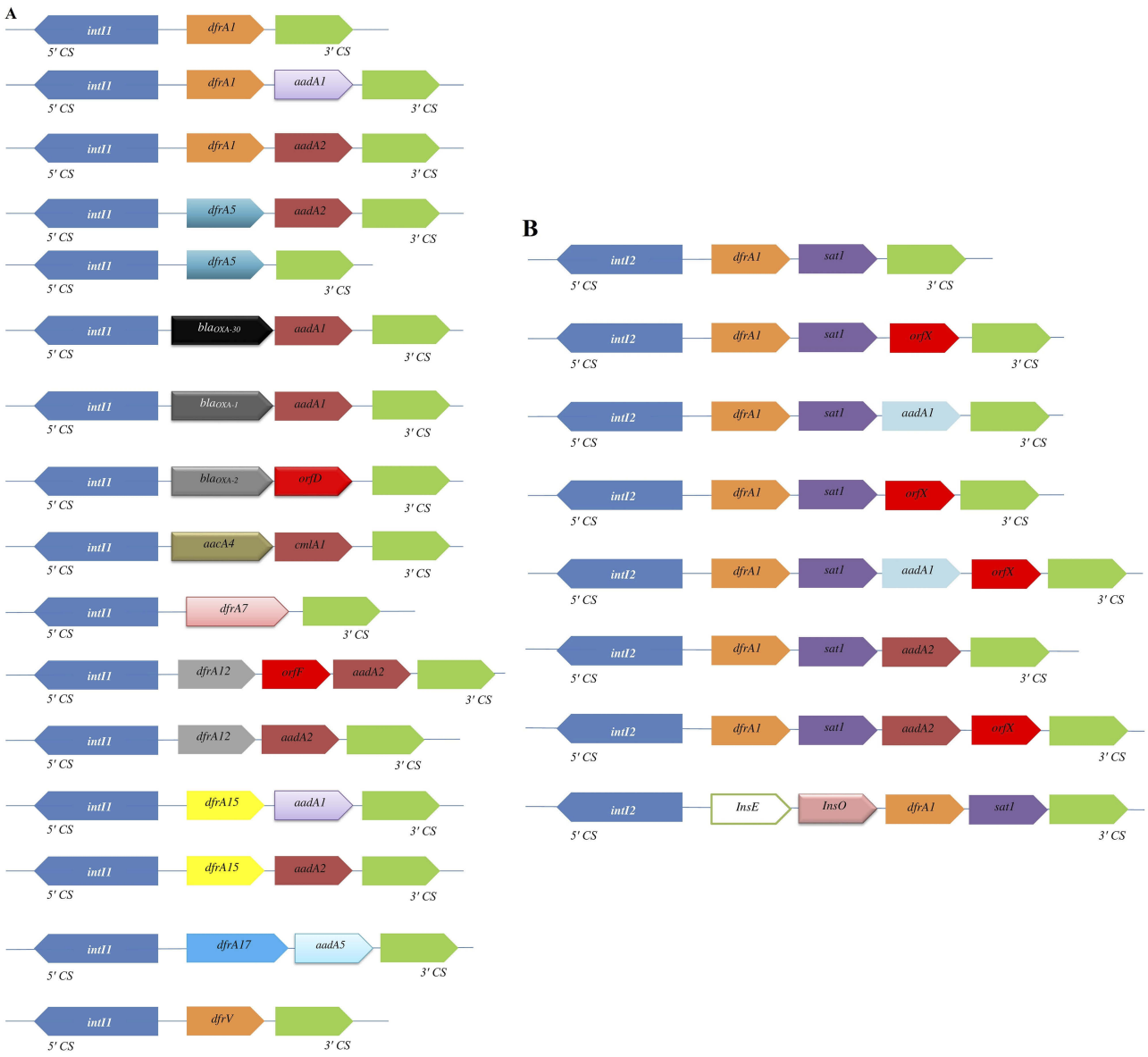

Figure 4 Variable regions of class 1 (**A**) and class 2 (**B**) integrons reported in different geographic area. Horizontal arrows indicate transcriptional orientation of genes.

by primer walking, and a native 6,779 bp plasmid was identified with presence of the *dfrA14* gene in a *sul2–strA'–dfrA14–'strA–strB* gene arrangement in *S. sonnei* strains, suggesting that *dfrA14* was associated with a small nonconjugative plasmid.[124] Class 2 integrons within a *dfr1–sat2–aadA1* cassette array were predominant in *S. sonnei* isolates from outbreaks cases in Taiwan, while class 2 integrons were absent in sporadic cases.[89]

A large study conducted in South Korea analyzed 122 *S. sonnei* isolates collected from stool samples in different parts of the country from 1991 to 2000. Resistance to trimethoprim was associated with *dfrA1* and *dfrA12*. *dfrA1* was found as a gene cassette of Tn7 located in chromosomes, while *dfrA12* was located in conjugative R plasmids as a gene cassette of class 1 integrons. Tn7 was not detected in *S. sonnei* isolates recovered from the 1980s, while in this study Tn7 was found in all *S. sonnei* isolates. The authors proposed that *S. sonnei* isolates carrying Tn7 were responsible for outbreaks of shigellosis in different parts of South Korea in the 2000s.[98]

Resistance To Sulfonamides

Since the first report of resistance to sulfonamides was found in both *S. sonnei* and *S. flexneri* isolates recovered from the early 1970s in South Korea,[98,125] resistance to this antibiotic has been identified in 94% of *S. sonnei* strains

from the 1980s and 100% of isolates from the 2000s and 2010s in different parts of the world.[2,50,118] Sulfonamide resistance is mediated by *sul1*, *sul2*, and *sul3* genes, and they are common in *Shigella*.[89] The *sul1* gene is highly frequent among *Shigella* isolates, because it is part of the 3′-conserved sequence region of class 1 integrons. Also, *sul3*, linked to an unusual 3′-conserved segment, is associated with class 1 integrons.[89,126] According to one study, *sul2* is one of the three sulfonamide-resistance genes, and is usually located on large transmissible plasmids or small non-conjugative plasmids and was first detected on a small nonconjugative plasmid of *E. coli*.[90] Since then, this gene has been found mostly on plasmids in *Shigella* isolates from humans in South Korea,[98] Taiwan,[89] Australia,[127] and Bangladesh.[128]

Several studies have suggested that *sul* genes are linked to other resistance genes. The *sul1* gene is often identified together with other antimicrobial-resistance genes located on gene-cassette arrays in variable regions of class 1 integrons. Class 1 integrons differ from class 2 in their excise gene cassettes, capacity to integrate, and presence of *sul1* in 3′ conserved region (3′CS). In Brazil, *sul1* (sulfonamide-resistant) was identified in two (3%) MDR *Shigella* samples, which were also positive for class 1 integrons.[122] In previous studies on MDR *S. sonnei* isolates obtained in South Korea, resistance to sulfamethoxazole was mainly associated with *sul1*, located in 8.4 kb of nonconjugative R plasmid.[98]

Antibiotic-resistance gene clusters containing *strA*, *strB*, and *sul2* are widespread among Gram-negative bacteria, particularly in *Shigella* isolates.[124] Iqbal et al[128] found *sul1*, *sul2*, *sul3*, integron 1, and integron 2 genes in all MDR *S. flexneri* 2a strains, and also found that *sul2* was absent in all sulfamethoxazole-sensitive strains (n=54), while it was present in all sulfamethoxazole-resistant strains (n=146). However, in this study, no change was observed in expressions of *sul1*, *sul3*, integron 1, or integron 2 genes in sulfamethoxazole-resistant and -sensitive *S. flexneri* 2a strains. Interestingly, curing of this 4.3 MDa plasmid resulted in loss of *sul2* and susceptibility to sulfamethoxazole in paired strains, suggesting involvement of *sul2* and this plasmid in resistance to sulfamethoxazole. In the same study, 24 *S. sonnei* isolates were detected to carry an atypical class 1 integron associated with *sul3*. The *estX–psp–aadA2–cmlA1–aadA1–qacH* cassette array of *sul3*-associated class 1 integron has been found to encode an esterase, a lipase, putative phosphoserine phosphatase/resistance to streptomycin, chloramphenicol, and quaternary amines.[23]

Macrolide Resistance

The American Academy of Pediatrics and the Infectious Diseases Society of America have recommended azithromycin as a medication for treatment of shigellosis in children, and also the WHO introduced it as second-line treatment in adults.[129] The Centers for Disease Control and Prevention has observed resistance to azithromycin in approximately 3% of *Shigella* cases tested. Resistant outbreaks involving *Shigella* spp. isolates with reduced susceptibility to azithromycin (RSA) are more recent phenomena and continually detected in Asia, North America, the US, Australia[130,131] and other geographic regions.[132–134] According to updated Clinical and Laboratory Standards Institute guidelines, if MICmeasured by broth microdilution is ≤16 and ≤8 μg/mL, then epidemiological cutoff values denote susceptibility for *S. sonnei* and *S. flexneri* wild-type, and if MIC is ≥32 and ≥16 μg/mL, then susceptibility is confirmed for non–wild-type, respectively.[135] Recently, several reports have suggested that resistance to azithromycin in *Shigella* spp. isolates is associated with presence of *mphA* or *ermB* plasmid-mediated genes or by both genes.[133,136] Macrolide resistance is mediated by four main mechanisms: enzymatic inactivation by phosphotransferases encoded by *mph* genes or esterases encoded by *ere* determinants; target-site modification by an rRNA methylase encoded by *erm* genes; punctual mutations in *rplV* encoding L22 ribosomal protein, *rplD* encoding L4 ribosomal protein, and *rrlH* (23S rRNA); and drug-resistance mediated by efflux pumps, such as OmpA, OmpW, mefA, and msrA.[137,138] All macrolide-resistance mechanisms can mediate resistance to azithromycin and erythromycin. The *mph*(A) gene was first identified in an *E. coli* isolate from Japan.[139] Since then, this gene has been recognized among *Pseudomonas* spp., *Aeromonas* spp., *Stenotrophomonas* spp., *Shigella* spp., and other enteropathogens.[137] Dissemination and acquisition of macrolide *mphA* resistance mechanisms in *Shigella* spp. has been shown to be mainly due to spread of plasmids from *E. coli*.[140] All the discovered *mphA*-associated plasmids have been identified in *E. coli* isolates, indicating their role as a repository from which antimicrobial resistance to *Shigella* spp. may appear.[135] This phenomenon has been previously been described in *E. coli* donating *mphA* to *S. sonnei*.[141]

Additionally, *Shigella* strains with RSA have been found mostly in strains recovered from men who have sex with men (MSM; 68.8% or higher) from the Montreal region. In this study, complete sequence analysis of six selected plasmids from different serotypes of *S.*

flexneri and *S. sonnei* emphasized the role of IS*26* in dispersal of RSA.[130] Also, in a study conducted in Taiwan, a series of clonally related azithromycin insusceptible *Shigella* spp. isolates was reported in relation to MSM.[136] Various gene-transfer systems (mobile genetic element acquisitions) are involved in acquiring antibiotic-resistance genes, such as transposons, integrons, and conjugative plasmids. The IS*26*–*mphA*–*mrx*–*mphR* (A)–IS*6100* gene cassette has been characterized in a clinical strain of *S. boydii* carrying the p2246–CTXM plasmid. Insertion sequences of IS*6100* and IS*26* have been found in the neighborhood of *mphA* in an *S. sonnei* strain recovered from France. Indeed, in addition to plasmid mobilization, dissemination, and acquisition of RSA among *Shigella* spp. and serotypes has also potentially occurred through IS*26* mobilization.[130,137]

IncFI and IncFII plasmids have been shown to carry the azithromycin-resistance gene *erm*.[135] The IncFI plasmid containing an IS*CR3* insertion sequence surrounds both *ermC* and *ermB* genes and carries a $bla_{\text{CTX-M-24}}$ gene downstream of an IS*Ecp1* element. The IncFII plasmid carries *ermB* and *ermC* genes downstream of an IS*6* transposase. Both plasmids share significant DNA homology with other previously sequenced plasmids found in *S. sonnei* and *S. flexneri* serotype 3a isolates associated with development of the disease in MSM.[135]

Recently, a plasmid carrying azithromycin-resistance genes, namely pKSR100 (conjugative R-plasmid) in *S. flexneri* serotype 3a has been described to be involved in intercontinental spread of RSA among MSM-associated outbreak lineage.[142] pKSR100-like plasmids have been found to be predominantly related to MSM-associated outbreak lineage in Australia and elsewhere.[131] There are a considerable number of studies on the prevalence of RSA among *Shigella* isolates throughout the world, particularly in MSM, and demonstrated global dissemination of a multi-resistant plasmid, highly associated with MSM, which is present across different continents.[131,142]

Biofilm Formation–Mediated Resistance

Recently, much attention has been given to biofilm formation in bacteria, because microbial cells grown in biofilms are less sensitive to antimicrobial agents and more resistant to environmental stress such as dehydration and oxidation. Microbial infections caused by biofilm-associated *Shigella* spp. are global health challenges.[143] Biofilm formation is regulated by a multifactorial process, with cellular adherence, exopolysaccharide secretion, and numerous gene regulations controlling detachment of bacteria from mature biofilm, and is mainly related to quorum sensing and social networking in the microbial world.[144,145] Ellafi et al investigated biofilm formation by *Shigella* strains grown in different NaCl concentrations, and they showed that all the isolates produced biofilm. According to their study, biofilm formation is a protective system under different environmental stress conditions.[146] Recent studies have demonstrated that bile salts increase the capacity of *S. flexneri* strains to adhere to and penetrate epithelial cells.[147,148] Indeed, extended exposure of *Shigella* to bile salts occurred in cases of increased biofilm formation, and thus it is an important resistance mechanism for *Shigella* sp. Similar biofilm phenotypes have been observed for *Campylobacter, Listeria*, and *Vibrio*, demonstrating that bile salt–induced biofilm production is conserved among members of the Enterobacteriaceae family.[19,148] Also, biofilm formation has been shown to require the presence of glucose, whereas biofilm diffusion requires the elimination of bile salts from the medium.[19] Bacteria in the form of biofilm can be 100,000 times more resistant to antimicrobial agents than planktonic forms of bacteria in the same species.[143] During biofilm formation, the effect of *shf, mdoH, VpsT*, and *LuxR*-like genes and OpgH protein expression has been confirmed among enteric bacteria, as well as *Shigella*.[143,145] Another study described biofilm-formation potentials and pathological behaviors of various mutants *S. flexneri* strains with an incomplete inner core of LPS containing only Kdo moieties.

Interestingly, 1rfaC (also called waaC) mutant, with an incomplete inner core of LPS due to deficiency in Hep biosynthesis, shows strong biofilm-formation ability and considerably high invasiveness and adhesiveness to human epithelial cells compared LPS-mutant strains. However, this strategy is successful in conferring high-level resistance only in bacterial species with a deficiency in Hep synthesis of LPS.[149] The relationship between biofilm formation and pathogenicity, as well as virulence factors and antimicrobial properties, has not been thoroughly studied in *Shigella* spp., and further studies are needed.

Therapeutics

Treatment for *Shigella* infections is recommended to prevent spread of infection to others and to shorten disease duration. According to current WHO guidelines and a systematic review, the use of fluoroquinolones (first-line,

preferably ciprofloxacin), cephalosporins (second-line), and β-lactams (second-line) for 7–10 days is recommended for treatment of shigellosis.[4,111] In regions known to have high rates of resistance to ciprofloxacin, azithromycin may be considered appropriate second-line therapy. Cefixime is also a good alternative, although its use should be balanced with respect to risk of developing antimicrobial resistance and spread of ESBL.[111] Conventional antibiotic therapeutics against shigellosis have become increasingly inefficient, due to the increase in number of MDR strains. However, no well-designed in vitro or in vivo combinations of antimicrobial agents have been performed to evaluate different antibiotic-class regimens for treating infections caused by *Shigella*. There has only been a related study on an antimicrobial-sensitivity case series reporting resistance in different regions and treatment outcomes for infections caused by *Shigella*.[4,111] In general, it has been observed that there is a decrease in susceptibility to first- and second-line agents. As such, there is an urgent need for development of novel therapeutic strategies for treatment of MDR *Shigella* infections.[111]

Alternative Therapeutics

Natural And Organic Products

Natural products are small molecules produced naturally by microbial agents, plants, and animals that have been demonstrated to be useful in treating *Shigella* infections. Biotherapeutic agents (preferably probiotics) have been suggested in prevention of antibiotic-induced diarrhea, and are also an alternative therapeutic choice for treatment of gastroenteritis infectious.[150] Bacteria and yeast are the most frequent microorganisms used as probiotics. Several mechanisms have been suggested for antimicrobial activity of bacteria toward enteric bacterial pathogens, including production of undissociated organic acids, organic acid molecules, and bacteriocin, competition for adhesion sites, and coaggregation with pathogens.[151]

Pretreatment of cells with bacterial components and products obtained from *Lactobacillus rhamnosus* and *L. acidophilus* results in interference with *Shigella* adherence and internalization into host cells and leads to an absence of IL8 expression, substantiating attenuation of inflammatory response during aggregate pretreatment. Lactobacilli have been shown to regulate cytokine production and stimulate the immune system.[152] Time-kill methodology has shown that viability of *S. sonnei* decreased after contact with cell-free culture supernatants of lactobacilli, which could be potentially used as probiotic strains in food industry.[151] Zhang et al selected a total of 91 lactobacilli for antimicrobial activity against *Shigella* isolates, among which 16 lactobacilli displayed potent antibacterial activity against *S. sonnei* strains. The nature of these antimicrobial agents was studied and found to be dependent on production of organic acids.[153] Also, other studies have indicated that lactobacilli and lactic and acetic acid bacteria possess high activity against MDR *Shigella* pathogenic strains, and that they can be the best candidate for probiotics.[152,154,155] *Saccharomyces boulardii* is a thermophilic and nonpathogenic yeast showing antagonistic activity against several bacterial pathogens, such as enterohemorrhagic and enteropathogenic *E. coli, Vibrio cholera, Salmonella typhimurium, and S. flexneri*.[156]

In one study, the effect of aqueous ethanol extract of *Euphorbia prostrata* was investigated in vitro and in vivo on bacterial growth of *S. dysenteriae* type 1 and found to be effective against *Shigella* isolates, with MIC and minimal bactericidal concentration of 3,500–12,000 μg/mL.[157] The preventive role of orally administered *Aloe barbadensis* Miller (*Aloe vera*)–supplemented probiotic lassi (APL) was determined for *S. dysenteriae* infection in mice, with a significant ($P<0.05$) decrease found in *Shigella* counts (log CFU/mL) and immunoprotective effects of APL against *S. dysenteriae*.[158] Antivirulence activity of a boiling black tea (*Camellia sinensis*) extract was shown to reduce expression of virulence traits by *S. dysenteriae*, as shown by decreased bacterium-survival strategies, and also an enhancement was found in innate immunoresponse against *Shigella* isolates.[159] Antishigellosis activity of *Picralima nitida* Stapf (Apocynaceae) extract has been found to be effective against *S. dysenteriae* type I strains, and MIC and minimal bactericidal concentration were 800 and 6,400 μg/mL, respectively.[160] In vitro antibacterial activity of methyl gallate isolated from *Terminalia chebula* has been shown to cause total disintegration of outer and inner membranes and leakage of cytoplasmic contents of MDR *S. dysenteriae*. Viable intracellular *S. dysenteriae* reduced in a time-dependent manner in the presence of methyl gallate and decreased to zero within 20 hours.[161] In another study, antimicrobial activity of thyme oil and ciprofloxacin and their synergistic effects were evaluated, and the combination displayed differing degrees of effects on microbial cell formation based on results obtained from scanning electron microscopy and transmission electron microscopy. In vitro and in vivo synergy between them showed maximum growth inhibition in *S. flexneri*.

Bacterial loads in infected colons reduced as a result of treatment with thyme oil, while the conventional drug failed to heal colon ulcers. Also, it decreased penetration of lamina propria by inflammatory cells.[162]

Antishigellosis activity of a *Crinum jagus* water–ethanol extract was found to be effective against *S. flexneri*, with inhibition of diameter by 18.90 (0.39 mg/mL) and 25.36 (200 mg/mL) mm, respectively. Indeed, *Crinum jagus* extract drastically decreased ($P<0.01$) diarrheal stool emission and microbial load and also reduced IFNγ, IL2, IgM, IgA, and motilin blood levels in *S. flexneri*–induced diarrheic rats.[163] An in vitro study on bovine lactoferrin recognized it as a coadministered adjuvant therapeutic in antibiotic therapy against *Shigella* isolates. Some strains of *Shigella* show a twofold or more decrease in their ampicillin MIC values in the presence of bovine lactoferrin.[164] In vitro data showed that antibacterial activity of gallic acid inhibited the effect on biofilm formation and reduced the number of viable *S. flexneri* strains. Indeed, gallic acid inhibited biofilm formation in *S. flexneri* by regulating expression of the *mdoH* gene. *mdoH* is essential for glucosyltransferase activity and osmoregulated periplasmic glucans synthesis, as they both contribute to biofilm formation and develop antibiotic resistance in pathogenesis. Inhibition of *mdoH* can help in treatment of *S. flexneri* biofilm.[143] Organic acids, such as citric, acetic, lactic, and malic acid, are natural substances categorized as "generally recognized as safe" according to the US Food and Drug Administration. They have antimicrobial activity and are widely used to inactivate bacteria. Results of a study showed that organic acids, carvacrol, and their combination were useful against *S. sonnei*. However, *S. sonnei* was shown to decrease to 4.53 and 3.25 log CFU/mL using 0.5% w:v malic, lactic acid, respectively, indicating the synergistic effect of combination therapy.[165] A previous study reported the effects of dithiocarbamate transition-metal complexes on survival and recovery of pathogenic bacteria, and they are very attractive and novel pharmaceutical targets for control and management of antibiotic-resistant bacteria, as well as *Shigella* isolates.[166]

Novel Therapeutic Strategies For Shigella Treatment

Nanoparticles

Nanoparticles (NPs) have gained growing importance in recent years and shown broad-spectrum antibacterial activity against pathogenic bacteria, due to their bactericidal characteristics.[167] NPs usually destroy bacterial targets with a damaging effect on membrane load cells and their integrity, along with generation of free oxygen radicals. Commonly, they can be delivered efficiently as antimicrobial agents. Recently, copper oxide NPs have been recognized as an antimicrobial agent for treatment of *Shigella*. MIC and minimum bactericidal concentration of copper oxide NPs were 2,500 μg/mL and ≤5,000 IU/mL, respectively, in treatment of *S. sonnei* using 33 nm NPs. The study also showed that smaller copper oxide NPs had stronger antibacterial effects than larger NPs at a specific time and concentration.[168] Iron is a biocompatible element, and can be directly used for treatment of many types of microbial pathogens. In one study, antimicrobial properties of Fe_2O_3 and Ag–Fe_2O_3 NPs against *S. dysenteriae* strains was evaluated, and both Fe_2O_3 and Ag–Fe_2O_3 NPs were shown to have antimicrobial effects, with antimicrobial activity of Ag–Fe_2O_3 NPs much more than that of Fe_2O_3 NPs alone.[169] The bactericidal effect of iron oxide NPs has been determined, with values of 50–100 μg/mL against Gram-positive and Gram-negative bacteria, as well as *S. dysenteriae* and *E. coli*.[167] The use of nanoantibiotic formulations is another strategy for treatment of drug-resistant *Shigella*. Mukherjee et al reported on the synthesis of a nanosized form of tetracycline by loading it in calcium phosphate NPs and showed that this treatment significantly decreased incidence of colon-length shortening, mushy-stool excretion, weight loss, and microbial colonization in gastrointestinal tracts of *Shigella*-infected mice. Immunohistological research has shown that as a result of tetracycline–calcium phosphate NP treatment, changes in morphology and level of inflammatory cytokines IL1β, IFNγ, and TNFα in intestinal tissue of mice caused by shigellosis were reverted to almost normal characteristics.[170] Silver NPs (AgNPs) are characterized by their broad-spectrum bactericidal toxic effects against broad-spectrum bacterial pathogens. Omara et al tested AgNPs against pathogenic *Salmonella* and *Shigella* strains recovered from layer-poultry farms. AgNPs at a concentration of 16 μg/mL were found to have both bacteriostatic and bactericidal effects against *Salmonella* and *Shigella* isolates.[171] Although NPs have shown high antibacterial activity during in -vitro and in -vivo experiments, future studies and judiciously performed clinical trials are required to achieve a better understanding of their potential side effects and clear regulatory guidelines.

Phage Therapy

Bacteriophages (phages) are the most abundant organisms killing bacteria a through lysis mechanism. They can be recovered from various environments. Phage therapy has earned increasing attention due to several advantages, including high specificity to target bacteria without effects on normal microflora of the human body, replication at infection site, bactericidal activity against antibiotic-resistant bacteria, and fewer side effects than other therapies. Phages are self-limiting, because phages remain at a shallow level on target sites after killing bacterial targets.[172] Phage therapy of shigellosis was discovered by a French microbiologist named d'Herelle, who described efficacy of phages in curing symptoms of dysentery.[173] Use of phages for treatment of MDR *S. dysenteriae* recovered from wastewater has been investigated as an alternative to antibiotics.[172] Another study showed that a virulent phage named pSb1 was able to infect all the *S. boydii* strains and had productive lytic activity against them. Also, results indicated that pSb1 might be a member of an N4-like phage group and might have potential applications as an alternative option for treatment of shigellosis.[174] Regular targeting of only a subgroup of strains within one bacterial species or closely related species without causing distortions in the gut microbiota is one of the benefits of phage treatment over antibiotic therapy in treatment of shigellosis.[175] Numerous animal have studies demonstrated that phages are able to survive in experimental animals with dysentery. Despite no reports on significant undesirable reactions during the long history of phage therapy in humans,[176] phage treatments still need to overcome admission constraints in the main medical repertoire.

Vaccine Strategies

Varieties of candidate vaccines have been developed to prevent infection by *Shigella* spp., most of which are currently under evaluation for safety and immunogenicity. However, there is no licensed vaccine available against this pathogen. At present, studies in humans and animals have shown that protection by vaccination is possible. Potential candidates for *Shigella* vaccines include glycoconjugate vaccines, such as recombinant glycoconjugate, synthetic glycoconjugate, *O*-polysaccharide covalently linked to immunogenic carrier proteins,[177,178] virG-based live attenuated (WRSS1, WRSs3, WRSf3, WRSf2G12, WRSf2G15and WRSd1),[179,180] recombinant outer-membrane proteins,[181] live attenuated vaccines,[182,183] invasion-plasmid antigens B, C, and D,[184] DNA-based vaccines, Ty21a typhoid vaccine expressing *Shigella* LPS,[185] recombinant probiotic–based candidates,[186] and whole-cell-killed and *Shigella* trivalent inactivated whole-cell and heat-killed multiserotype *Shigella*,[187,188] as well as novel antigen candidates, such as triacylated S-LPS, subcellular complexes purified from virulent cultures (Invaplex),[189] GMMA protein particles,[190] and an OMV-NP vaccine (Table 3).[191]

Live attenuated strains such as *S. dysenteriae* type 1 WRSd1, *S. sonnei* WRSS1, WRSs2, WRSs3, and some whole-cell-killed and novel antigen candidates are now developing, and were safe and immunogenic in a phase I trial (more details are presented in Table 3). Among possible *Shigella* candidate vaccines, GMMA protein particles, live attenuated *Shigella flexneri* 2a SC602, and *S. dysenteriae* type 1 SC599 strains have entered phase II clinical evaluation, and only glycoconjugate candidates have already undergone Phase III trials, with other formulations still under development in patients with shigellosis.[192,193] As shown in Table 3, many studies have demonstrated humoral response as a main value of an immunoresponse to vaccination, and also fever and transient diarrhea have been repaorted as the most frequent complications in relation to some vaccine candidates in clinical investigations (Table 3). Finally, it seems that development and evaluation of multivalent candidates may provide a means for protection against serogroups/serotypes of *Shigella* in future.

Conclusion

In this review, antibiotic-resistance mechanisms and therapeutic strategies have been summarized regarding *Shigella* infection. Antimicrobial resistance in *Shigella* spp. is multifactorial in that it can occur through innate, acquired, or adaptive mechanisms, and infections resulting from it are exceedingly difficult to treat. Drug resistance among *Shigella* spp. occurs as a result of selective pressure and horizontal resistance-gene transmission. Accordingly, there is an urgent need to comprehensively learn and understand mechanisms of drug resistance among *Shigella* isolates, in order to develop antishigellosis drugs. The multifarious nature of antibiotic-resistance mechanisms contributes in an increase in the number of MDR strains and causes conventional antibiotic therapeutics to be highly inefficient against shigellosis. Despite intensive research efforts in the last few decades related to determination of antimicrobial resistance, researchers have not been able to find the best solution to control MDR isolates. Also, direct contributions to antibiotic resistance by many antimicrobial-resistant mechanisms remains unknown, showing a need for continuous monitoring of a broader range of associated mechanisms contributing

Table 3 Overview Of *Shigella* Vaccines In A Aifferent Phase

Class/type	Investigational Vaccines	Delivery Systems	Target Antigen (Type)	Valued Immunoresponse	Status And Results	Limitations	Development Phase	Reference
Live vaccine								
Live-vaccine candidate strain	*Escherichia albertii* strain DM104	Intranasal	Whole-cell *E. albertii*	Humoral	Completed, efficacy, protection in guinea pigs	Unknown	Preclinical	198
Attenuated vaccines								
Live attenuated strains	*S. flexneri* 2a WRSf2G12, WRSf2G15	Ocular	*virG*, *set*, *senA*, *sbB2* genes	Humoral	Ongoing, protection in guinea pigs	Needs balancing immunogenicity with reactogenicity	Preclinical	176
	S. dysenteriae type 1 WRSd1	Oral	*virG* gene	Humoral	Efficacy when given orally	Transient diarrhea	Phase I	199
	S. flexneri 2a (SC602)	oral	*virG*, *iuc* genes	Humoral	Completed, efficacy, protection	ND	Phas IIB	200
	S. dysenteriae type 1 SC599 *S. sonnei* WRSS1	Oral Oral	*virG*, *ent*, *fep*, *stxA* genes *virG* gene	Humoral Humoral	Completed, efficacy, protection Phase I, ongoing, protection in adults	Unknown Children elicited lower mucosal immunoresponses than adults	Phase II Phase I	201 177
	S. sonnei WRSs2, WRSs3	Oral	*virG*, *senA*, *senB*, *msbB2* genes	Humoral	Ongoing, need for human challenge models for the efficacy of the vaccine	Fever, transient diarrhea	Phase I	176
	Trivalent of *S. flexneri* serotypes	Intranasal	*guaBA* gene	Humoral	Ongoing, prevention in guinea pigs and needs further development	Unknown	Preclinical	179
	S. flexneri 2a CVD 1208S	Oral	*guaBA*, *set*, *sen* genes	Humoral and cellular	Completed, efficacy, prevention and induces diverse T-CMI responses in human volunteers	ND	Phase II	180
	RNA-binding protein mutants of *S. dysenteriae* type 1 and *S. sonnei*	Ocular and oral	*hfq* gene and *ipaBCDA* plasmid	Humoral	Ongoing, induced protective immunity	Animals vaccinated in the eye showed fewer symptoms	Preclinical	181

(*Continued*)

Table 3 (Continued).

Class/type	Investigational Vaccines	Delivery Systems	Target Antigen (Type)	Valued Immunoresponse	Status And Results	Limitations	Development Phase	Reference
Hybrid and live attenuated vectors	*Salmonella enterica* serovar Typhi strain, Ty21a	Oral	O-antigen biosynthesis gene	Humoral	Completed, efficacy, protection in mice	Unknown	Preclinical	182
	S. flexneri 2a	Intranasal and oral	*virG*, *aroA* genes	Humoral	Ongoing, efficacy and future investigation need for more attenuated recombinant mutant strains	Fever and diarrhea	Phase II	202
	Live transconjugant *Shigella* hybrid (LTSHΔstx) strain	Oral	*stx* gene	Humoral and cellular	Completed, efficacy and good protection in mice	Unknown	Preclinical	203
	Escherichia coli K12	Oral	pWR110–R64drdII invasiveness plasmid	Humoral	Stopped, causes mild diarrhea in human primates	Fever, mild diarrhea to frank dysentery	Preclinical	204
	E. coli K12, EcSf2a-3, EcSf2a-5	Oral	*virG*, *aroD genes*	Humoral	Stopped, EcSf2a was immunogenic but also reactogenic and thus not sufficiently attenuated in the guinea pig	Not sufficiently attenuated	Preclinical	204
Inactivated vaccines								
Whole cell–killed vaccines	*Shigella* with a truncated O-SP	Intranasal	*wzy* gene	Humoral	Completed, efficacy, protection	ND	Preclinical	205
	S. flexneri 2a	Oral	Inactivated whole cells	Humoral and cellular	Completed, safety, robust immunoresponse	ND	Phase I	206
	S. sonnei	Oral	Inactivated whole cells	Humoral	Completed, efficacy, protection	ND	Phase I	205
	Trivalent of *Shigella* whole cells	Intranasal	Formalin inactivation of *S. flexneri 2a*, *S. sonnei*, and *S. flexneri* 3a	Humoral	Completed, efficacy, protection from lethality in guinea pig infection model	Unknown	Preclinical	184
	Heat-killed multiple serogroups/serotypes of *Shigella* (HKMS)	Oral	*S. dysenteriae* 1, *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 6, *S. boydii* 4 and *S. sonnei*	Humoral and cellular	Completed, efficacy, protection in rabbit infection model	ND	Preclinical	185

(*Continued*)

Table 3 (Continued).

Class/type	Investigational Vaccines	Delivery Systems	Target Antigen (Type)	Valued Immunoresponse	Status And Results	Limitations	Development Phase	Reference
Subunit vaccines	IpaDB fusion proteins	Intradermal	Invasion plasmid antigens B and D (IpaB and IpaD)	Humoral	Ongoing, protective efficacy using a mouse pulmonary infection model	ND	Preclinical	178
	rIpaB domain rGroEL	Lung	*S. flexneri* IpaB, *S. Typhi* GroEL	Humoral	Immunogenic and protective efficacy against *S. flexneri, S. boydii* and *S. sonnei* in BALB/c mouse infection model	ND	Preclinical	207
Glycoconjugate candidates	Bioglycoconjugates	Intramuscular	*S. dysenteriae* 1 LPS exoprotein A	Humoral	Ongoing, protection, additional serotypes will be tested shortly	ND	Phase I	208
	Lipid-linked *S. dysenteriae*–type 1 O-polysaccharide	Intramuscular	O-SP Ag	Humoral	Ongoing, need future studies using synthetic saccharides of different size	Low level of antibodies	Preclinical	209
	O-polysaccharide covalently linked to immunogenic carrier proteins	Subcutaneous	O-SP Ag	Humoral	Ongoing studies	Unknown	Preclinical	174
	Synthetic oligosaccharides	Intramuscular	*S. flexneri* 2a O-SP Ag tetanus toxoid	Humoral	Ongoing, need future studies for the development of multivalent glycoconjugate vaccines	Unknown	Preclinical	209
Novel antigen candidates	Artificial Invaplex (recombinant IpaB and IpaC proteins with purified *Shigella* LPS)	Intranasal	LPS, IpaB, and IpaC Ag	Humoral	Efficacy, protection in mice, need future testes for determination of safety and immune response in humans	ND	Preclinical	186
	Triacylated S-LPS	Parenteral	Partial alkaline deacylation of S-LPS	Humoral	Completed, efficacy, protection with robust humoral immunoresponse	Unknown	Phase I	108
	GMMA protein particles	-	Outer-membrane particles from *S. sonnei* and *S. flexneri* 2a	Humoral	Completed, good safety and immunogenicity profiles in healthy adults	ND	Phase IIa	187
	Δ*tolA*-OMV vaccine	Intramuscular	Outer-membrane vesicles, disruption of *tolA* gene from *S. boydii* 4	Humoral	An ongoing, potentially cost-effective vaccine in the mouse infection model	ND	Preclinical	210
	OMV-nanoparticle vaccine	Intranasal	*Shigella* outer-membrane vesicles	Humoral	Ongoing, need future studies for the development of a multivalent vaccine	ND	Preclinical	188

(*Continued*)

Table 3 (Continued).

Class/type	Investigational Vaccines	Delivery Systems	Target Antigen (Type)	Valued Immunoresponse	Status And Results	Limitations	Development Phase	Reference
Recombinant probiotic–based candidates	*Lactococcus lactis* bacterium like particles	Intranasal	IpaB and IpaD	Humoral	Ongoing, prevention in adult and infant mice	Unknown	Preclinical	[83]
	L. lactis	Subcutaneous	*ompA* of *S. dysenteriae* type 1	Humoral	Ongoing, efficacy of provoked immunoresponses in affording protection from *Shigella* needs to be evaluated	Unknown	Preclinical	[83]

to development of MDR strains. Most candidate vaccines cause an improvement in host immunity along with prevention of infection, but some have low efficiency and host challenge, which must be thoroughly overcome before being used against *Shigella* infection. More understanding on the host–microbe relationship is needed to develop novel therapeutic strategies to fight shigellosis. Development of innovative therapeutic and alternative strategies is also required for prevention and treatment of *Shigella* infections.

Author Contributions

Both authors conceptualized and designed the review, contributed towards data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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