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Shigellosis and Development of Multiple Antimicrobial Resistance Mechanisms of *Shigella* spp.

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Shigellosis is a serious public health issue. Millions of people suffer from this deadly food and water borne disease each year. The main manifestations of affected persons are bloody diarrhea with excessive dehydration. The causative agent of this disease is the bacteria Shigella spp. which has four serogroups. Though Shigella flexneri and Shigella dysenteriae are the dominant serogroups in developing countries, reports of other serogroups, namely Shigella boydii and Shigella sonnei, in the food contaminations are available. There are seasonal variations of Shigella infection throughout the world. In Asian subcontinent, monsoon and post monsoon times are the ideal for infection. The transmission of the bacteria in human is usually caused by feco-oral route or by contaminated food and water. There are several groups of antibiotics like foscomycin, macrolide, amiglycoside, tetracycline etc. which were used before. But they are now become useless as Shigella spp. is getting resistant against those drugs. The quinolone groups of antibiotics like ciprofloxacin, ofloxacin, norflxacin, ceftriaxone etc. are the important drugs for the cure of the disease shigellosis but prevalence of drug resistant strains of Shigella spp. against those drugs are a great concern nowadays. The occurrence of plasmid mediated quinolone resistance genes (PMQR), efflux pump proteins and effective mutations at drug binding region of gyrA etc. are the major mechanisms for the development of drug resistance.

Keywords: Drug resistance; Efflux proteins; Pathogenicity; Serogroup; Virulence factors.

Shigellosis is an infectious gastrointestinal disease. It is caused by Shigella serogroups of four types. They are responsible for the diarrhea in children and adults with morbidity and mortality specially in developing countries[1]. Infection due to the *Shigella spp*. were estimated to be about 170 million per year with about 1 million deaths in developing countries, 69% of them are children under 5 years[2]. Although this number has decreased but it has remained one of the important heath threat around the world. The children and young adults of developing countries of South Asia, Southeast Asia and Africa are mostly affected by *Shigella* infections[3]. A large number of people suffer from gastroenteritis in Bangladesh every year[4]. The burden of shigellosis is mainly associated with the poor sanitization, poor healthcare, contaminated water and food. Infection is common by feco oral route[5]. The genous Shigella having four serogroups (*Shigella flexneri, Shigella dysenteriae, Shigella sonnei* and *Shigella boydii*). The occurrence of most common

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Shigella serogroups differs geographically. In underdeveloped countries *Shigella dysenteriae* and *Shigella flexneri* are common and generally spread by environmental surface water sources[6]. On the other hand, *Shigella sonnei* and *Shigella boydii* are reported mostly in industrialized countries and usually spread through food sources. Antimicrobial and oral rehydration therapy are recommended to treat this disease. World Health Organization (WHO) recommended different antibiotic such as ceftriaxone, azithromycin, ciprofloxacin, pivmeccillinam, for the treatment of shigellosis but the indiscriminate use of antibiotics results in the emergence of multidrug (MDR) resistant *Shigella spp*. which makes shigellosis a global threat[7]. Particularly resistance to quinolones and cephalosporin makes the situation more challenging[8]. Various antimicrobial resistance mechanism in *Shigella spp*. have been described by the researcher such as extrusion of drug by activating efflux pump, decrease in cellular permeability, overexpression of drug-modifying and inactivating enzymes or target modiûcation by mutation[9]. Thus the recurrent changes in antimicrobial resistance pattern among the *Shigella spp*. poses the complication in recommending standard drug for effective treatment of shigellosis. The current study was done to review the emergence of antimicrobial resistance among the *Shigella spp*.

Shigellosis as an infectious acute dysentery

Infectious diseases are a major burden and the common cause of death worldwide. More than millions of people die each year in developing countries due to this disease among them most of are the children[2]. Dehydration by excessive fluid and electrolyte loss in stool results in acute dysentery which is the main reason of death. In developing countries diarrheal disease is not only a major health issue but also a risk factor to the travelers who travel to these countries. Treatment and preventive measures including fluid replacement and improvement of hygiene decrease the mortality rate from about 5 million to approximately 1.5 million deaths throughout the past two decades[10]. However, increased frequency of multi-drug resistant bacteria posed the problem more challenging[7,8,11]. *Vibrio cholerae,* multiple serovars of *Salmonella enterica*, different serotypes of Shigella, *Campylobacter spp*. and enteropathogenic *Escherichia coli* strains including *Escherichia coli* (ETEC) with the enteric viruses including rota viruse are the most important organisms that are frequently associated with diarrhea[12]. Acute watery diarrhea, dysentery and persistent diarrhea are the different types produced by those pathogens. Among the enteric pathogens, *Shigella spp*. is the most pathogenic organism that causes bacillary dysentery or shigellosis. Infection occurs by feco-oral route with the contaminated food and water[5]. Shigellosis is manifested by fever, abdominal cramps including the passage of loose stools mixed with blood and mucous. The annual number of shigellosis was estimated to be about 170 million episodes at the end of the last century with about 1 million deaths in developing countries[2]. Global projections suggest that there are 165 million episodes of shigellosis per year, of which 99 percent occurs in less than 5 years of age[13]. Although the number has reduced, shigellosis still remains one of the most important endemic diseases in the world [2].

Overview of *Shigella spp*.

Kyoshi Shiga in 1896 first isolated the *Shigella dysentery* at the Kitasato institute, Japan. He isolated a Gram negative bacillus from stool sample which showed positive agglutination reaction against sera of patient recovering from acute dysentery. Primarily he proposed the name of this organism as *Bacillus dysentericus* in 1897, later he changed it to *Bacillus dysenteriae* in 1898[14]. During his research he described the production of toxic factor by the organism that now known as Shiga toxin. Few years later German microbiologist Kurse (1900) isolated two similar organisms from patients' stool samples but they were serologically different from *Bacillary dysenteriae*. Over the two decades additional three groups of related organisms were identified and taxonomically placed in the Shigella and named as *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* to honor the Shiga, Flexner, Boyd and Sonne. The genus *Shigella* was first termed in the 1930 edition of *Bargey's Manual of Determinative Bacteriology* [15].Shigella belongs to *Enterobacteriaceae* which are small, nocapsulated, non-motile, non sporulating facultative anaerobic Gram negative bacilli. Genus Shigella has four major groups with several serotypes. They are - *Shigella dysenteriae* (13

serotype), *Shigella flexneri* (15 serotype), *Shigella boydii* (18 serotypes), *Shigella sonnei* (One serotype)[16].

Shigella can also be grouped into different serogroups including A, B, C and D depending on the basis of common specific polysaccharide antigens present on the cell surface. Whole genome sequence of *E. coli* and all four *Shigella spp*. revealed that they share a common DNA backbone of approximately 3.9 Mb, interrupted by *E. coli* specific and Shigella specific sequences. *S. dysenteraie* and *S. flexneri* are the most common serotypes found in developing countries. *S. dysenteraie* type 1 usually cause epidemic and pandemic bacillary dysentery with severe form of disease and produce potential lethal complications due to the production of Shiga toxin. In Latin America, Asia and Europe *S. dysentariae* type 1 causes epidemic dysentery in 1960. Asian countries such as Bangladesh (1972-78, 2003), Sri Lanka (1976), Maldives (1982), Nepal (1984-85) and Bhutan (1984-85) reports the dysentery epidemic. In India epidemics due to Shigella infections were reported from different parts of the country including eastern India (1984), Andaman and Nicobar Island (1986), Chandigarh (2003), Vellore (1972-73,1997-2001) , West Bengal (2002-03) in different times[2]. Frequent and occasional Shigella infections were reported during summer and winter from Israel [17]. Emergence of multidrug-resistance is the major problem in developing countries. In India especially in Kolkata *S. dysenteraie* type 1 became resistant to common antibiotics which results in high restriction in treatment[18]. Occasionally *S. dysenteraie* type 2 infections were reported and sometimes exceeded type 1 in the frequency of isolation. Other serotypes were encountered rarely among the diarrheal patient's sample.

In developing countries mostly *S. flexneri* causes endemic. The serotypes 1b, 2a, 3a, 4a, and 6 were encountered in industrialized countries[19]. Several uncommon serotypes and sub-serotypes of *S. flexneri* were also isolated from different countries like Bangladesh, Egypt and Russia[20,21]. Outbreak of *S. flexneri* 2a in India as well as in Taiwan and United State were also reported[22,23].

Outbreak due to *S. sonnei* is very rare in developing countries. There is no report of *S. sonnei* outbreak from India. Although it cause sequential outbreak but the mixture of genes with different origin found on *S. sonnei* plasmid due to the insertion of elements and number of other open reading frame. Shigella enterotoxin 2 prevalent in *S. sonnei* and a virulence gene *sat* has also been reported in this serotype[24].

S. boydii is mainly reported from Indian subcontinent and which is least prevalent in developing countries[25]. *S. boydii* is mainly spread throughout the World by travelers. It is the second dominant serogroups in India followed by *S. flexneri*. New serovar of *S. boydii* has been reported from Bangladesh in 2005[26].

Across the world the incidence of *Shigella spp.* depend upon the seasonal pattern. During summer or early autumn Shigella infection were most common in US and Europe. Kagalwalla et.al in 1992 reported the most of the Shigella serotypes were isolated during April-May in Saudi Arabia. Increase incidence of Shigella was reported form Bangladesh during post-monsoon season of August-November and pre-monsoon from the month of April - May of each year. In India specially in Kolkata Shigella infection was prevalent throughout the year with high isolation rate during the summer and early monsoon months[8,27,28]. *Shigella spp*. were detected more in June to July, i.e. during rainy season in Laos and Guatemala. In Hungary July to September was the peak period for the isolation of *S. flexneri* and *S. sonnei*[29].

Transmission and epidemiology

Shigellosis is endemic throughout the world, although one century is past after K. Shiga's remarkable discovery but still it remains a global health problem. World Health Organization (WHO) estimated that 164.7 million cases of shigellosis occurred per year of which 1.1 million cases result in death in developing countries due to the poor hygiene, limited access of clean water and malnutrition. Shigella required only 10 -100 organisms to cause infection[30]. It is transmitted by feco-oral route or by the contaminated food and water[30,31]. Transmission by house flies has also been documented[32]. Outbreaks due do the *Shigella spp*. have been reported from different parts of the world as well as from India. In 2007 an outbreak due to the *S. flexneri* in West Bengal has been reported which affect more than 461 people[33]. Another foodborne outbreak of *S. sonnei* infection was reported from

Pakaplole village, South 24 Parganas, India which affect more than 25 people in 2018[34]. In Iran an outbreak due to the *S. flexneri* 2a affect more than 700 prisoners in 2007[35,36,37]. Similarly in US an outbreak of shigellosis in 2006 was associated with the tomatoes[23]. MDR *Shigella spp*. outbreak has been reported from the Children Welfare Institute, China in 2015[4].

Antibiotic resistance profile of *Shigella spp*.

There are number of antibiotics recommended by WHO for the treatment of shigellosis which reduce the number of deaths per year but the extensive use of them led to an increase in isolation of multidrug-resistant *Shigella spp.* in several countries [7,11,19,38,39,40]. Still shigellosis remains one of the most important endemic diseases in the world. In recent day's treatment with ciproûoxacin [a ûuoroquinolone (FQ)] or one of the three second-line antibiotics, pivmecillinam, azithromycin and ceftriaxone (a third-generation cephalosporin), have been recommended. However reports are available of FQ-resistant Shigella isolates from India and other Asian countries which become a serious concern to treat shigellosis[41,42,43]. Antibiotic resistance genes may be located in plasmid, chromosome and mobile genetic elements like integrons and transposons of the bacteria (Table 1).Mutation in the drug target or genes associated with efflux system also cause the drug resistance (Table 2).

Centre for Disease Control and prevention (CDC) (https://wwwn.cdc.gov/narmsnow/) reports that year wise antibiotic resistant pattern among the isolated *Shigella spp.* is a serious threat (Fig.3). There are various mechanisms which result in resistance to antibiotics to the *Shigella spp*. including extrusion of drugs by activating efflux pump, decrease in cellular permeability, modifications of target sites by mutations and over expression of enzymes which modify or inactivate the drugs[44,45]. The possible mechanisms are listed below-

Function of outer membrane permeability

Cell wall of microorganisms is served as first barrier for penetration of antimicrobial drug into the cell. Some modification or changes in the membrane result in porin loss which increase the minimum inhibitory concentration (MIC) to the antibiotics[46]. Most of the antibiotics which are used to treat Shigella infections should be able to penetrate through cell membrane to reach intercellular accumulation and target site. Quinolone group of antibiotics such as nalidixic acid, ofloxacin, ciprofloxacin interfere with DNA gyrase and topoisomerase IV to inhibit DNA replication. Aminoglycoside antibiotics including streptomycin and spectinomycin bind with ribosomal subunits and inhibit protein synthesis.â-lactam antibiotics such as cephalosporin and penicillin target the penicillin binding protein and inhibit the cell wall biosynthesis. Mutations or absence of a ~39 kDa porin in the membrane of Gram negative bacteria as for example *Shigella spp*. slowdown the penetration of â-lactam (Aztreonam and Dianionic moxalactam) and hydrophilic antibiotics such as penicillin and piperacillin[44,46]. Three mutant strain of *S. dysenteriae* isolated in India showed resistance to imipenem and the study reported that the resistance towards the imipenem is associated with the permeability of outer membrane proteins. It is also reported that the resistance to colicin E_2 among the *S. flexneri* strains associate with LPSs of the outer membrane[47].

Role of efflux system

Activation of efflux pump plays an important role in antibiotic resistance phenotype of *Shigella spp*. to expel the toxic compounds from the cells. Efflux system can be grouped into five families such as- the major facilitator super family, small multidrug resistance family, resistance-nodulation-division family, multidrug extrusion family and ATP binding cassette family[48]. In *Shigella spp*. a triplet complex namely AcrAB-Tol C, belonging to resistance-nodulation-division family of efflux system reduce the level of quinolone accumulation inside the cells which results in the resistance to quinolones[49]. It is also reported that drug efflux pump such as *mar*A, *tolC*, *YdhE* and *mdfA* also confer resistance to quinolones[45].

Resistance to β-lactam antibiotics

Class A β-Lactamase

Extended-spectrum â-lactamase (ESBLS) are the enzymes belongs to the Amber class A that confer resistant to most of the antibiotics such as penicillin, carbapenems and cephalosporin. The first report for isolation of ESBL producing strain was from Bangladesh in 2004[50]. Apart from that different β-lactamases of the Amber class A such as TEM, SHV and CTX-M have been reported in *Shigella spp*. These enzymes are

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	References
β-Lactams	Class A β-lactamases	blaSHV 2,11,12	P,C	India, Argentina,	80-82
		blaPER-2	-	Argentina	80
		blaTEM-1,1b,15,17			
		blaTEM-19,20,52	I, P	Lebanon, China, South Korea	83-85
		blaCTX-M-1-123	P,C	China, Turkey, Argentina, South Korea, India, Israel, Japan	82,86-89
	Class B β-lactamases	blaIMP-like	P	India, France	52
		blaKPC	-	Senegal, France	90
		blaVIM-like	-	India	52
	Class C β-lactamases	blaCMY-2,59	C, P	China, Mexico, India,	53
		blaDHA-1	C, I, P	China, India, Israel	86
	Class D β-lactamases	blaoxa-1,2,5,30	I, P	Mozambique, Chile, China, India, US, Egypt, Djibouti, Spain, Greece, Denmark, Peru, Iran	87,91
Quinolones	Plasmid-borne resistance	qnrA	P	Iran	54
		qnrB,4,19	P	India, Switzerland	60,92
		qnrC	P	India	93
		qnrS,I	P	Pakistan	69
		aac-(60)-Ib-cr	P	India	92
	Efflux pump	qepA	p	China	60
Fosfomycin	Fosfomycin resistance enzymes	fosA3	P	China	64
Aminoglycosides:		strA,B	MGE	India, Australia, Chile, Pakistan, South Korea	67
streptomycin	Adenyltransferase	aadA1,2,5	I, P	Senegal, Bhutan, India, Taiwan	65,90,94
Tetracycline	Effux pumps	tetA,B,G	C, P	Mozambique, Taiwan, Iran, Spain, South Korea	9,94,95
Trimethoprim	Dihydrofolate	dfrA1,5,7,8,12,13	I, P	Spain, Taiwan, Senegal,	9,90,91,94,7

	reductases	dfrA14,15,16,17		Mozambique, India, China, Iran, South Korea, Peru, France, Chile, Australia	
		dfrV	I, P	China, India	
Sulfonamides	Plasmid-borne resistance	sul1	I, P	Australia, India	65,67
Phenicols	Chloramphenicol	catA-like	P	Taiwan, Mozambique, India	90
	acetyltransferase genes	catP	P	Pakistan	69
	Effux pumps	cmlA1	I, P	Taiwan	94
Colistin	Plasmid-borne resistance	mcr-1	P	China, Vietnam	73
Macrolide	Enzymatic inactivation	mphA	P	Palestine, Switzerland	60
	rRNA methylase	ermB	P	Vietnam	

Abbreviation: P, plasmid; C, chromosome; I, integrin; —, unknown; MGE, mobile genetic element.

responsible for selective hydrolysis of ceftriaxone, cefotaxamine and ceftazidime[51]. Till now several reports from different parts of the world like Canada, Turkey, Israel, Argentina, China and India showed that *Shigella spp.* harbor different ESBL encoding genes (Table 1).

Class B β-Lactamase

Class B β-lactamase can hydrolyze carbapenem and other â-lactam. A study reported that, a metallo-â-lactamase (MET 1) encoded by a plasmid mediated gene IMP-3 in *S. flexneri* conferred resistance against sulfonamide and kanamycin. *S. sonnei* and *S. flexneri* isolated from Andaman and Nicobar Island in India showed the presence of bla_{VIM} and bla_{IMP} which conferred the resistance to carbapenem[52].

Class C β-Lactamase

Ceftriaxone and cephalosporin has been recommended to treat the MDR *Shigella spp*. However *Shigella spp.* resistant to those antibiotics has also been reported. Class C β-Lactamase, known as AmpC-type enzymes are encoded by both chromosomal and plasmid genes. CMY-2, a plasmid encoded AmpC β-lactamase firstly identified in *S. sonnei* isolates which was obtained from a dysentery outbreak in Taiwan and there after CMY-2 have been reported in several countries like China, Iran, Costa Rica and India[53-55]. Different AmpC genes are listed in table1.

Class D β-Lactamase

Resistance to cloxacillin, ampicillin, cephalothin, oxacillin mainly mediated by class D β-lactamase or OXA-type β-lactamase[56]. OXA-type β-lactamase encoding gene bla_{OXA} have been identified in integrons and plasmids in different Gram negative bacteria including *Shigella spp*. especially in *S. flexneri*[57]. bla_{OXA-1} and bla_{OXA-30} are differed from each other by having a single mutation at codon 131,containing Tn2603 and Tn1409 transposons respectively.

Quinolones and fluroquinolones resistance

Resistance to fluroquinolones due to chromosomal target site mutations

Quinolones are used to treat shigellosis for a very long time across the world[58]. This group of antibiotics mainly consists of ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, nalidixic acid etc. There are various genes present into the chromosomes as well as plasmids of the bacteria Shigella which confer resistance against these

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	References
gyrA	57	Asn'!Lys	AAT'!AAA	*S. ûexneri*	China	99
	69	Gln'!Trp	—	*S. sonnei*	India	93
	80	His'! Gly	CAT'!GGT	*S. dysenteriae*	Belgium	99
	83	Ser'!Leu	TCG '!TTG	*S. boydii*	China, Bangladesh, India	92
	180	Asp'!Met	—	S165/15	India	27
	83	Ser'!Leu	—	S148/17	India	
	181	Glu'!Lys	—	S74/15	India	
	87	Asp'!Asn	—	S138/16	India	
	192	Phe'!His	—	S28/14	India	
	25	Ile'!Met	—	S124/16	India	
	39	Glu'!Lys	—	S49/15	India	
gyrB	517	Gln'!Arg	CAG'!CGA	*S. ûexneri*	China	61
parC	64	Ala'!Asp	GCC'!GAC	*S. ûexneri,*	India, China	93
	64	Ala'!Cys	GCC'!TGC	*S. sonnei*	China	93
	80	Ser'!Ile	AGC'!ATC	*S. dysenteriae*	China, Bangladesh, India	92
	85	Ala'!Ser	GCG'!TCG	*S. boydii*	Switzerland	60

drugs. They can be mainly classified into plasmid mediated quinolone resistance genes (PMQR) and quinolone resistance determining regions (QRDR). Efflux pump attributor proteins also confer resistance to quinolones of the bacteria. *gyrA, gyrB, parC* and *parE* are the corresponding subunits of DNA gyrase and topoisomerase IV, which are encoded by *gyrA*, *gyrB*, *parC* and *parE* gene respectively (Table 1).Quinolones bind with QRDR region of DNA gyrase and mutation in that region causes reduced susceptibility to the quinolones. The most mutations have been found in between Ala 67 and Gln 07 in several studies[59]. A very recent study reported the mutations at the position 83 (Ser-83-Leu) and 87 (Asp-87-Asn) of *gyr*A in the *Shigella spp*. isolated from environmental water samples of in and around Kolkata have higher MIC to quinolones[27] . Some researchers reported that a single mutation in *gyrA* cannot decrease the susceptibility to quinolone and for that further mutation in *parC* and *gyrA* region are needed[60,61]. Amino acid and nucleic acid changes in QRDR region of *gyrA, gyrB, parC* and *parE* in *Shigella spp*. are shown in table 2.

At *parE* two novel mutations at codons 408 and 458 have recently been identified among the *Shigella spp.* isolated in India (2011) and in China (2016)[28,62]. Mutation in codon 408 is associated with resistance to nalidixic acid but not to ciprofloxacin whereas mutation at codon 458 conferred resistance to both nalidixic acid and ciprofloxacin.

Plasmid mediated resistance

Presence of plasmid mediated gene namely *qnr* (*qnrA*, qnr*B*, *qnrC*, *qnrD*, *qnrS*, qep, *aac*[6'] -*lb-cr*) at the plasmid-mediated quinolone resistance region (PMQR) is also one of the main reason to gain resistance to the quinolones among the *Shigella spp*. A study from China reported that *aac(6')-lb-cr* and *qep*A positive showed a high level of resistance to quinolones[49]. Gene *aac (6')-lb-cr* encodes an acetyltransferase which reduce the activity of quinolones. Although the mutation in the QRDRs of DNA gyrase and topoisomerase IV genes

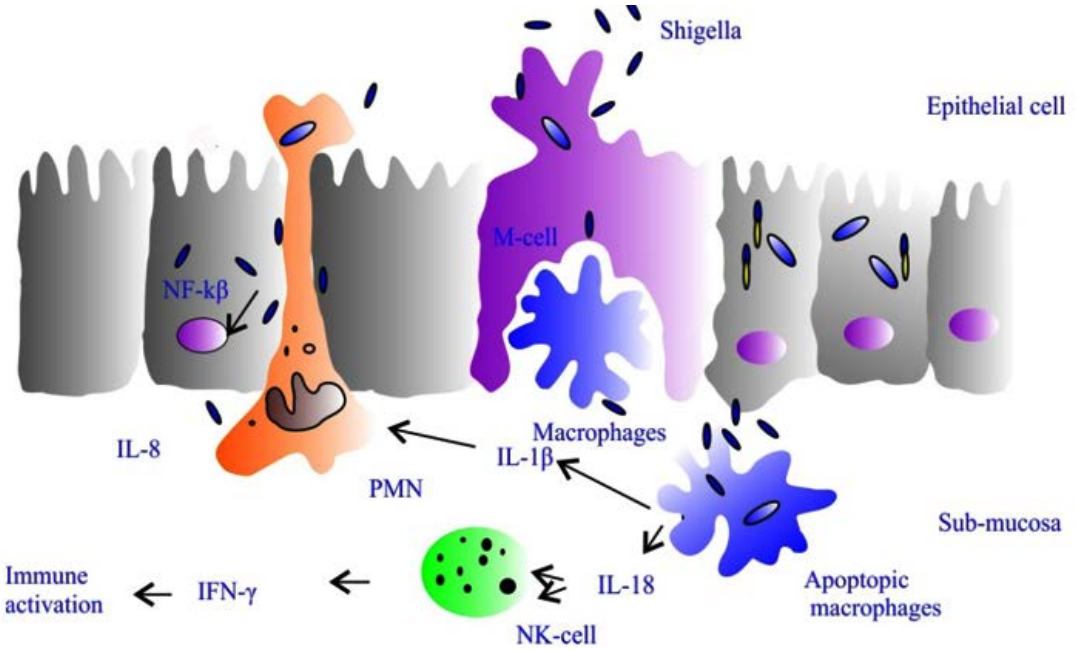

Fig. 1. Mechanism of Shigella pathogenesis: *S. flexneri* encounters host macrophages after crossing the epithelial barrier (EC) and entry into M cells. The bacteria avoid degradation into the macrophages and enter into EC from basolateral side and move into cytoplasm. The PMN are attracted concomitantly by proinflammatory signals and EC with the activation of NK cells. As a result, the PMN disintegrates the EC lining with immense intensification of infection and tissue destruction which facilitate the invasion of more bacteria. Finally Shigella is killed through PMN mediated phagocytosis

is the main cause of resistance to fluroquinolones but PMQRs may expedite in selection of isolates showing higher levels of resistance through extra chromosomally encoded mechanisms and admit reduced susceptibility to fluroqinolones[63] .

Fosfomycin resistance

Fosfomycin inhibit cell-wall biosynthesis by inactivating the MurA enzymes[64]. Although fosfomycin have been used to treat microbial infections for four decades but fosfomycin resistance have also been found in several enteropathogens including *Shigella spp*. Resistance to fosfomycin occurred primarily by two mechanisms: mutations in the *uhpA/T* and *glpT* genes which encode proteins responsible for two carrier dependent system associated with the uptake of fosfomycin ; fosfomycin modifying enzymes containing two kinase(FomA, FomB) and three metallo enzymes (FosX, FosA and FosB). For the first time fosfomycin modifying enzymes was reported from China among the isolated strain of *S. flexneri* from patient' samples[64] .

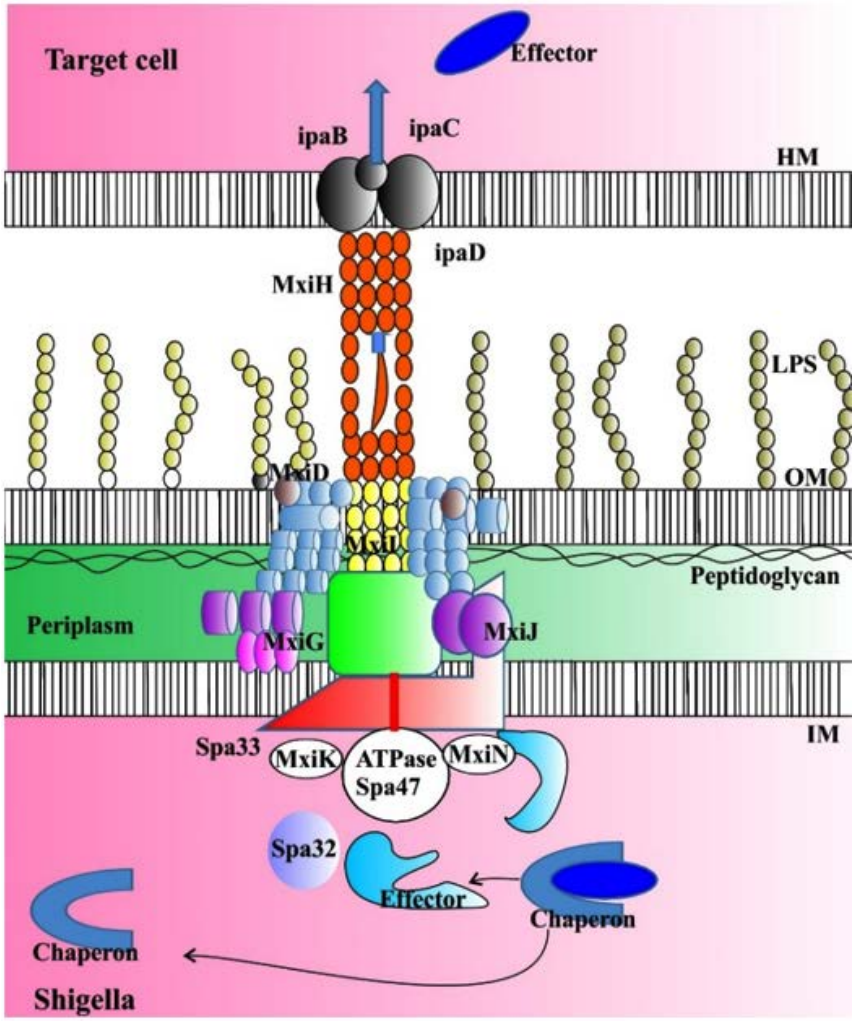

Fig. 2. Molecular architecture of the *S. flexneri* Mxi-Spa T3SS: The T3SS comprises of four main parts. The basal body that extents the bacterial inner membrane (IM), the periplasm and the outer membrane (OM). There is cytoplasmic ring consists of proteins in the T3SS which help in the transport process and facilitate the recognition of substrates, substrate unfolding and chaperone release. The LPS which is the major bacterial surface antigen and the peptidoglycan (PGN) molecules are located respectively on the surface and in between the inner and outer membrane

Aminoglycoside resistance

Aminoglycosides are being used to treat various bacterial infections for long time. They are associated with different kinds of mechanisms such as enzymatic inactivation, ribosomal modifications and active efflux pump. Out of these mechanisms, aminoglycoside modifying enzymes are the most common in clinical settings[65,66]. Aminoglycoside adenyltransferase (*aadA* gene cassettes) is very significant in *Shigella spp*. which confers resistance to streptomycin and spectinomycin[66,67]. Different types of *aadA* gene cassette have been identified in enterobacteriaceae but *aadA*1 and *aadA*2 has high prevalence among the isolated *Shigella spp.*[66]. Genes *strA* and *strB* which encode aminoglycoside phosphotransferase are also well dispersed among the plasmid (IncFII and pNV-Y394) of the isolated *Shigella spp*.[65,68,69] . A study from India reported that 100% and 88% of *S. dysenteriae* and *S. flexneri* harbored *strA* genes which confer resistance to streptomycin[70] .

Tetracycline resistance

Roberts et al. reports five tetracycline-efflux genes-*tet*(A), *tet*(B), *tet*(C), *tet*(D) and *tet*(G) with one ribosomal protection protein encoded by *tet*(M) that have been identified among the *Shigella spp.* Various *tet* genes flanked by transposases were identified in a ~20.4 kb of genomic island encoding MDR genes[71]. Identical MDR cassette was firstly identified in *S. flexneri* 2a YSH6000 strain which is referred to as Shigella resistance locus-pathogenicity island[71]. Moreover presence of the MDR genes also reported in the *E. coli* plasmid pRs225 with similar arrangement which suggests that *tet* genes might be dispersed among the other species by horizontal gene transfer. *S. dysenteriae* isolated from dysentery outbreaks in different parts of India showed that *tet*(B) was more common (90%) than *tet*(A) among the isolates[72].

Phenicol resistance

Phenicols have used for past few years to treat Shigella infection but the treatment is more challenging now a days due to resistance to these antibiotics. Resistance to chloramphenicol in *Shigella spp*. is associated with the *cat* genes [*catA* (*catA1,catA2, catA3*) and *catB* (*catB2, catB3, catB7, catB8)]* encoding chloramphenicol acetyltransferase, activation of efflux pump by *cmlA* (*cmlA*1, *cmlA*4, *cmlA*9) genes and/or by fluorinated and unfluorinated phenicols (*flor*) by major facilator-superfamily proteins[73]. About 96 Shigella were isolated from diarrheal patients sample in Pakistan and out of them 69 (72.9%) were resistant to chloramphenicol[11].

Colistin resistance

Colistin (polymixin E) interacts with the outer membranes of Gram negative bacteria. The gene responsible for colistin is a plasmid-mediated polymyxin resistance gene namely in *mcr*-1 have been identified in the *Shigella spp.*[74]. Gene *mcr*-

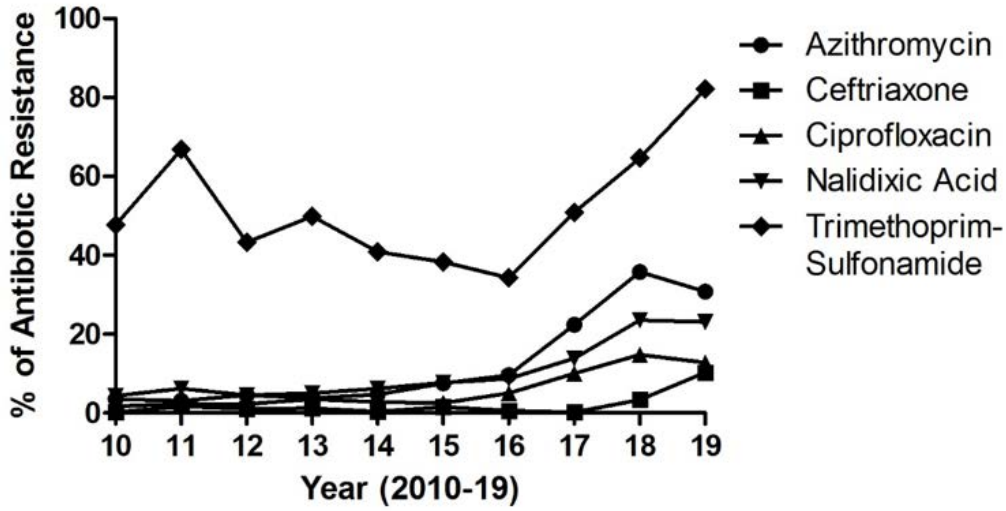

Fig. 3. Antibiotic resistance among *Shigella spp.*: Year wise antibiotic resistant pattern among the *Shigella spp*. isolated from 2010 to 2019

1 produce a phosphatidyl ethanolamine which modify the lipid A on cell membranes and reduce the affinity for colistin and related polymixins and consequently reduce the antibacterial activity of these drugs[75]. Isolated *Shigella spp*. having *mcr*-1 gene showed four to eight fold increase in the MIC of polymixin B[76]. *mcr-1* have been identified in *S. sonnei* isolated from Shanghai (2010-2012) which were resistant to polymixin B (MIC 4-8 µg/ml)[76].

Sulfonamide and trimethoprim resistance

Spreading of trimethoprim-sulfonamide resistance among the *Shigella spp*. across the different parts of the world make this drug ineffective to treat shigellosis[30]. This is mainly due to mutational or recombinational changes in target enzymes (dihydropteroate synthase and dihydrofolate reductase respectively). The genes responsible for encoding dihydropteroate synthase and dihydrofolate reductase are *sul* and *dfr* respectively. Almost 42 types of *dfr* genes have been detected among different groups of bacteria which confers resistance to trimethoprim and 12 of them have been identified among the trimethoprim resistant *Shigella spp*. Gene cassettes within class 1 integrons among the Shigella plasmid or chromosome often encodes resistance to trimethoprim (*dfrA*), streptinomycin (*aadA*) and ampicillin (*oxa-1*)[77]. 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*. Resistance to trimethoprim is mainly associated with the presence of *dfrA1* genes occurring in a cassette in both class 1 and class 2 integrons. This integron associated antibiotic resistance may transfer to other species via plasmid conjugation. Gene cassette array carried by class 1 integron have been recognized in *S. sonnei* isolated from Chaina, Vietnam and Australia[66,77]. The genes responsible for sulfonamide resistance are *sul1*, *sul2* and *sul3*, very common in *Shigella spp*. Different studies from the different parts of the world showed that number of *Shigella spp*. especially *S. sonnei* isolated from 2000 onwards have 100% resistance to the sulfonamides.

Macrolide resistance

Currently WHO recommends azithromycin as a second line treatment for shigellosis but now a days this antibiotics are becoming resistant day by day. CDC reports that approximately 3% of the all tested *Shigella spp*. is getting resistant to azithromycin. Possibly there are four mechanisms which mediate the resistance to these drugs including enzymatic inactivation by phosphotransferase encoded by *mph* gene or esterase encoded by *ere* determinant; target site modification by rRNA methylase encoded by *erm* genes; punctual mutation in *rpIV* encoding L22 ribosomal protein, *rplD* encoding L4 ribosomal protein and *rrlH* (23 rRNA); drug resistance mediated by efflux pumps including OmpA, OmpW, mefA and msrA[78]. Reduced susceptibility to azithromycin among the isolated *Shigella spp*. have been continuously reported from different parts of the world such as Asia, North-America, Australia and US[79].

Thus frequent shifts in antimicrobial resistance profiles of Shigella isolates caused difûculty in recommending standard drugs for effective treatment of the disease.

CONCLUSION

Shigellosis is one of the most important health concerns in countries that yet to overcome many socioeconomic challenges. There are several virulence factors and enterotoxins which confer the pathogenicity of the bacteria Shigella, the causative agent of this disease. There were several antibiotics like ampicillin, tetracycline, foscomycin, trimethoprim, sulfonamide, macrolide group which now became ineffective. Only some fluoroquinolone group of antibiotics is active to combat the disease. But indiscriminate use of antibiotics and other risk factors help to develop a number of antibiotic resistant strains of *Shigella spp.* into the environment. This difficult situation demands the discovery of new and better drugs. So, it is very much essential to search for the actual mechanisms of developing drug resistance into the bacterial cells. In this review, we summarized the recent advancement of knowledge regarding different drugs used to combat the disease, latest pattern of drug resistance, development of antibiotic resistance mechanisms of *Shigella spp.* for better understanding of the host microbe interaction and to build up new strategy to combat this disease.

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Ethics approval

N/A

Conflicts of interest

All authors declare that they have no conflict of interest.

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