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Virulence Factors and Pathogenicity development of *Shigella* , Review

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Abstract
Shigella is a main cause of gastroenteritis and it is responsible for 5 to 10 % of diarrheathrough the world.The natural hosts of *Shigella* are typically humans and other primates, but it has been shown that thehost range of Shigella has expanded to many animals. Although *Shigella* is becoming a major threat to animals,there is limited information on the genetic background of local strains. *Shigella* is the major cause of bacillary dysentery world-wide. It is divided into fourspecies, named *S. flexneri*, *S. sonnei*, *S. dysenteriae*, and *S. boydii*, which are distinctgenomically and in their ability to cause disease. Shigellosis, the clinical presentationof *Shigella* infection, is characterized by watery diarrhea, abdominal cramps, and fever.*Shigella*'s ability to cause disease has been attributed to virulence factors, which areencoded on chromosomal pathogenicity islands and the virulence plasmid. However,information on these virulence factors is not often brought together to create a detailedpicture of infection, and how this translates into shigellosis symptoms. Firstly, *Shigella*secretes virulence factors that induce severe inflammation and mediate enterotoxiceffects on the colon, producing the classic watery diarrhea seen early in infection.Secondly, *Shigella* injects virulence effectors into epithelial cells via its Type III SecretionSystem to subvert the host cell structure and function. This allows invasion of epithelialcells, establishing a replicative niche, and causes erratic destruction of the colonicepithelium. Thirdly, *Shigella* produces effectors to down-regulate inflammation and theinnate immune response.

Key words : Virulence Factors , Antibiotic Resistance , *Shigella* , Pathogenicity .distinct gnomically

Introduction

Diarrheal diseases caused by bacterial, viral, or parasiticpathogens are a major public health problem. Estimations bythe World Health Organization (WHO) indicate that theworld population suffered from 4.5 billion incidences of diarrheacausing 1.8 million deaths in the year 2002 (334). Approximately99% of the cases occurred in developing countries,where poor hygiene and limited access to clean drinking waterpromote the spread of enteric diseases. Malnutrition and thelack of appropriate medical intervention contribute to the highmortality rate, especially for young children.Species of the genus *Shigella* are among the bacterial pathogensmost frequently isolated from patients with diarrhea. Five to fifteenpercent of all diarrheal episodes worldwide can be attributedto an infection with *Shigella*, including 1.1 million fatal cases(136). Two-thirds of all episodes and deaths occur in children
under 5 years.
Shigellosis or blood dysentery is widespread in underdevelopedor developing regions with poor hygiene andlimited access to clean drinking water and has become aserious threat to public health . Shigellosis is causedby non-motile, facultative anaerobic gram-negative bacilliof the Enterobacteriaceae family, including *S. dysenteriae*,*S. flexneri*, *S. boydii*, *and S. sonnei*.*Shigella*species have high effectiveness in invasive systems thatenable bacteria to invade and multiply within the humanintestinal epithelia, ultimately leading to severe inflammatorycolitis, which is referred to as bacillary dysenteryor shigellosis .Various virulence factors located on chromosomes orlarge virulence inv plasmids are recognized as crucialfactors related to the pathogenesis of shigellosis.Moreover, these different virulence factors are associatedwith the colonization of intestinal cells and intracellularinvasion, which may partly explain why various manifestationsare detected in the clinic, such as intestinal inflammatoryresponses and watery diarrhea. Bacterial cell-to-cell movement and dissemination within epithelialcells of the intestine are allowed by the iphH gene, which isencoded by chromosomal DNA and/or recombinant plasmids,while ial, which is encoded by plasmids (invasion-associatedloci), enables Shigella bacteria to penetrate intestinalepithelial tissues . The chromosomal genes set1A andset1B encode *Shigella* enterotoxin 1 (ShET-1) , which iseasily detected in all S. flexneri 2a isolates.

OspB: Promotes PMN Migration, Inflammation, andCell Proliferation

Like most effectors, OspB is found in the four Shigellaspecies, and has homolog in Salmonella species .Although its biochemical function is unknown,it is thought that OspB plays a role in the activation ofextracellular-signal-regulated kinases (ERK) and p38 MAPKpathways, resulting in phosphorylation of phospholipase A2and the generation of eicosanoids. OspB is capable of nuclearlocalization for activation of MAPK signaling pathways. Thiscontributes to inflammation and PMN migration, possiblyinducing hepoxilin A3, an arachidonic acid derivative, and apicalsecretion of IL-8, a PMN chemoattractant .An ospB− mutant had a 60% decrease in PMN migration anda 30% decrease in ERK1/2 activation 90 min post-infectionwhen compared to wild-type Shigella .Furthermore, showed that an ospB−knockout displayed significantly reduced onset and severityof symptoms in the guinea pig keratoconjunctivitis model ofinfection (Sereny test). However, OspB also activates the masterregulator of cell growth mTOR via a direct interaction withthe cellular scaffold protein IQGAP1, which also interacts withmTOR activators ERK1/2. This seems to restricts the spreadof S. flexneri in cell monolayers, possibly by enhancing cellproliferation in infected foci .

OspC1: Promotes PMN Migration and Inflammation

OspC1 is part of the ospC family. There is 96% identity betweenospC2, ospC3, and ospC4, but only 74% identity between thesethree ospC genes and ospC1 . Thislevel of similarity may indicate redundancy. However, ospC4is a pseudogene and different functions have been identifiedfor OspC1 and OspC3 (discussed later). Tagged OspC1 isfound throughout the host cytoplasm, localizing primarily tothe nucleus . An ospC1− knockoutshowed a significant decrease in the amount of neutrophilrecruitment to the epithelial cells in PMN migration assays,which was restored to wild-type levels on complementationwith a plasmid expressing ospC1. showedthat this increase in PMN migration correlated with increase inthe phosphorylation of ERK1/2 pathways mediated by OspC1.An ospC1− knockout showed a decrease in phosphorylation ofERK1/2 compared to wild-type levels but no reduction in IL-8 secretion. OspC1 plays a role in *Shigella* virulence in vivoas an ospC1− knockout had reduced amounts of swelling andinflammation in the Sereny test, with clearance of infection after 2 days .

OspZ: Promotes PMN Migration and Inflammation

In S. flexneri 2a, an ospZ− knockout has no effect on theSereny test. However, an ospZ− knockout caused a significantdecrease in PMN migration. The knockout also had 63 and53% ERK1/2 phosphorylation and NFkB activation, respectively,when compared to wild-type S. flexneri .OspZ therefore plays a role in the migration of PMN leukocytesacross the epithelial barrier. However, discovered that S. flexneri species, excluding S. flexneri serotype6, contain a stop codon at amino acid 188, forming a truncatedprotein lacking an IDSYMKmotif at position 209. The full length
OspZ proteins in the remaining Shigella species were foundto have an immunosuppressive function through prevention ofNFkB activation. Finally, an OspZ homolog, NleE, is found inenteropathogenic Escherichia coli (EPEC), and both NleE andOspZ can substitute for each other.

Serine Protease Autotransporters ofEnterobacteriaceae

Serine Protease Autotransporters of Enterobacteriaceae(SPATEs) are a family of proteases which catalyse theirown secretion via the Type V secretion pathway. Shigella hasthree known SPATEs, not all of which are found in each species.Their secretion is thermoregulated (37◦C) and pH-dependent. They have different proposed activities relevantto intestinal penetration: induction of mucin secretion andcleavage (Pic), destabilization of focal adhesions via cleavage offodrin (SigA), and, through unknown targets, enterotoxicity,fluid accumulation and epithelial desquamation (SigA and SepA).

Shigella Enterotoxin 1 and Shigella Enterotoxin 2:

Enterotoxic Activity in the JejunumShigella enterotoxin 1 (ShET1) and Shigella enterotoxin 2(ShET2) are virulence determinants proposed to mediate earlyfluid secretion in the jejunum to establish infection in the colonand produce to the characteristic watery diarrhea seen early inshigellosis. The shared name is due to their similar propertiesas enterotoxins, as there is no homology between ShET1 and ShET2.ShET1 is encoded by set1A and set1B genes on the Shigellachromosome as part of the SHI-1 PAI, and only present inS. flexneri 2a isolates .The two subunits are proposed to form a holo-ABtypetoxin complex in an A1-B5 configuration, producing a55 kDa complex . The holotoxin mayfollow a secretion mechanism similar to that of the choleraholotoxin, via the Sec pathway and Type II secretion. Whenion transport across a cultured epithelium was measured in anUsing chamber, a set1AB− knockout had 60% lower Isc (shortcircuit current) in comparison to wild-type strains. The effect on Isc of ShET1 was also dose-dependent,and washout of ShET1 produced no change in Isc, indicatingirreversible binding of ShET1 to epithelial receptors . However, the set1A and set1B genes overlap withthe pic gene but are divergently transcribed. Therefore, theadditional pic− knockout may have caused these effects. complemented the pic/set1AB mutant with picand set1AB individually, showing that pic has a more significantcontribution to restoring Isc levels to wild-type, although set1ABcomplementation also produced a significant increase in Isc. Figure 1 and table 1 showed role of virulence factors and genes.

Adhesion to the Colonic Epithelium at theBasolateral Surface
Lipopolysaccharide: Glucosylation for T3SSAccessibility

The lipopolysaccharide (LPS) is a common feature of Gramnegative pathogens, triggering the host immune response andinflammatory reactions during infection. LPS modification byglucosylation is thought to contribute to Shigella adhesion andinvasion by revealing the T3SS for efficient activation uponcontact with the host cell. Showed thatglycosyltransferase gtrA− and gtrB− mutants had only a partialconversion of the O-antigen serotype, and a gtrX− mutant hadno conversion at all. A mutation in the gtr operon leads to areduced ability to invade, and this invasion is restored when thegtr operon is reintroduced . The reductionin O-antigen length by glucosylation enhances accessibility ofthe T3SS for contact with the host epithelial cell to initiateinvasion.IpaB: Binds CD44 at the Basolateral Surface **,** OpsE1/E2: Bile Salts-Dependent Adhesion **,** IcsA (VirG): Polar Adhesion.

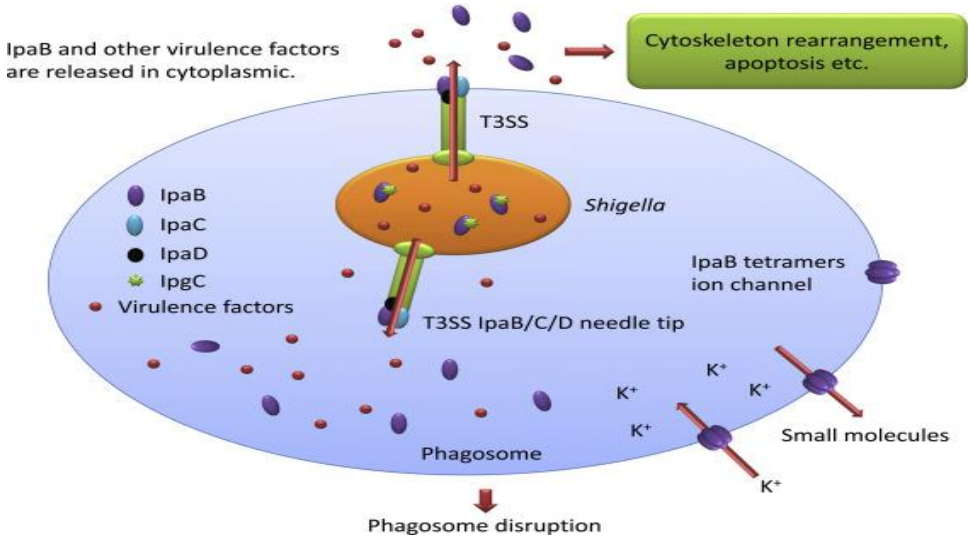

Figure-1: Shown role virulence factors in *Shigella*

Table-1: Shown genes of virulence factors in *Shigella*

PAI	Gene(s)	Protein function	References
SHI-1	*sigA*	Putative enterotoxin	Al-Hasani et al., 2009
	pic	Intestinal colonization	Navarro-Garcia et al., 2010
	set1A, set1B	ShET1 enterotoxin	Fasano et al., 1997
SHI-2	*iucA–D*	Siderophore, complexes with iron	Vokes et al., 1999
	iutA	Bacterial receptor for iron-siderophore complex	Vokes et al., 1999
	shiA–G	Novel ORFS, ShiA involved in reduction in host inflammatory response	Ingersoll et al., 2003
SHI-3	*iucA–D*	Siderophore, complexes with iron	Purdy and Payne, 2001
	iutA	Bacterial receptor for iron-siderophore complex	Purdy and Payne, 2001
SHI-O	*gtrA, gtrB, gtr*	Serotype conversion and O-antigen modification	Allison and Verma, 2000
Stx-phage P27	*stxAB*	Shiga toxin	Yang et al., 2005

Table 3- Genes location of virulence factors in *Shigella*

SPATE	Gene location	Putative function	Role in infection	References
Pic	SHI-1 (opposite *set1AB*)	Cleavage of mucin	Penetrate colonic mucus layer to access epithelium	Gutierrez-Jimenez et al., 2008
		Mucin secretagogue	Mucus-containing dysentery in shigellosis	Navarro-Garcia et al., 2010
SigA	SHI-1	Cytopathic activity	Cleavage of fodrin to destabilize links between actin cytoskeleton and membrane proteins, detachment of focal adhesions	Canizalez-Roman and Navarro-García, 2003; Al-Hasani et al., 2009
		Enterotoxic activity	Fluid accumulation	Al-Hasani et al., 2000
SepA	Virulence plasmid	Enterotoxic activity	Fluid accumulation	Benjelloun-Touimi et al., 1995
		Epithelial desquamation	Disease progression	Coron et al., 2009

Lysis of the Double Membrane Vacuole
Vps and VacJ: Proposed ABC Transporter

The vpsABC operon is found on the *Shigella* chromosome,and consists of VpsA, a possible ATP-Binding Cassette(ABC) transporter protein, and VspB and VspC, proposedtransmembrane proteins. Both vpsC− and vspA− knockoutshad a defect in plaque formation but were similar to wildtypestrains in their capability to invade, indicating that theyplay a role in intercellular spread . VacJis also encoded on the chromosome, and a vacJ− knockoutis incapable of escaping into the recipient cell cytoplasm,suggesting that VacJ also plays a role in intercellular spread .Describe aVps/VacJ ABC transporter, which maintains asymmetry oflipids in the outer membrane and in the context of Shigellainfection is required for lysis of the double membrane vacuole.Transformation of vps/vacJ knockouts with a plasmid expressing pldA, a phospholipase in other Gram negative bacteria, wasable to restore the maintenance of outer membrane lipidasymmetry but was unable to lyse the double membranevacuole, indicating that these two functions of the proposedVps/VacJ ABC transporter are separate .Another substrate may therefore be transported across themembrane to induce vacuolar lysis, however this is yet to bediscovered.

Virulence Determinants inDisease-Causing Species

S. flexneri has a relatively stable genome, and acquired thevirulence plasmid early in its evolution. S. flexneri is capable ofpersisting in water for several months, similarly to Vibrio cholera. This may explain its epidemiologicalprevalence, as access to human hosts for months at a timecould facilitate the endemics seen in countries with poor watersanitation. Speculatively, it may cause the most disease asS. flexneri 2a harbors the SHI-1 PAI, which encodes Pic, SigA, andShET1. Pic may confer an advantage for scavenging nutrients,therefore other strains may have a metabolic disadvantage whencompared to S. flexneri. The importance of Pic and ShET1 in pathogenesis hasalso been highlighted by . The deletion ofpic and set1AB, in addition to ospD3, in a guanine autotrophic(guaAB−) background produced an increasingly attenuatedvaccine, with none of the 14 volunteers developing diarrhea. The truncated OspZ found in S. flexneri hasa pro-inflammatory role, compared to its anti-inflammatory inthe remaining sub-species, which may confer an inflammatoryadvantage for initial establishment of infection .

Conclusion

Gene expression of virulence genes in *Shigella* play important role in spreading pathogenicity like*sigA, invE* and *virF* genes showed that thisclassical regulatory pathway of *Shigella* virulence factorgene expression can play a major role in the pathogenesisof this bacterium. This bacterium consider originating from harmless enterobacterial relatives,the structure and function of the major virulence factors,including a T3SS and cognate effector proteins, as well as theinteractions of *S. flexneri* with various host cells. All these mechanisms of virulence factors production under control quorum sensing signals.

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