

## **Shigellosis in Disasters: its detection, treatment, and methods to deal with it in disasters**

Mohammad, Heiat<sup>1</sup>. Isa, Malmir<sup>2</sup>. Hossein, Aghamollaei<sup>3</sup>. Mohammad Raza, Rezaeimehr<sup>4</sup>. Hamid, Kooshki<sup>5</sup>. Atefeh, Yaali Jahromi<sup>6</sup>

**Introduction:** Shigellosis, known as a bacillary diarrhea, is an infectious disease that begets by a gram negative bacterium called *shigella*. *Shigella*, as an invasive pathogen, has a powerful ability to cause diarrhea even with a small number of bacteria. It is one of the most important and potentially dangerous agents which can cause outbreaks in disasters. Investigation on *Shigella*, its prevention, treatment, modes of detection and also hygienic guidelines can reduce shigellosis related consequences in disasters.

**Methods:** This is a review study in which to find the required articles we searched through related databases like PubMed and Elsevier. Following keywords were used in this regard: bacteria species, growth conditions, virulence factors, diagnosis, treatment of shigellosis. Obtained data were categorized, analyzed and divided into different sections.

**Results:** Findings about bacterial morphology, growth properties, antigen patterns, clinical symptoms and diagnosis of Shigellosis, treatment, outbreak prevention in the crises and view points of some experts were separately classified.

**Conclusion:** Public and strategic educations about this pathogenic agent can decrease the backwash of epidemic events. Preparation of a plan to draw social preparation against shigellosis is can be of effective strategies to combat shigellosis in disasters.

**Keywords:** Shigellosis, Treatment, Detection, Disasters

---

1 - Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences

2 - Baqiyatallah University of Medical Sciences

3 - Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Corresponding author

4 - Jahrom University of Medical Sciences

5 - Nano Biotechnology Research Center, Baqiyatallah University of Medical Sciences

6 - Jahrom University of Medical Sciences

**Introduction**

Enterobacteriaceae account as a wide group of non-spore and Gram negative bacteria which some of them normally live in the human intestine [1]. Numerous of bacteria include *Escherichia coli*, *Salmonella*, *Klebsiella*, *Proteus*, *Entrobacter* and *Shigella* are the main members of this family. Despite normal flora of human intestine is composed of some of this group like *E. coli*, but the presence of some other members such as various strains of *Shigella* can cause disease in human [2 ,3]. For the first time, Shiga, a Japanese scientist, could separate *shigella* from the environment. First report of shigellosis, a disease caused by *Shigella*, referred to 4<sup>th</sup> century AD. As shigellosis can rapidly contaminate a lot of soldiers and refugees, it must significantly be mentioned from the viewpoints of disasters and military [4 ,5]. At recent 100 past years, *S. dysenteriae* serotype 1 was introduced as the essential cause of epidemic diarrhea with different mortality rate through the world. [2 ,4]. During 30 past years several wide pandemics caused by *S. dysenteriae* serotype 1 has been reported in Central America, Bangladesh, south and central Asia, Middle East and South Africa. There is not still enough and exact information about the number of patients all over the world [6]. Nonetheless some estimations and also conservative survey suggested that approximately 90 million of people annually involve to diarrhea which more than 100 thousand of them will die. Most of these mortality cases occur among children who live in developing countries[7]. According to the data reported by WHO, *Shigella* causes severe disease in passengers and military personnel in the industrial countries. On average, more than 18 thousand shigellosis cases are reported annually in the United State. Children, elderly and weak people are the most competent persons to catch this disease. Patients with immune deficiency disease such as HIV positive persons are severely encountered to disease [8] However according to statistical surveys, people who live in developing countries have the more risk for catching disease and the mortality rate in such country is more than industrial ones so that annually about 5 to 15 percent of patients will die [9]. *Shigella* is one of the most important and potentially dangerous agents which cause disease outbreak in disasters, so investigations about *Shigella* and its related issues like prevention, treatment, and detection methods and also hygiene guidelines, can reduce shigellosis damages in the disaster events. Therefore our main goal in this review article is to illustrate some problems and collect some categorized information about said points.

**Methods:**

In this review article we tried to extract data around the main concept through the known databases include PubMed, Elsevier, Scopus and EMBASE until 2013 to get relevant articles. During this process some of the most important keywords such as *Shigella*, treatment, prevention, pathogenicity, virulence mechanism, detection etc were used. Regardless to any categorization, about 80 full articles and 40 Abstracts were found from mentioned sources and subsequently different methods and information were classified and compared with each other. Then the main collected points were summarized, analyzed and divided into different sections. In order to more analysis, obtained results about bacterial morphology and growth properties, antigen patterns, clinical symptoms and diagnosis of Shigellosis, treatment, outbreak prevention in the crisis condition etc, were presented to some experts and their view points were embedded in article. Finally, all income items and findings were grouped in different content.

**Results:*****Morphology, Growth properties and Stains of Shigella***

*Shigella* is bacilli shaped, non-motile, non-spore and flagella and non-capsulate bacteria. Short coco-bacilli shape of bacteria can be observed in the fresh culture. It is 1-6 micrometer in length and 0.3-1 micrometer in diameter [2, 4]. While *Shigella* is an aerobic bacterium it can be alive in anaerobic conditions. The antigen pattern of *shigella* is very extensive and complicated. This bacterium has a lot of joint antigen with other genus of enterobacteriaceae. *shigella* doesn't have the H antigen. Its O-antigen is made of lipopolysaccharide. The polysaccharide site of O-antigen is very exclusive and makes it different from other types of antigens. [2, 4] Based on O-antigen part of lipopolysaccharide, *Shigella* genus divided into four groups include group A (*S. dysenteriae*), group B (*S. flexneri*) group C (*S. boydii*) and group D (*S. sonnei*) [10, 11]

***Shigella stability***

Various types of *Shigella* strains are more sensitive to physical and chemical agents rather than *salmonella* or other enteric bacteria. *Shigella dysenteriae* is more sensitive rather than other strains.

*Shigella sonnei* is more resistant among others against cold and dryness. Incubation for one hour at 55°C and phenol 1% for 30 min can kill the *Shigella* bacterium. *Shigella* cannot tolerate the high acidic concentrations. Also high concentration of bile salt will block the growth rate. *Shigella* is sensitive to light and dryness while low heat and humid conditions is suitable for its growth so that it is able to be alive in water with normal temperature for six months. Dried diarrhea reproductive bacilli on bed sheet can be alive for six weeks. *Shigella* can be alive for about 10 days in soil. If feces have the alkaline pH, bacteria will be able to be alive for some days but in acidic pH those are rapidly killed. Pasteurization and colorization can generally kill and remove *Shigella* contamination [2].

### ***Bacterial pathogenesis factors***

Before addressing the details of virulence factors, we refer to a series of key points. Cellular pathogenesis and clinical manifestation of shigellosis is the sum of a large number of bacterial virulence factors. The main part of molecular mechanism of *Shigella* is invasion to the host cells and intracellular survival. The factors which controls this part are coded on both virulence plasmid and the chromosome [5, 12, 13] Some infectious islands are in *Shigella's* Chromosomes and plasmids named PAI. The islands are usually between different Insertion sequences that can transfer genes from plasmid to chromosome and vice versa, making various varieties [14, 15]. So-called SHI are the PAIs on chromosomes. They have some types like SHI-1, SHI-2, SHI-3, SHI-O and SRL. Some of Pathogenic proteins factors encoded by plasmid include: 1) *virF* (virulence F): The position of this gene is almost 50 kb far from the plasmid replication initiation origin. This gene encodes a 30kD protein that is responsible for invasion and is necessary for regulating the expression of virulence genes. 2) *virG*: This gene encodes a 130kDa protein placed in the outer membrane of bacteria. This protein acts as a cell surface antigen that is responsible for concentrating the F-actin and puts the actin filaments at the end of the bacterium to form a long tail. 3) *virB*: *virB* gene is activated by the *virF* gene production. *virB* gene produce a 33kD protein that regulates the transcription of genes in the *ipa* operon [16, 17].

4) *icsA* (Inter cellular spear): This gene encodes a 100kDa protein that expands infection among cells. 5) *icsB*: encodes a protein that appears on the bacterial surface and is required for cell to cell spreading. 6) *ipaA, B, C, D* (invasion plasmid antigen): Productions of these genes are important antigens and the host of Shigellosis will have a strong humoral immune response against these antigens. These antigens are present on the cell surface. They include: a) *ipaA*: encodes a 78kDa protein. Activity of this protein is partly unknown, but its role in actin depolymerization in the host cell recently has been established. b) *ipaB*: encodes a 62kDa protein that after inducing by IpaD is called to be recruited on the cell surface and after making a complex with IpaC creates a pore in the host cell membrane. c) *ipaC*: encodes a 43kDa protein that together with IpaB and IpaA form a complex interacting with the host cell membrane and stimulate cellular signal cascades to the entry of bacteria through endosom. d) *ipaD*: encodes a 37kDa protein applying IpaB on the bacterial surface and begins invasion process [18].

Lipopolysaccharide is suggested as an antigen of *Shigella* and is considered as a component of bacterial virulence factors. Lipopolysaccharide is a complex combination of lipids and polysaccharides. Its molecular weight is about 10kDa and is usually hydrophobic and its toxicity is due to lipid A [19, 20]. Shiga toxin composed of A and B subunit, which is as a set of hexamer with molecular weight of about 70kDa.

#### ***Populations that are at risk of shigellosis***

All people could be susceptible to this disease. However, children in kindergarten centers, native Caucasus, international travelers, gay men, and patients with HIV infection are at highest risk of disease [4, 21]. Populations of insects such as mosquitoes and flies are abundant in regions that are as well as a group of people who are living in camps or are at risk does, because the disease can be transmitted from person to person and the most transfer mediums are food, fingers, feces and flies. Swimming in contaminated water with this bacteria, using equipments suspected to be Infected specially items associated with food can increase the risk of catching the disease.

***Clinical symptoms of Shigellosis***

Disease caused by *Shigella* is called Shigellosis. Shigellosis is an infectious disease that affects a part of the small intestine. Most Symptoms of *Shigella* infection include diarrhea, fever, nausea, vomiting, cramps and abdominal pain, blood, mucus or pus in the stool. Sudden onset of *shigella* is associated with abdominal pain, fever and watery diarrhea that just occurs 4 hours after bacterial spreading in the ileum and colon.

Daily, 300-200 mg serum protein is also lost in the feces. Its symptoms will be observed between 1 to 7 days after entry of bacteria. The diagnosis is based on blood and feces. Neutrophil in stool smear is a reliable sign of infection. [2]. Bacteria produce toxins that usually attack the epithelial cells of the colon and causes wounds and inflammation in the intestinal wall. These toxins may have two roles, firstly, the toxin acts like enterotoxin and produce watery diarrhea, which occurs early in the disease, secondly, after local invasion of bacteria in the gut due to inhibition of protein synthesis and cytotoxic properties, the cells are destroyed and the wound is created. In this situation, diarrhea with blood and mucus is observed. Toxin binds to receptors on the surface of epithelial cells and activates adenylate-cyclase, so that water and electrolytes flow into the intestine. Endotoxin has clinical effects such as hemorrhage, falling blood pressure, leukopenia, and hypoglycemia. Adrenal gland is vulnerable to the toxin [4, 21]. During diarrhea, *Shigella* can be found in stool with approximate rate of  $10^6$ - $10^8$  [4, 21]. After bacterial colonization in the intestine the number of bacteria increases to ten billion bacteria per cubic centimeter in stool.

***Laboratory Diagnosis of Shigellosis***

Samples are excreted feces, mucus and blood. The best samples for diagnosis achieves in rectum sigmoid by using of a swab. Stool samples could also be used for laboratory diagnosis. For this purpose direct method of stool examination, stool culture and serological tests are used. These bacteria have the ability to shift to Viable But Non-Culturable (VBNC) phase and thus can hide itself from the perspective of conventional diagnostic systems [22]. Laboratory Diagnosis of Shigellosis includes:

1) Direct stool examination: The direct stool examination cannot distinguish between diarrhea producing bacteria from each other. Infection source can be detected via identical methods include a cytology method of stool and the presence or absence of ameba or other parasites. In the hemorrhagic bacillary dysentery, approximately 90% of cells existed in stool consist of polymorph nuclear cells while in the amebial diarrhea and *salmonella* diarrhea there is lower rate of PMNs and more monocytes. 2) Stool culture: In the primary stage of the disease we can isolate the diarrhea producing bacteria from stool culture. Stool culture must be performed immediately after defecation because *shigella* will die due to the effects of stool's coliforms and its lower pH.

If immediate culture is not available the stool sample must be preserved in the glycerol buffer up to prepare instruments. Different kind of media such as deoxicolat citrate, salmonella *Shigella*, Mc conky etc can be use For culture [4, 21]. 3) Serological examination: The healthy serum can contain antibodies against *Shigella*. Therefore in the cases that increasing of Antibody titer among two tests performed in 10-15 days interval is important. Recently the indirect hemagglutination methods in which LPS connects to RBC membrane has been suggested.

### ***Shigellosis prevention and Treatment***

*Shigella* transmitted between humans by foods, feces and contaminated instruments. Most of *Shigella* infections were seen in the children at the age of less than 10 years old. In the militaries the use of drugs for shigellosis prevention was performed in the restricted times. Unfortunately, due to the wrong chemoprophylaxis, the drug resistant mutants were rapidly revealed. Since the human is a major host of pathogenic *Shigella*, for this reason the prevention methods for this organism from its sources must be associated with these parameters. 1) Sanitary control of water, foods, dairy products, from its and wastes. 2) Isolation of patients. 3) Identification of carriers special persons that work with foods [23, 25]. Nowadays, effective vaccines against Thyphoidal fever and *Vibrio cholera* are available but there are not available a suitable vaccines against pathogenic agent such as *Campylobacter*, human calsiviridae, entrotoxigenic *E. coli* and *Shigella*. Since several years ago many studies about producing an efficient vaccine that acts as an anti-shigellosis were performed. Some oral attenuated vaccines such as bivalent *Shigella flexeneri* type 2a and bivalent *Shigella sonnei* were made. The successful function rate of these vaccines was approximately 61%-65% for bivalent *Shigella flexeneri* type 2a vaccine and 57%-72% for bivalent *Shigella sonnei* vaccine. Other Types of these

vaccines such as a live attenuated *Shigella flexneri* type 2a (SC602) and *Shigella dysenteriae* type1 (SC599) were also prepared which had a mutation in *toxA* (*stx*), *int*, *iuc*, *icsa* (*virG*). Final evaluation of these vaccines did not show a good results in human [26]. In the other hand recombinant vaccines were also developed. In 2000 Turbifil and his coworkers isolated and characterized the subunits of invasion protein complex (IpaA, B, C). Then they analyzed their immunogenicity effects in the mouse and guinea pig. results showed the evaluated antibody titer against LPS and Ipa [27]. In 2006 a vaccine composed of IpaB, IpaC, IpaD that called invaplex passed the animal tests and showed a good results in the challenge with *Shigella* [24]. The latest investigation to produce a vaccine against *Shigella* refers to year 2008. When researchers established an invention that showed the IpaD polypeptide and its functional derivatives can produce anti-IpaD which is severely able to prevent *shigella* invasion [28]. Shigellosis treatment principally associated with oral rehydration solutions contain enough salt (sodium) to increase the level of ions decreased due to diarrhea. Oral rehydration for many of the patient was satisfied. Furthermore it may that some of the patients need intravenous rehydration. In many cases, the disease can be healed after 4 to 8 days without the use of any antibiotics. For shigellosis treatment, in the cases of patients that are very young or very old use of antibiotics such as ampicillin, ciprofloxacin, nalidixic acid and trimethoprim are suggested. When disease is in a very acute form and the risk of morbidity is high, in addition of the last antibiotics the use of ampicillin not amoxicillin is necessary. For shigellosis treatment anti-diarrhea drugs such as diphenoxilate and loperamide cannot be used, because they increase the period of infection. Researcher reports reveal that the use of 1 unit (2.5 gr) tetracycline orally can be useful for acute cases of dysentery. At present, ampicillin is a selective drug for shigellosis treatment that must be consumed 2 gr per day for 5 days in adults. For children this drug must be consumed 100mg per 1 kg of body weight. Cotrimoxazole can be used as an appropriate alternative for ampicillin [7, 23, 24].

#### ***Outbreak prevention in the crisis condition***

According to the top passages the importance of identification, prevention and treatment of *Shigella* completely advise. For these reason to prevention of shigellosis outbreaks in the crisis condition follow points must be considered. 1) Education: People must be known the primary knowledge about shigellosis. They must be known shigellosis symptoms, transmission ways, outbreak prevention and control disease .they must help each other to

understand those risks. In other words the knowledge is the best way for prevention of shigellosis outbreak. People that have enough knowledge about disease live in lower risk. 2) Consumption of Packed Food: Although using the pattern of water consumption and packed and hygienic food does not seem economic, it can be a perfect economic measurement in preventing infectious agents such as *shigela* which can be transmitted through infecting food. In such situation preparation and prediction of packed food is a significant measure for urgent use. However in any situation it is recommended not to use unsafe specially open and unpacked food. 3) Vaccination: Vaccination of exposed groups in the areas which has a high possibility of creating unexpected adventure can restrain *shigella's* outbreak. If vaccination is impossible, preparation and prediction of necessary vaccine & anti-serums can keep exposed people safe in urgent situation. 4) Preparation of Individual equipment: Equipments such as water Filtration instrument and chlorine tabs can be useful to be ensured of water safety. Aid teams must be equipped of special dressing, disinfecting agents, and sampling devices. 5) Preparing disinfecting agents: Predicting the preparation of disinfecting agents in order to disinfect major sources of infection like water tanks in a wide volume can be a measure toward decreasing the risks and number of patients due to shigellosis. 6) Drugs' preparing: Predicting and preparation of necessary drugs is a vital measurement for the treatment of people and patients. The suitable amount of stock and the area of drug stores must be pre-determined. These predictions can lead to performance of useful and quick measurements in order to access the drugs. 7) Environment Improvement: Development and expansion of urban and rural environment in order to prevent food and water pollution and hygienic burial of rubbish can lead to the decrease of people infection to diarrhea. 8) Empowering and Promoting Safety Points: Empowering and promoting safety points within a country which follows personal & social safety promotion will be a big step in conflict to probable application of biologic agents. Using bio-safety rules in all hygienic clinical centers and preventing illegal people traffic into lab. Centers & hospitals can increase safety coefficient. 9) Empowering Hygienic Remedial Systems: In order to diagnose, prevent, and remedy, the hygienic remedial system must be empowered. If the hygienic remedial system of a country is more powerful and advanced, preventing, diagnosing, and treatment of infecting agents such as *shigela* will be performed in less time and low cost. Below is the list of vital measurements which are important factors to empower the hygienic remedial system. 10) Making labs equipped: Prediction and making country's labs equipped toward quick, accurate, and

proper diagnosis of the infectious agent can announce the danger at least possible time. Performing the test of periodic sensitivity of the infectious agent's antibiotic resistance in the labs and reporting their results to responsible centers is a kind of readiness toward conflict to the disease. The preventive measurements can be performed through labs' caution and prevent the danger of society health via daily tests of drinking water, food, sampling, and hygienic control of the environment regarding the existence of pathogen's microorganisms before spreading the infectious agent. Nowadays the significance of using quick diagnosis methods of biologic factors and their toxin such as PCR and ELISA and LFA systems is completely cleared for all hygienic remedial systems of the world countries and also follows gallant advances. Many bacterial and fungal agents and toxins which are transmittable through food can be diagnosed via these methods. Researchers with the aim of accessing to technical knowledge of producing quick diagnosis kits numerously must be performed by researchers and research centers.

11) Prediction of quarantine and isolation: Necessary prediction for patients' isolation and even quarantine of injured and the patients with *shigella* diarrhea far from normal ones will be urgent and prevent spreading infection.

12) Epidemiology Studies: Epidemiologic survey in different areas toward disease assessment and creating applicable systems in order to report cases to clinics immediately is a kind of preventive measurement toward infection control in a crisis.

13) Informing: The country's hygienic remedial system including state and private sectors, welfare, etc. must have the ability of encountering possible adventures and infection outbreak via systematic contact. Informative system requires cooperation of coherent organs such as the organizations related to public hygiene, medical researches' centers, administrative of hygienic services and their network, occupation groups, medical & hygienic equipment makers, broadcasting, press, and international organizations. Connecting infectious sectors with each other and receiving momentarily reports and accurate executing of rules and hygienic standards under the survey of infection experts in conflict to any possible adventure will depend on suitable organizing of medical team. While an unexpected adventure happening, giving proper & enough information at a suitable time to people is necessary to prevent any toll and decrease their panic and fray. It is obvious that the decreasing mortality amount, preventing adventure occurrence, and decreasing crude reaction of people against these adventures are some of the aims of this program.

**Conclusion:**

*Shigella* is an important productive factor of inflammatory acute diarrhea and a gram negative bacterium without Spore which can create disease with a low amount. Disregarding hygienic principles, polluted water and food sources, which will be created in a crisis easily, are the major factors of spreading this bacterium and infection accordingly. Training exposed people before the adventure occurrence is the most important principle of encountering to infection. As *shigella* is one of the most dangerous and spreading factors of making disease in most unexpected adventures. Making people informed of this factor and its derived diseases can be a preventive measurement toward decreasing the number of affected ones. However adjusting a compiled program for continuous training of people of the society is a prompt affair. Subjects like using packed food, prediction and preparation of disinfecting agents, observing personal hygiene, preparing individual equipment and the guidance of their usage, preventing the spread of infection to the others, required drugs and the way of their use are such cases which must be notified in training. Local and country authorities must also be trained and these training should be repeated and updated on a regular program. Performing maneuver can determine strength and weakness points. Developing hygienic remedial system is the most duty of authorities. In order to decrease the amount of toll and cost in crisis situation, improving the environment and water nets, drug prediction in sufficient amount and determining a suitable place for their storage, adjusting bio-safety principles, and making them local must also be performed before the crisis. Developing quick systems for *Shigella* diagnosis, based on molecular methods and making lab and clinical centers equipped of these diagnosis system is necessary for timely reaction to *Shigella* outbreaks. Moreover, creating networks in order to immediate report of suspect and confirmed cases to the related authorities is necessary to decrease epidemic situations.

**References:**

1. Ryan, K.J. and C.G. Ray, Sherris medical microbiology 2010: McGraw Hill Medical.
2. Kay, B., J. Clemens, and K. Kotloff, Generic protocol to estimate the burden of shigella diarrhoea and dysenteric mortal. World Health Organization Geneva. Tex Book, 1999.
3. Potter, J.F., Water recreation and disease: Plausibility of associated infections: Acute effects, sequelae and mortality, by Kathy Pond, 2005. London and Seattle: IWM publishing in association with WHO, 239pp., ISBN 9-241-56305-2, £ 75, US 150 (hardback), for IWA members £56.25, US 112.50. The Environmentalist, 2006. 26(4): p. 329-329.
4. Jonsson, J., et al., Late detection of a shigellosis outbreak in a school in Madrid. Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin, 2005. 10(10): p. 268-70.
5. Mims, C.A., et al., Medical microbiology 1993: Mosby Europe Limited.
6. Ram, P.K., et al., Part II. Analysis of data gaps pertaining to Shigella infections in low and medium human development index countries, 1984-2005. Epidemiology and infection, 2008. 136(5): p. 577-603.
7. Ojha, S.C., et al., A Pentaplex PCR Assay for the Detection and Differentiation of Shigella Species. BioMed Research International, 2013. 2013.
8. Angulo, F.J. and D.L. Swerdlow, Bacterial enteric infections in persons infected with human immunodeficiency virus. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 1995. 21 Suppl 1: p. S84-93.
9. Todar, K., Todar's online textbook of bacteriology 2006: Kenneth Todar, University of Wisconsin-Madison Department of Bacteriology.
10. Baron, S., T.L. Hale, and G.T. Keusch, Shigella. 1996.
11. Bodhidatta, L., et al., Establishment of a *Shigella sonnei* human challenge model in Thailand. Vaccine, 2012.
12. Sansonetti, P.J., Rupture, invasion and inflammatory destruction of the intestinal barrier by Shigella, making sense of prokaryote-eukaryote cross-talks. FEMS microbiology reviews, 2001. 25(1): p. 3-14.
13. Sasakawa, C., et al., Molecular alteration of the 140-megadalton plasmid associated with loss of virulence and Congo red binding activity in Shigella flexneri. Infection and immunity, 1986. 51(2): p. 470-5.
14. Buchrieser, C., et al., The virulence plasmid pWR100 and the repertoire of proteins secreted by the type III secretion apparatus of Shigella flexneri. Molecular microbiology, 2000. 38(4): p. 760-71.
15. Schmidt, H. and M. Hensel, Pathogenicity islands in bacterial pathogenesis. Clinical microbiology reviews, 2004. 17(1): p. 14-56.
16. Islam, D., et al., In situ characterization of inflammatory responses in the rectal mucosae of patients with shigellosis. Infection and immunity, 1997. 65(2): p. 739-49.
17. Tobe, T., et al., Temperature-regulated expression of invasion genes in Shigella flexneri is controlled through the transcriptional activation of the virB gene on the large plasmid. Molecular microbiology, 1991. 5(4): p. 887-93.
18. Heiat, M., et al., Cloning and expression of n-terminal region of IpaD from Shigella dysenteriae in E. Coli, J Paramedical Sciences, 2010. 1(4): p. 12-17.
19. Guichon, A., et al., Structure-function analysis of the Shigella virulence factor IpaB. Journal of bacteriology, 2001. 183(4): p. 1269-76.

20. Sansonetti, P.J., Genetic and molecular basis of epithelial cell invasion by *Shigella* species. *Reviews of infectious diseases*, 1991. 13 Suppl 4: p. S285-92.
21. Goosney, D.L., D.G. Knoechel, and B.B. Finlay, Enteropathogenic *E. coli*, *Salmonella*, and *Shigella*: masters of host cell cytoskeletal exploitation. *Emerging infectious diseases*, 1999. 5(2): p. 216.
22. Heiat, M., et al., Application of extreme environmental conditions to resuscitation of viable but non culturable *E. coli* DH5 $\alpha$ . *European Journal of Experimental Biology*, 2012. 2(6): p. 2033-2037.
23. Jawets, E. and J. Melnick, REVIEW OF MEDICAL MICROBIOLOGY, 16a. edición, 1984, Lange Medical Publications.
24. Kaminski, R.W., K.R. Turbyfill, and E.V. Oaks, Mucosal adjuvant properties of the *Shigella* invasin complex. *Infection and immunity*, 2006. 74(5): p. 2856-66.
25. Coimbra, R.S., et al., Molecular and phenotypic characterization of potentially new *Shigella dysenteriae* serotype. *Journal of clinical microbiology*, 2001. 39(2): p. 618-21.
26. Coster, T.S., et al., Vaccination against shigellosis with attenuated *Shigella flexneri* 2a strain SC602. *Infection and immunity*, 1999. 67(7): p. 3437-3443.
27. Turbyfill, K.R., A.B. Hartman, and E.V. Oaks, Isolation and characterization of a *Shigella flexneri* invasin complex subunit vaccine. *Infection and immunity*, 2000. 68(12): p. 6624-32.
28. ALLAOUI, A., et al., *Shigella* Ipad protein and its use as a vaccine against *Shigella* infection, 2008, WO Patent WO/2008/044,149.