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Invasion Gene (virF and ial) Profiling of *Shigella flexneri* from Pediatric Diarrheal Infections in Al-Diwaniyah

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Abstract *Shigella flexneri* is among the common causes of bacillary dysentery in children, especially in developing countries. The intracellular invasion and replication of *Shigella* within intestinal epithelial cells are regulated by a number of virulence-associated genes, the most important of which are virF and ial, situated on a large virulence plasmid. One hundred stool specimens were obtained from pediatric patients suffering from acute diarrhea attended Al-Diwaniyah, Iraq. *Shigella flexneri* was confirmed through microbiological and biochemical tests among a portion of the isolates from which genomic DNA was extracted for molecular studies. PCRs were carried out as described for the virF and ial genes using specific primers. The virF gene was detected in 40% isolates, and the ial gene in 30% of isolates, and the combination of both genes was noted in few percentages of isolates. These results reveal significant genetic diversity in circulating strains and emphasize the potential of molecular screening for highly virulent isolates.The aim of the study was to detect the presence of virF and ial genes in *Shigella flexneri* isolates to assess genetic variability and identify potentially more virulent strains. Comprehension of the distribution of invasion associated genes may strengthen epidemiological monitoring and might have potential implications to improve diagnostic methods and preventive measures for pediatric shigellosis.

Keywords*: Shigella flexneri*, *virF* gene, *ial* gene, pediatric diarrhea, PCR

Introduction

Shigellosis is a major public health problem world-wide, especially in areas with poor sanitation and high population density. This extremely transmissible variant of bloody stool is induced by species of Shigella and has been particularly prevalent in children under the age of five year [1, 2]. Of the four Shigella species identified, *S. flexneri* is the most commonly isolated in low- and middle-income countries and causes the majority of endemic disease [3,4].The World Health Organization has placed *Shigella* spp. on its priority pathogen list because of the rising antimicrobial resistance (AMR) and the high disease burden in children [5]. Shigella infections are listed as causing more than 4 million cases and 100,000 deaths, annually around the world [6]. Shigellosis can manifest clinically as mild, watery diarrhea or severe dysentery and is primarily determined by the virulence properties of the infective strain [7].Invasion of colonic epithelial cells and intracellular survival are both critical phases in the pathogenesis of *S. flexneri*. This invasive behavior is largely facilitated by a massive 220 kb virulence plasmid, which harbors a number of genes that contribute to the function of a type III secretion system (T3SS), allowing direct delivery of effector proteins into host cells [8,9]. The best-known virulence markers are the virF and ial genes.VirF is the product of the virF gene and is a positive transcriptional regulator of both the invasion and ial defines a hierarchy in the invasion of host cells by *S. flexneri*, gene loci. It is considered as the master of the regulatory cascade mediating the activation of factors that serve essential functions such as virB, ipaB and ipaC used to penetrate the host cell [10,11]. On the other hand, the ial gene (invasion-associated locus) is present on the invasion plasmid, and is thought to play an important role in the maintenance of an invasive phenotype, notably being involved in both intracellular multiplication and intercellular spread [12,13].Because of their close correlation with virulence, virF and ial have been suggested as molecular markers for both diagnostic and

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epidemiological uses [11,14]. PCR assays for detection of these genes provides a quick, highly specific means to identify invasive Shigella, differentiating them from non-invasive enteric bacteria and thereby facilitating more focused public health efforts [9,15].Monitoring virulence gene occurs more important With the growing threat of multi-drug resistant *Shigella flexneri* isolates and the lack of a licensed vaccine, Knowledge on frequency and distribution of invasion-associated genes in clinical isolates is of particular importance for understanding bacterial pathogenicity and is a key factor to diagnose and control such infections.Accordingly, the current study was aimed at detecting and profiling of the invasion's associated virulence genes (virF& ial) among clinical isolates of *S. flexneri* that recovered from pediatric diarrhea patient in Al- Diwaniyah city within Iraq. By traditional PCR methods.

Materials and Methods

Ethical Approval

This research was authorized by the Scientific and Ethical Committee in the Department of Biology, College of Education, University of Al-Qadisiyah (Approval number: 35- 25/1/2025). Written institutional consent was not necessary for the acquisition and processing of clinical specimens. Nonetheless, oral informed consent was sought from the parents or legal guardians of all children before stool collection. Throughout the observation, patient privacy and data confidentiality were kept confidential.

Sampling and Bacteriological Identification

One hundred stool samples were collected from children with acute diarrhoeal disease in the city of Al-Diwaniyah. Specimens were collected in sterile containers and transported at 4°C to the microbiology laboratory. The samples were all analyzed within 2 h after collection. Isolates were inoculated on selective medium, MacConkey and Xylose Lysine Deosycholate(XLD) agar, and were aerobically incubated at 37°C for 24 h. Colonies with morphological features indicative of Shigella, such as the pale color on MacConkey agar and the pink colonies on XLD agar, were chosen for additional testing.

Morphological and Biochemical Features

Presumptive Shigella colonies were examined microscopically refer to Gram staining. Furthermore, the isolates were conscious using the standard biochemical tests in agreement with the standard diagnostic technique including catalase and oxidase test, TSI agar slant reaction, methyl red test, indole production, Voges-Proskauer test, citrate utilization, urease activity, lactose fermentation, and all media and reagents used were ready according to manufacturer instruction and then incubated properly.

Genomic DNA Extraction

The genomic DNA of the ten *Shigella flexneri* isolates were extracted by a commercial genomic DNA extraction kit (Taiwan) according to the manufacturer's instructions. DNA extraction quality and quantity were confirmed with the Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). The DNA samples were stored subsequently at –20°C for later analysis.

PCR Amplification of virF and ial Genes

Conventional PCR was used to detect the virulence genes virF and ial. The primers used, product sizes, annealing temperatures, and references are presented in Table 1.

Table 1. PCR primers used in the study

Gene			Product Size (bp)	Annealing Temp (°C)	Reference
virF	F (5'-3')	CCTCAGAATAGGAGTGT TGAA	331	55	(16)
	R (5'-3')	TCTTAGTTACTCTGTAAACAC			
ial	F (5'-3')	CTGGATGGTATGGTGAGG	320	58	(17)
	R (5'-3')	GGAGGCCAACAATTATTTCC			

PCR Reaction Mixture

Polymerase chain reaction (PCR) was carried out in a final reaction volume of 25 µL. Each reaction contained 12.5 µL of 2X PCR Master Mix (Bioneer, Daejeon, South Korea), 1 µL of forward primer and 1 µL of reverse primer (each at a concentration of 10 pmol/µL), 2 µL of genomic DNA template, and 8.5 µL of nuclease-free water to complete the volume. All reagents were mixed gently and briefly centrifuged before thermal cycling (Applied Biosystems Veriti™, Thermo Fisher Scientific, USA).

Thermal Cycling Conditions

Amplification of the target genes was performed in a thermal cycler using the following cycling parameters: an initial denaturation step at 95°C for 5 minutes, followed by 35 amplification cycles consisting of denaturation at 95°C for 30 seconds, annealing at 55°C for virF or 58°C for ial for 30 seconds, and extension at 72°C for 45 seconds. The program concluded with a final extension step at 72°C for 5 minutes to ensure complete product elongation (16, 17).

Gel Electrophoresis

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PCR products were resolved on a 1.5% agarose gel stained with Safe Stain to visualize the DNA bands. Electrophoresis (Scie-Plas, UK) was performed at 100 volts for 45 minutes in 1X TAE buffer. A 100 bp DNA ladder was included alongside the samples to estimate fragment sizes. DNA bands were visualized using a UV transilluminator (Scie-Plas, UK) and photographed for documentation.

Results

Gram staining was performed in microscopical examination on suspected Shigella colonies. The isolates were Gram-negative, non-motile, non-spore-forming short rods. Biochemical identification was done according to routine practices. The catalase and oxidase positive isolates, were examined. They fermented the TSI with an alkaline slant and acid but (K/A), with no H_2S production and gas. Other characteristics were a positive test with methyl red (MR), but test-negative for indole production, Voges-Proskauer test, citrate utilization, urease activity and lactose fermentation. Ten isolates whose morphological and biochemical characters matched to *S. flexneri* were confirmed by biochemical tests as S. flexneri, as showed in table 2

Table 2. Morphological and biochemical characteristics of *Shigella flexneri* isolates

Test	Result
Gram stain	Gram-negative short rods
Motility	Non-motile
Spore formation	Non-spore-forming
Catalase	Positive
Oxidase	Positive
TSI reaction	K/A (Alkaline slant / Acid butt)
H_2S production	Negative
Gas production	Negative
Methyl Red (MR)	Positive
Indole	Negative
Voges-Proskauer	Negative
Citrate utilization	Negative
Urease activity	Negative
Lactose fermentation	Negative

Based on culture characteristics and biochemical testing, 10 isolates (10%) were confirmed as *Shigella flexneri*. Genomic DNA was successfully extracted from all 10 isolates and used as templates for PCR analysis to detect the presence of the virulence-associated invasion genes virF and ial.PCR results revealed that the virF gene was detected in 4 out of 10 isolates (40%), specifically in

isolates 1, 2, 3, and 4. The ial gene was detected in 3 out of 10 isolates (30%), observed in isolates 3, 4, and 5. Notably, isolate 4 tested positive for both genes, suggesting a high virulence potential.The distribution of the two genes among the isolates is shown in Table 3 and Figure 1, 2.

Table 3. Distribution of virF and ial genes among *S. flexneri* isolates

Isolate No.	virF	ial
1	+	-
2	+	-
3	+	+
4	+	+
5	-	+
6–10	-	-

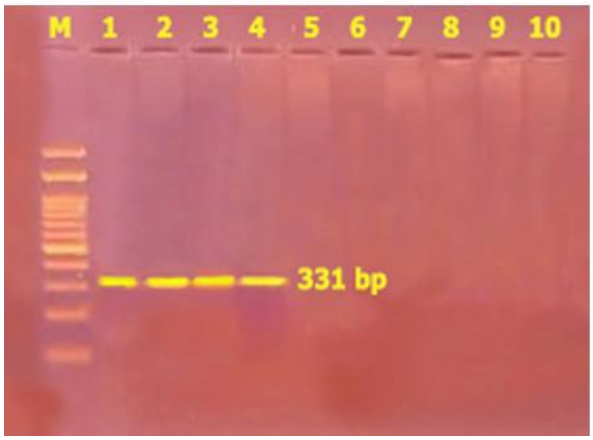

Figure 1. PCR amplification of the virF gene (331 bp) in *S. flexneri* isolates. Agarose gel electrophoresis showing PCR results for ten clinical isolates. Clear bands at 331 bp were observed in lanes 1–4, indicating positive amplification of the virF gene. Lanes 5–10 showed no bands. Lane M represents the 100 bp DNA ladder used as a molecular size marker.

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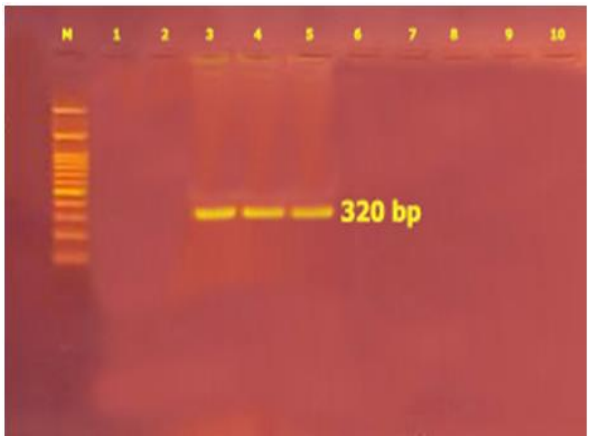

Figure 2. PCR amplification of the ial gene (320 bp) in *S. flexneri* isolates. Agarose gel electrophoresis showing PCR results for ten clinical isolates. Lanes 3–5 show positive bands at 320 bp, indicating the presence of the ial gene. Lanes 1–2 and 6–10 show no amplification. Lane M represents the 100 bp DNA ladder used as a molecular size marker.

Discussion

The molecular investigation of virulence genes in *Shigella flexneri* isolates provides valuable insights into the invasive capacity and pathogenic potential of circulating strains. In this study, the detection of the virF gene in 40% and ial in 30% of isolates reveals significant variability in the distribution of these key invasion-associated markers among pediatric clinical strains.The virF gene encodes a regulatory protein that acts as the transcriptional activator of the invasion regulon. It initiates the expression of *virB*, *ipaBCD*, and other genes required for type III secretion system (T3SS) functionality and epithelial invasion. Its presence is generally considered a hallmark of fully virulent *Shigella* strains [18]. The finding of virF in only 4 out of 10 isolates could have been due to the genetic instability of the virulence large plasmid or loss of the gene because of sub-culturing and environmental stresses as reported elsewhere [19, 20].The ial gene is involved in the intracellular multiplication and cell-to-cell spread of the bacterium, and is thus essential for tissue invasion and bacterial persistence [21]. The infrequency of isolates containing ial in the present study agrees with earlier findings that this gene is lost or mutated sporadically in certain *S. flexneri* populations [22]. While false negative PCR could arise from primer mismatches or gene rearrangements, the likelihood of this was minimized in our study due to the careful selection of gene–specific primers which have been previously tested and validated. Thus, the lack of amplification in some isolates probably indeed results from true lack of the target gene, not from technical failure [23].The presence of both virF and ial in only one isolate indicates that many of the circulating strains are likely to be devoid of an intact repertoire of invasion determinants, which may be mediated through plasmid loss or genetic silencing. This is similar to the previous study in Sudan were a small number of Shigella isolates harbored both invasion determinants [24]. Concomitant presence of virulence genes among the isolates had been associated with high disease severity in the clinical symptoms and increase in duration of disease, underpinning the diagnostic value of screening for multiple targets [25].In comparison with studies from Bangladesh and India, where the prevalence of virF and ial often exceeds 70%, the lower rates observed in this study may be attributed to geographic variation, sample size, or differences in population immunity and environmental pressures [26,27]. The serotype distribution of S. flexneri also affects virulence gene profiles; for instance, some serotypes are known to lack the invasion plasmid entirely or possess incomplete virulence gene clusters [28].Moreover, the results underscore the dynamic nature of *S. flexneri* genetics. The virulence plasmid is known to be unstable and can be lost even in vivo under antibiotic pressure or immune stress, reducing the invasive phenotype but potentially contributing to asymptomatic carriage and under-detection [29]. This raises public health concerns as non-invasive but plasmid-negative strains can still participate in transmission and reservoirs.The diagnostic implications of these findings are notable. While conventional biochemical testing confirms species identity, molecular detection of virulence genes like virF and ial adds a critical layer of information about pathogenic potential. Several studies recommend multiplex PCR targeting these genes alongside *ipaH*, *set1*, and *sen* to increase diagnostic sensitivity and specificity [30,31].Furthermore, the absence of these genes in some strains does not imply avirulence, as other chromosomal and plasmid-borne factors such as *icsA*, *virA*, *ipaH*, and *sat* may contribute significantly to invasion and immune evasion [32]. Future work using whole genome sequencing (WGS) would allow for more comprehensive profiling of virulence determinants and antimicrobial resistance patterns.

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In the local context of Al-Diwaniyah and similar Iraqi settings, limited molecular surveillance and under-reporting of *Shigella* virulence markers hinder timely responses to outbreaks. Incorporating molecular diagnostics into routine microbiological workflows is strongly recommended to inform targeted treatment, particularly in pediatric populations with high morbidity risk.Finally, understanding the prevalence and variability of *S. flexneri* virulence genes like virF and ial can aid in shaping vaccination strategies. Several vaccine candidates currently under investigation include components derived from these genes or their regulated proteins. Hence, regional molecular data are essential to support the design of locally effective vaccines.

Conclusions

This study highlights the molecular detection of the *virF* and *ial* invasion-associated genes among clinical isolates of *Shigella flexneri* collected from pediatric diarrheal cases in Al-Diwaniyah, Iraq. The results demonstrated that a subset of the isolates harbored these critical virulence genes, with *virF* present in 40% and *ial* in 30% of strains. The limited co-occurrence of both genes suggests considerable genetic variability among circulating isolates, potentially influenced by plasmid instability or regional strain dynamics. These findings reinforce the utility of molecular diagnostics in characterizing the pathogenic potential of *Shigella* and emphasize the importance of continuous surveillance of virulence determinants to support public health responses and vaccine development strategies.

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Conflict of interest

No conflict of interest is found for the present study.

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References

1. Muzembo BA, Kitahara K, Mitra D, Ohno A, Khatiwada J, Dutta S, Miyoshi SI. Burden of Shigella in South Asia: a systematic review and meta-analysis. Journal of Travel Medicine. 2023 Jan;30(1):taac132.
2. Pakbin B, Brück WM, Brück TB. Molecular mechanisms of shigella pathogenesis; recent advances. International Journal of Molecular Sciences. 2023 Jan 26;24(3):2448.
3. Connor TR, Barker CR, Baker KS, Weill FX, Talukder KA, Smith AM, Baker S, Gouali M, Pham Thanh D, Jahan Azmi I, Dias da Silveira W. Species-wide whole genome sequencing reveals historical global spread and recent local persistence in Shigella flexneri. elife. 2015 Aug 4;4:e07335.
4. Zhu Z, Wang W, Cao M, Zhu Q, Ma T, Zhang Y, Liu G, Zhou X, Li B, Shi Y, Zhang J. Virulence factors and molecular characteristics of Shigella flexneri isolated from calves with diarrhea. BMC microbiology. 2021 Dec;21:1-2.
5. Bengtsson RJ, Simpkin AJ, Pulford CV, Low R, Rasko DA, Rigden DJ, Hall N, Barry EM, Tennant SM, Baker KS. Pathogenomic analyses of Shigella isolates inform factors limiting shigellosis prevention and control across LMICs. Nature Microbiology. 2022 Feb;7(2):251-61.
6. Scott TA, Baker KS, Trotter C, Jenkins C, Mostowy S, Hawkey J, Schmidt H, Holt KE, Thomson NR, Baker S. Shigella sonnei: epidemiology, evolution, pathogenesis, resistance and host interactions. Nature Reviews Microbiology. 2025 May;23(5):303-17.
7. Raso MM, Arato V, Gasperini G, Micoli F. Toward a Shigella vaccine: opportunities and challenges to fight an antimicrobial-resistant pathogen. International journal of molecular sciences. 2023 Feb 28;24(5):4649.
8. Mattock E, Blocker AJ. How do the virulence factors of Shigella work together to cause disease?. Frontiers in cellular and infection microbiology. 2017 Mar 24;7:249163.
9. Bravo V, Puhar A, Sansonetti P, Parsot C, Toro CS. Distinct mutations led to inactivation of type 1 fimbriae expression in Shigella spp. PLoS One. 2015 Mar 26;10(3):e0121785.
10. Di Martino ML, Falconi M, Micheli G, Colonna B, Prosseda G. The multifaceted activity of the VirF regulatory protein in the Shigella lifestyle. Frontiers in Molecular Biosciences. 2016 Sep 29;3:61.
11. Fan W, Qian H, Shang W, Ying C, Zhang X, Cheng S, Gu B, Ma P. Low distribution of genes encoding virulence factors in Shigella flexneri serotypes 1b clinical isolates from eastern Chinese populations. Gut Pathogens. 2017 Dec;9:1-9.
12. Marquart ME, Picking WL, Picking WD. Soluble invasion plasmid antigen C (IpaC) from Shigella flexneri elicits epithelial cell responses related to pathogen invasion. Infection and immunity. 1996 Oct;64(10):4182-7.

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ISSN P: 1818-5746
E: 2313-4429
qjvms.qu.edu.iq

13. Cruz CB, Souza MC, Serra PT, Santos I, Balieiro A, Pieri FA, Nogueira PA, Orlandi PP. Virulence factors associated with pediatric shigellosis in Brazilian Amazon. BioMed research international. 2014;2014(1):539697.
14. Binkhamis K, Alangari S, Juma F, Althawadi S, Al-Qahtani AA, Bohol MF, Alshahrani FS, Alotaibi F. Shigella flexneri Outbreak at a Rehabilitation Center: First Report from Saudi Arabia. InHealthcare 2025 Apr 23 (Vol. 13, No. 9, p. 971). MDPI.
15. Garcia-Williams AG, Logan N, Marsh ZA. Shigella. InFoodborne Infections and Intoxications 2021 Jan 1 (pp. 221-236). Academic Press.
16. Wang SJ, Chen JH. A rapid and specific PCR method for the detection of Shigella spp. in spiked samples. Journal of Food and Drug Analysis. 2012;20(1):17.
17. Vargas M, Gascon J, Jimenez De Anta MT, et al. Prevalence of Shigella enterotoxins 1 and 2 among Shigella strains isolated from patients with traveler's diarrhea. J Clin Microbiol. 1999; 37:3608–11.
18. Batalha F, Cordeiro IB, Taquita P, Verçosa JV, Cavalcante A, Santos I, Figueiredo D, Souza RM, Dantas P, Orlandi P. Proteomic Analysis of Virulence Profiles in Clinical Strains of Shigella flexneri in Manaus–Amazon State.
19. Moses AE, Udo EE, Akpanudo EI, Moses EA. Detection, risk factors and antibiogram of Shigella dysenteriae and Shigella flexneri in young children with acute gastroenteritis in Uyo Metropolis, Nigeria. World Journal of Biomedical Research. 2023;10(1):1-1.
20. Mohamadi F, Pourakbari B, Hosseinpour Sadeghi R, Sotoudeh M, Mahmoudi S, Mamishi S. Changing Epidemiology and Antimicrobial Resistance of Bacterial Childhood Diarrhea: Insights from a 7-Year Study in an Iranian Referral Hospital. Foodborne Pathogens and Disease. 2025 Apr 1;22(5):309-15.
21. Shrestha SK, Shrestha J, Mason CJ, Sornsakrin S, Dhakhwa JR, Shrestha BR, Sakha B, Rana JC, Srijan A, Serichantalergs O, Sethabutr O. Etiology of acute diarrheal disease and antimicrobial susceptibility pattern in children younger than 5 years old in Nepal. The American Journal of Tropical Medicine and Hygiene. 2022 Dec 5;108(1):174.
22. Ndungo E, Bhaumik U, Liang Y, Chen WH, Travassos MA, Tapia MD, Kotloff KL, Levine MM, Pasetti MF. Shigella humoral immunity during the first 2 years of life in children from endemic areas. mBio. 2025 May 14;16(5):e00555-25.
23. Begum K. *Plasmid analysis of shigella strains isolate in Bangladesh with special reference to Drug resistance and invasive character* (Doctoral dissertation, © University of Dhaka).2025.
24. Zakir Hossain AK, Zahid Hasan M, Mina SA, Sultana N, Chowdhury AM. Occurrence of shigellosis in pediatric diarrheal patients in Chattogram, Bangladesh: A molecular based approach. Plos one. 2023 Jun 15;18(6):e0275353.
25. Chung The H, Bodhidatta L, Pham DT, Mason CJ, Ha Thanh T, Voong Vinh P, Turner P, Hem S, Dance DA, Newton PN, Phetsouvanh R. Evolutionary histories and antimicrobial resistance in Shigella flexneri and Shigella sonnei in Southeast Asia. Communications biology. 2021 Mar 19;4(1):353.
26. Kerketta S, Rout UK, Kshatri JS, Kerketta AS, Paikaray AK, Dash A, Pradhan R, Padhi A, Patra T, Behera AK, Swain SP. An acute diarrheal disease outbreak in urban setting of Odisha, India. BMC Infectious Diseases. 2025 Feb 27;25(1):283.
27. Zhang W, Zhou CL, Hu Y, Lin L, Li J, Xu Y, Cui S. Dissemination of multiple drug-resistant Shigella flexneri 2a isolates among pediatric outpatients in Urumqi, China. Foodborne Pathogens and Disease. 2022 Aug 1;19(8):522-8.
28. Ghosh D, Chowdhury G, Samanta P, Shaw S, Deb AK, Bardhan M, Manna A, Miyoshi SI, Ramamurthy T, Dutta S, Mukhopadhyay AK. Characterization of diarrhoeagenic Escherichia coli with special reference to antimicrobial resistance isolated from hospitalized diarrhoeal patients in Kolkata (2012–2019), India. Journal of Applied Microbiology. 2022 Jun 1;132(6):4544-54.
29. Uddin MS, Rahman MM, Faruk MO, Talukder A, Hoq MI, Das S, Islam KS. Bacterial gastroenteritis in children below five years of age: a cross-sectional study focused on etiology and drug resistance of Escherichia coli O157, Salmonella spp., and Shigella spp. Bulletin of the National Research Centre. 2021 Dec; 45:1-7.
30. Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P, Zaidi AK. Shigellosis. The Lancet. 2018 Feb 24;391(10122):801-12.
31. Khan WA, Griffiths JK, Bennish ML. Gastrointestinal and extra-intestinal manifestations of childhood shigellosis in a region where all four species of Shigella are endemic. PloS one. 2013 May 17;8(5):e64097.
32. Yaghoubi S, Ranjbar R, Dallal MM, Fard SY, Shirazi MH, Mahmoudi M. Profiling of virulence-associated factors in Shigella species isolated from acute pediatric diarrheal samples in Tehran, Iran. Osong public health and research perspectives. 2017 Jun 30;8(3):220.