

Multidrug Resistant *Shigella* Associated with Class 1 Integrase and Other Virulence Genes as a Cause of Diarrhea in Pediatric Patients

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Abstract

Background: *Shigella* is one of the most serious pathogens associated with bloody diarrhea in children. The empiric antibiotic therapy of enteric illness with blood streaked stool leads to emergence of multi drug resistant (MDR) *Shigella*. The condition gets exacerbated by presence of integrons that facilitate the horizontal spread. Virulence genes associated with MDR *Shigella* modulate the patient outcome, particularly in children. **Objectives:** The present study was aiming at isolation of MDR *Shigella* from children with diarrheal sickness and characterization of those isolates as regarding presence of class 1 integrase and other virulence genes. **Methods:** Four hundred and ninety patients under the age of five suffering from diarrheal illness were examined for presence of *Shigella* in their stool specimens. MDR *Shigella* was determined using the antibiotic susceptibility testing by disc diffusion method; those isolates were tested for presence of class 1 integrase by PCR. Multiplex PCR assay was used to determine the presence of virulence genes, *virA*, *ial*, *sen*, *set1A*, *set1B*, *sat*, *ipaBCD*, *ipaH* and *stx* in the MDR *Shigella* isolates. **Results:** The isolation rate of *Shigella* from pediatric patients was 5.3%. Most of the isolated *Shigella* (57.7%) were from infants between 12 and 23 month. 73.1% of the identified *Shigella* were MDR. *intI1* gene was present in 78.9% of MDR isolates. Multiplex PCR revealed that *ipaH* and *ipaBCD*, *virA*, *sat*, *ial*, *set1A* and *set1B*, *sen* were detected in 94.7%, 78.9%, 73.7%, 68.4%, 42.1%, 36.8% of the MDR *Shigella* isolates respectively. **Conclusion:** The MDR isolates represented a considerable percentage of *Shigella* detected in pediatric patients. Presence of *intI1* gene in most of MDR *Shigella* reflects the higher possibility of resistant strains spread. Existence of a variety of virulence genes in those isolates is an important indicator of serious disease outcome.

Keywords

MDR, *Shigella*, Integrase, Diarrhea

1. Introduction

Shigella is one of the most important pathogens associated with a serious food born diarrheal disease termed shigellosis [1]. It accounted for about 5% to 15% of diarrheal cases [2]. Human is the only reservoir of this bacterium that was found to be the cause of about 163 million cases of diarrhea in developing countries every year [3] and 1.1 million mortality per year all over the world [4], of which children under the age of ten years are mostly affected [5]. 61% of deaths caused by *Shigella* enteric illness are among pediatrics under the age of five [6].

Diarrhea streaked with blood in pediatrics is seriously considered as an important manifestation of enteric disease associated with invasion which usually leads to high morbidity and mortality outcomes; in developing countries, the commonest bacteria that mostly recovered from fecal samples of children suffering from blood streaked diarrhea are *Shigella* [7].

Beside the rehydration therapy, diarrheal diseases caused by *Shigella* usually require antibiotic administration to decrease the period of the disease and limit its spread among close associates; resistance to trimethoprim-sulfamethoxazole and ampicillin have been continuously emerged by *Shigella* isolates due to their excessive usage; ceftriaxone and flouroquinolones are suggested by the WHO for management of dysentery caused by *Shigella* in pediatric patients [8], and also they are efficient against *Shigella* isolates exhibiting multidrug resistance in immunosuppressed children [9]. Ceftriaxone is the antibiotic of choice for management of *Shigella* infection in localities at which resistance to flouroquinolones is frequent; however, azithromycin is suggested by the American Academy of Pediatrics as a substitutive therapy for dealing with *Shigella* dysentery specially caused by multidrug resistance isolates [10].

The most important predisposing factors that encourage *Shigella* species to be more resistant are the empirical therapy of blood streaked diarrhea as shigellosis, also the frequent antibiotic administration for such enteric diseases in spite being listed among causes of restricted suggestions of antimicrobial therapy as advised by the WHO [11].

Antimicrobial insensitivity in the enteric bacteria including *Shigella* is a serious problem as it records an elevated concern worldwide [12]. *Shigella* isolates also have the ability to gain resistant genetic determinant by horizontal spread and the situation becomes more aggravated by the appearance of multi drug resistant (MDR) *Shigella* species, particularly among patient suffering from diarrheal diseases [13].

Presence of genetic movable elements like transposons, insertion sequences (i.e integrons) and contagious plasmides is frequently linked to the multidrug

resistance. The relation between integrons presence and the appearance of MDR isolates was initially documented in 1980s [14].

Functionally the integron consists of three components, *intI* gene which encodes the integrase enzyme that can recombine the genetic cassettes and change them to competent genes [15], *attI* that contains antimicrobial resistance genetic cassettes [14], and the P_c promoter that controls the genetic cassettes transcription [16]. It has been documented that *Shigella* species exhibiting multidrug resistant criteria contain integrons of class 1 and class 2 [17].

Virulence factors of *Shigella* together with the patient immune response usually modulate the clinical presentation of shigellosis particularly in children. The disease spectrum is generally ranged from self limiting diarrhea which is usually mild, to severe diarrheal disease characterized by presence of blood and accompanied with fever and extra intestinal problems [13]. Intestinal cells invasion is the most important virulence character exhibited by *Shigella* isolates, it is modulated by *ipaH*, *ipaA*, *ipaB*, *ipaC* and *ipaD* genes carried by *inv* plasmid [18]. On the other hand, *ial* genes harbored by the same plasmid was found to be very important in cell to cell passage [19].

The chromosomal genes encoding *Shigella* enterotoxin 1 (*set1A* and *set1B*) are responsible for diarrhea in its watery phase. Enterotoxin 2 produced by *Shigella* species is encoded by *sen* gene and also carried by the invasion plasmid. The genus *Shigella* harbored also some members of class 1 serine protease auto-transporters of *Enterobacteriaceae* as secreted autotransporter toxin (*sat*). Cell invasion and spread are also mediated by *virA* placed on virulent plasmids [20].

Shigella dysenteriae serotype 1 express *stx-1* and *stx-2* shiga toxins which are chromosomally encoded [21] and responsible for the harmful vascular injury to the central nervous system, kidney and colon [22] and this explain the life threatening complications of infection by this type of *Shigella* species [23].

Previous studies related to *Shigella* isolation in Egypt were performed mainly to study the epidemiology of this organism and to concern certain serotypes. The aims of the present study were to isolate MDR *Shigella* from stool specimens of children with diarrheal diseases attending outpatient clinics of Mansoura University Children Hospital (MUCH) and to characterize those isolates as regarding presence of class 1 integrase and other virulence genes being important determinants of *Shigella* resistance and disease severity particularly in children.

2. Methods

The study plan:

The research protocol was agreed by the Medical Institutional Review Board in Faculty of Medicine, Mansoura University with code number: R.19.10.655, informed consent for every one of the included patients was taken.

During the time period extending from February, 2017 to August, 2019, a cross sectional descriptive study was conducted on 490 children under the age of five attending the outpatient clinics of MUCH and suffering from diarrheal

illness. Twenty six *Shigella* species were isolated from those patients; then the MDR isolates were determined and evaluated for presence of class 1 integrase and other virulence genes.

Patients' criteria and pathological samples:

Stool specimens were collected from all studied cases fulfilling the inclusion criteria which were diarrhea with passage of stool in 24 hours for at least three times, being children under the age of five, not admitted at hospital and they had not received any antibiotic therapy. Stool samples were immediately transferred to the department of Medical Microbiology and Immunology at the Faculty of Medicine, Mansoura University where they were processed in the Microbiology Diagnostic and Infection Control Unit (MDICU).

Sample processing and identification of *Shigella* isolates [24]:

Stool samples were inoculated on tubes of selenite broth media which were incubated for 24 hours at 37°C for enrichment. Next day, subculture was done on Salmonella Shigella (SS) agar, Xylose Lysin Desoxycholate (XLD) agar and Hekton Enteric (HE) agar media, *Shigella* isolates appeared as non lactose fermenting colonies on the plates were selected for further identification by the standard biochemical reactions and the API 20E (*Bio-merieux SA, Montalieu Vercica and France*)

Antibiotic susceptibility of *Shigella* isolates:

The disk diffusion technique was used to assess the antibiotic sensitivity for all isolated *Shigella* according to the CLSI, 2014 [25]. MDR *Shigella* isolates were selected as being resistant to unrelated three or more classes of antimicrobials [26].

Detection of class 1 integrase and virulence genes in the MDR *Shigella* isolates:

All the MDR *Shigella* isolates were checked for presence of Class 1 integrase by PCR using the primer pair (fw 5'-ATGGCCGAGCAGATCCTGCACG-3' and rv 5'-GCCACTGCGCCGTTACCACCGC-3') for detection of 899 bp *intI1* gene product with the following conditions: 5 minutes at 94°C for initial denaturation, then 35 cycles (30 seconds at 94°C, 40 seconds at 60°C and 1 minute at 72°C). A step for final extension was achieved at 72°C for 7 minutes. the PCR was performed with a volume of 50 µL reaction mixture including *Taq* DNA polymerase (1U), 100 ng DNA, 200 µM dNTP, 1 µM primers, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3) [17].

Multiplex PCR assay was performed for all MDR *Shigella* isolates for detection of virulence genes including (*virA*, *ial*, *sen*, *set1A*, *set1B*, *sat*, *ipaBCD*, *ipaH* and *stx*) using primers listed in Table 1. The test was conducted according to the previously described method [27] using 20 µL volum of reaction mixture including 0.5 µL (10 mM) deoxynucleotides, 0.5 µL DNA template, 0.5 µL primer of each examined gene, 0.5 µL (50 mM) MgCl₂, 0.5 µL (5 U/µL) *Taq* DNA polymerase, 2.0 µL 10× PCR buffer and 13 µL distilled water (2 µL water was added In *set1A/set1B*). The amplification conditions of the multiplex PCR were initial

Table 1. Primers used for detection of virulence genes by multiplex PCR.

The targeted gene	Primer sequence	Melting temperature (°C)	band Size, bp	Reference
<i>virA</i>	F-CTGCATTCTGGCAATCTCTTCACATC R-TGATGAGCTAACTTCGTAAGCCCTCC	58	215	[28]
<i>ial</i>	F-CTGGATGGTATGGTGAGG R-GGAGGCCAACAATTATTTCC	58	320	[28]
<i>sen</i>	F: 5'-ATGTGCCTGCTATTATTTAT-3' R: 5'-CATAATAATAAGCGGTCAGC-3'	60	799	[28]
<i>set1A</i>	F: 5'-TCACGCTACCATCAAAGA-3' R: 5'-TATCCCCCTTTGGTGGTA-3'	55	309	[28]
<i>set1B</i>	F: 5'-GTGAACCTGCTGCCGATATC-3' R: 5'-ATTTGTGGATAAAAAATGACG-3'	55	147	[28]
<i>sat</i>	F: 5'-ACTGGCGGACTCATGCTGT-3' R: 5'-AACCCCTGTAAGAAGACTGAGC-3'	55	387	[29]
<i>ipaBCD</i>	F: 5'-GCTATAGCAGTGACATGG-3' R: 5'-ACGAGTTCTGAAGCACTC-3'	60	612	[30]
<i>ipaH</i>	F: 5'-TGGAAAACTCAGTGCCTCT-3' R: 5'-CCAGTCCGTAAATTCATTCT-3'	58	423	[31]
<i>stx</i>	F: 5'-CAGTTAATGTGGTTGCGAAG-3' R: 5'-CTGCTAATAGTTCTGCGCATC-3'	60	895	[28]

denaturation for 1 minute at 94°C, then 35 cycles of denaturation for 1 minute at 94°C, annealing for 90 seconds at 58°C, extension for 1 minute at 72°C and final extension for 7 minutes at 72°C.

Statistical Analysis:

Data analysis and processing were done using SPSS, version 21 software. Description of qualitative data was done using number and percent. Chi-square test was used to check the association between categorical variables, when expected cell count less than 5, Fischer exact test was used. For all statistical tests done the threshold of significance is fixed at 5%. The results were considered to be significant when the error probability was less than 5% ($p < 0.05$).

3. Results

Twenty six *Shigella* isolates were detected in the examined 490 stool specimens that were collected from patients under the age of five and suffering from diarrhea. The prevalence of *Shigella* among studied cases was found to be 5.3%. Of the isolated 26 *Shigella* strains, 15 (57.7%) were *S. flexneri*, 6 (23.1%) were *S. sonnei*, 4 (15.4%) were *S. dysenteriae* and 1 (3.8%) was *S. boydii*.

Shigella isolation rate was found to be higher in patients under the age of two years, as 57.7% of the isolated *Shigella* were detected in infants between 12 and 23 month. There wasn't any statistically significant difference regarding distribution of *Shigella* isolates between males and females ($P = 0.1$). Throughout the study time, most of the isolated *Shigella* (69.2%) were significantly detected in

patients during warm climate, May till October (**Table 2**).

Clinical data of the studied patients revealed that, fever is the most common sign associated with *Shigella* infection as it was observed in 65.4% of patients showed culture positive results for *Shigella* with a statistically significant value ($P = 0.04$). Vomiting, bloody stool, dehydration were other common clinical features that were found to be related to *Shigella* infection as they were respectively recorded in 53.8%, 30.8% and 26.9% of *Shigella* infected patients (**Table 3**).

All of the isolated *Shigella* were sensitive to imipemem as revealed by the

Table 2. Demographic and environmental factors affecting patients infected with *Shigella* as a cause of diarrhea.

		Patients with diarrhea positive results for <i>Shigella</i> (n = 26)		Patients with diarrhea negative results for <i>Shigella</i> (n = 464)		P value
		No	%	No	%	
Age (month)	0 - 11 n = 80	7	26.9	73	15.7	0.02*
	12 - 23 n = 175	15	57.7	160	34.5	
	24 - 60 n = 235	4	15.4	231	49.8	
Gender	Males n = 204	15	57.7	189	40.7	0.1
	Females n = 286	11	42.3	275	59.3	
Weather	Warm seasons n = 219	18	69.2	201	43.3	0.01*
	Cold seasons n = 271	8	30.8	263	56.7	

*Significant P value.

Table 3. Distinctive clinical features of patients infected with *Shigella* as a cause of diarrhea.

	Patients with diarrhea positive results for <i>Shigella</i> (n = 26)		Patients with diarrhea negative results for <i>Shigella</i> (n = 464)		P value
	No	%	No	%	
Dehydration	7	26.9	126	27.2	0.8
Blood in stool	8	30.8	78	16.8	0.1
Presence of fever	17	65.4	201	43.3	0.04*
Vomiting	14	53.8	316	68.1	0.1
Hospital admission	2	7.7	43	9.3	0.9
Abdominal pain	5	19.2	172	37.1	0.1
Convulsion episodes	1	3.8	13	2.8	0.7

*Significant P value.

antibiotic sensitivity testing. The isolates recorded also good sensitivity for amikacin (96.2%) and showed a sensitivity of 92.3% for ceftazidime, ceftriaxone and cefepime. Nalidixic acid and co-trimoxazole were the least effective antibiotics against the examined *Shigella* isolates as they recorded a sensitivity of 11.5% and 19.2% respectively (Table 4). Resistance to unrelated three or more classes of antibiotics was observed in 19 isolates, accounted for 73.1% of all studied *Shigella* and considered to be MDR isolates (11 were *S. flexneri*, 5 were *S. sonnei*, 2 were *S. dysenteriae* and 1 was *S. boydii*).

Fifteen isolates among the 19 MDR *Shigella* (78.9%) were found to harbor *intI1* gene as proved by PCR assay. *intI1* gene was most frequently detected in *S. flexneri* (11 isolates) followed by *S. sonnei* (3 isolates) and *S. boydii* (1 isolate).

Multiplex PCR revealed that 18 isolates of the 19 MDR *Shigella* (94.7%) were found to be positive for *ipaH* and *ipaBCD*, 15 isolates (78.9%) were proved to be positive for *virA* gene. *sat* gene was explored in 14 isolates (73.7%), 10 of 11 (90.9%) *S. flexneri*, two *S. dysenteriae* (100%), one *S. boydii* (100%) and 1 of 5 (20%) *S. sonnei*. *ial* gene was found to be harbored by 13 isolates (68.4%) of the studied MDR *Shigella*, it was found in 9 of 11 (81.8%) *S. flexneri*, 3 of 5 (60%) *S. sonnei* and 1 of 2 (50%) *S. dysenteriae*. *set1A* and *set1B* were found to be present in 8 isolates (42.1%), all of them were *S. flexneri*. *sen* gene was detected in 7 isolates (36.8%), 3 of 11 (27.3%) *S. flexneri*, 2 of 5 (40%) *S. sonnei* and two *S. dysenteriae* (100%). None of the examined MDR *Shigella* isolates exhibited presence of *stx* gene by the multiplex PCR assay.

4. Discussion

Shigella infection as a cause of diarrheal illness remains an important dilemma in

Table 4. The pattern of antimicrobial susceptibility testing of *Shigella* isolates.

	The studied <i>Shigella</i> isolates (n = 26)			
	Sensitive		Resistant	
	No	%	No	%
Ampicillin	9	34.6	17	65.4
Co-trimoxazole	5	19.2	21	80.8
Ceftazidime	24	92.3	2	7.7
Cefotaxime	20	76.9	6	23.1
Nalidixic acid	3	11.5	23	88.5
Ceftriaxone	24	92.3	2	7.7
Ciprofloxacin	19	73.1	7	26.9
Imipenem	26	100	0	0
Amikacin	25	96.2	1	3.8
Gentamicin	18	69.2	8	30.8
Chloramphenicol	15	57.7	11	42.3
Cefepime	24	92.3	2	7.7

developing communities, also it has been listed among the most frequent bacterial infections associated with enteric illness in developed countries [32].

Egyptian studies regarding *Shigella* are limited; epidemiology and serotyping were the most important aims of previous researches that had been conducted on Egyptian patients, particularly children [33] [34] [35]. Isolation of MDR *Shigella* from pediatric patients with diarrheal illness and its association with movable genetic elements (class 1 integrase) and other virulence genes were the main objectives of the present study.

The present study revealed a prevalence rate of 5.3% for *Shigella* as a causative agent of diarrhea in children, approximating that previously reported by Lluque *et al.*, 2015 [26], who could isolate *Shigella* from (69/1235) stool samples of children with diarrheal illness, recording 5.6% isolation rate. *Shigella* prevalence among Indian children documented a value of 6% versus 1% in adult which is parallel to the present results [13]. A lower isolation rate was observed in other study performed in Turkey; the authors reported that *Shigella* had been detected only in 1.6% of the studied pediatric cases suffering from diarrhea [7]. Recent study achieved in Iran showed higher percentage of *Shigella* species in diarrheal samples of the examined children (75/946, 7.9%) [27].

Previous researches conducted on patients suffering from diarrhea documented higher rate of *Shigella* isolation among children than adult patients; Antoine *et al.*, 2010 [18] performed their study on patients with different ages and they observed a prevalence rate of 2.6% for all patients, however; 5.1% was the documented prevalence among children under the age of five in their research. Children over the age of five were found to be more affected than children under the age of five as reported by Ghosh *et al.*, 2011 [36] (69% and 31% respectively).

The present study showed that 57.7% of *Shigella* isolates were from children in the second year of life which was in agreement with the previous Egyptian study that reported diarrhea related *Shigella* incidence with higher frequency in children after the age of six month reaching its peak at the age of two years [33]. The difference in *Shigella* prevalence among different studies usually reflects the variation in health care levels among various localities; however the common finding was the children higher prevalence that supports choice of children as the studied age group in the present research.

More than half of the isolated *Shigella* (57.7%) in the present study was *S. flexneri* species, supporting the previous finding of Abu-Elyazeed *et al.*, 2004, in their Egyptian study [33]; they reported *S. flexneri* as the most common species (55%) of *Shigella* isolated from diarrheal samples of pediatric patients; Antoine *et al.*, 2010, also found that *S. flexneri* accounted for 52.8% of the isolated *Shigella* [18]; similarly, Barrantes *et al.*, 2014, observed that *S. flexneri* represented 83% of the isolated *Shigella* [2]. On the other hand, *S. sonnei* was found to be the most prevalent *Shigella* species in other studies [7] [37] [27] reflecting the various species distribution in different localities.

The present study reported fever and vomiting as the most common clinical

presentations of children with *Shigella* associated diarrhea supporting the fact of high invasive capacity of this organism; this finding was in parallel with that previously reported in Egyptian studies [33] [34] and the Turkish study conducted by Özmert *et al.*, 2011 [7]. Other studies mentioned that bloody stool was the commonest presentation of children with *Shigella* associated diarrheal illness [13], reflecting the difference in the expression of several virulence factors of *Shigella* among various localities.

The examined *Shigella* isolates exhibited an elevated rate of resistance to most of the examined antibiotics. Previous reports documented the progressive increased resistance of *Shigella* to several antimicrobials leading to adverse outcomes [38]. The initial antibiotic therapy for *Shigella* infections is dependant mainly on ampicillin and co-trimoxazole [39], however the current study reported an elevated resistance for both antibiotics, 65.4% and 80.8% respectively which limits the therapeutic options, this was in agreement with previous researches that recorded high resistance to co-trimoxazole up to 96.5% [40] [41] [42].

Nalidixic acid recorded the highest rate of resistance for the examined isolates, 88.5%, which is in concurrence with previous data [43] [44], however a lower resistance rate was recorded in other studies (34.4%) [37]. The recorded percentage of ciprofloxacin resistance for the examined isolates (26.9%) reflects the possibility of emergence of quinolone resistance strains among *Shigella* which is in need for further analysis as it is considered to be alike finding in many studies [45] [46]. Imipenem remains the mostly efficient antibiotic against all of the isolates (100% sensitivity rate) putting it in the first position of treatment options of the isolated *Shigella* strains in the present study and it could be used as an alternative to amikacin (96.2% sensitivity) avoiding its unwanted side effects. The emergence and spread of clones resistant to antibiotics in different localities could clarify the various antimicrobial profiles of *Shigella* isolates that are mainly influenced by the pressure related to antibiotic usage.

Shigella isolates in the present work generally exhibited an elevated degree of antibiotic resistance as 73.1% of them were classified as MDR, preceding studies demonstrated higher antibiotic resistance rate than that currently recorded, 87%, 90%, 95.6%, 97.9% were the percentages of MDR *Shigella* isolates reported by previous researchers [2] [36] [47] [48], however, MDR *Shigella* isolates recorded a percentage of 70% that approximating our results by Sangeetha *et al.*, 2014 [13]. Generally, gaining of MDR criteria by *Shigella* species seems to be an important observation that should draw the attention as this may be a dangerous obstacle in patient management.

The wide dissemination of *Shigella* resistance is very important. It is usually mediated by movable determinant like integrons [14]. In the present study, *intI1* gene was found to be harbored by 78.9% of the MDR *Shigella* isolates approximating the prevalence that has been recorded by Zhu *et al.*, 2011 [47] (79.1%), however this percentage seems to be low in relation to other studies. Frank *et al.*,

2007 [17] observed the presence of *intI1* gene in 88.8% of *Shigella* isolates, Yang *et al.*, 2014 [24] recorded the presence of that gene in 90.3% of the examined MDR *Shigella*, in the same year, Barrantes *et al.*, 2014 [2], also observed an elevated prevalence of *intI1* gene among *Shigella* species (93%). The increasing prevalence of integrase gene in *Shigella* isolates is a reflection of the high possibility of further spread of resistance species. In other localities with different pattern of bacterial resistance and antibiotic policies, a lower prevalence of *intI1* gene has been recorded, 44.2% of *Shigella* isolates were found to be positive for *intI1* gene in an Indian study [48].

Virulence genes detection in the MDR *Shigella* is very important as they could be strongly associated with the resistance profile of those isolates. In the present research, testing of the MDR *Shigella* by the multiplex PCR assay revealed presence of *ipaH* and *ipaBCD* in most of the examined isolates (94.7%) which was in agreement with previous studies, Antoine *et al.*, 2010 [18] reported the presence *ipaH* in 90.2% of *Shigella* isolates. Other authors confirmed the occurrence of that gene in all of their tested *Shigella* strains [13] [27] [49] [50] [51]. In harmony with our results, *ipaBCD* was found to be harbored by all *Shigella* isolates that were previously examined [52] [53]. *ipaH* gene is a constant gene that it is expressed with many copies on chromosomes and plasmids giving an explanation for its existence in all *Shigella* isolates [18]. *virA* gene was found to be expressed by 78.9% of MDR *Shigella* isolates approximating results that has been previously reported [36]. Recently, Yaghoubia *et al.*, 2017 [27] recorded the presence of this gene in all *Shigella* isolates confirming its essential role in the cellular invasion. Regarding *sat* gene, it was initially reported in *E.coli* (uropathogenic strains). Recently, *Shigella* isolates were found to express that gene [20]. In a recent study conducted in 2017 [27], *sat* gene was only expressed in 28% of *Shigella* which is much lower than the gene percentage revealed in the present study (73.7%).

The gene *ial* is less stable being expressed mainly on *inv* plasmid; it may be exposed to deletion [54]; its percentage in the present work was 68.4% which is much lower than *ipaH* gene. In previous study performed in 2010 the prevalence of this gene was low (46.3%) [18]. However, the present results was in harmony with that recently reported in 2017 as 74.7% of the studied *Shigella* isolates were found to be positive for that gene [27], meaning that the capacity of *Shigella* to be more virulent as regarding cellular spread has been increased. *S. flexneri* isolates were the only species in the present study that found to be positive for *set1A* and *set1B* genes, which are tandem genes being expressed by the same isolates [13]. The same observation was recorded by recent research performed in 2016 [55], this gene confirmed a prevalence of 7% in a previously performed study [52] which was much lower than that observed in the present work.

The *sen* gene which is specific for enterotoxin 2 production was found to be expressed only by 36.8% of the MDR isolates approximating the preceding results documented by Casabonne *et al.*, 2016 [52] (40%) and Yaghoubia *et al.*,

2017 [27] (45.4%), higher prevalence of that gene was observed in other studies; as Hosseini *et al.*, 2015 [55] and Ghosh *et al.*, 2011 [36], confirmed its presence in 66.1% and 91.5% of the tested *Shigella* isolates respectively. Fortunately all of the examined isolates were found to be negative for *stx* gene, being the gene associated with serious complications of *Shigella* related diarrhea, these results matched the previously recorded data [13]. On the other hand *S. dysenteriae* and *S. flexneri* isolates were respectively found to be positive for this gene as proved in previous researches [27] [56], higher percentage of different *Shigella* species (21%) were proved to be positive for that gene in French study conducted in 2015 [57] as all of the examined patients were travellers who had been returned from other locality.

The current study was limited by the low number of the examined *Shigella* isolates as detection of class 1 integrase and other virulence genes was restricted only for the MDR species. Further studies are recommended to detect other resistant determinants in *Shigella* isolated from patients with different age groups which is very important to form a clear idea about the resistance profile of *Shigella* in Egyptian patients.

5. Conclusion

In the present study, *Shigella* has been accounted for 5.3% of pediatric cases with diarrheal illness. Although this prevalence seems to be low, more than two thirds (73.1%) of those isolates were proved to be MDR. The presence of *intI1* gene was documented in 78.9% of the MDR *Shigella* meaning that the probability of resistant species dissemination is considered. Virulence genes of various types were detected in the examined MDR isolates with substantial values reflecting the strong association between MDR criteria and presence of virulence genes which in no doubt affects the disease outcome, particularly in children.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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