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Molecular characterization of virulence and antimicrobial resistance profile of Shigella species isolated from children in Delta, Egypt

Fatma Ibrahim Sonbol
Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Egypt

Tarek Elsaeed Elbanna
Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Egypt

Maysaa El Sayed Zaki
Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt

Hind Awad Elderiny
Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Egypt
*Corresponding author email: awadhind91@gmail.com

***Abstract*---**Background: The ability of Shigella to invade, colonize, and kill host cells is affected by many virulence factors. The study aimed to confirm the identification and assess the presence of 12 virulence genes of the recovered Shigella spp. and 6 common antibiotic resistance genes particularly in *S.flexneri* isolates recovered from delta area –Egypt. Methods: A total of 62 *Shigella* isolates were collected from different hospitals. The detection of genes specific for the genus, serotypes, major virulence genes and genes encoding antibiotics resistance was performed by PCR. Results: A total of 62 (6.7%) isolates were positive for *Shigella* spp. virulence genes namely *ipaH & virA & sigA & icsA & sen* genes were detected in all species while *sat & sepA* were not detected in *S.boydii* . on the other hand, *pic* was not detected in the tested species isolates. The tested isolates also harboured all except *blaCTXM* antibiotic resistance gene. Conclusions: This study provide information about the prevalence of MDR character and the distribution of virulence & antibiotic resistance genes for effective control policies within this geographic region.

Keywords---Shigella, pathogenesis, virulence genes, resistance genes.

Introduction

Shigellosis (bacillary dysentery) continues to be a major public health burden, it is reported that it has remained to be responsible for annual increased number of mortalities induced by dysentery worldwide, particularly impacting children <5 years of age in developing countries (Ranjbar and Farahani. 2019). It is an acute invasive enteric infection leading to a spectrum of clinical manifestations ranging from mild watery diarrhea to severe colitis. *Shigella* is a member of the *Enterobacteriaceae* family that is classified into four serogroups; *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D) and is restricted to infecting humans impacting gastrointestinal mucosa.

Shigella is a host adapted to humans and nonhuman primates, is transmitted mainly via feco-oral route. Because as few as (10-100) organisms are required for the disease, shigellosis is easily transmitted. once *Shigella* comes in contact with intestinal epithelial cells, the type III secretion system (T3SS) is activated resulting in the release of effector proteins such as *ipaH* which is an important mediator of invasion in the intestinal epithelium. There are other virulence factors associated with the genus *Shigella*, such as *virA* which has been implicated in invasion and intercellular spreading. after cell invasion, *Shigella* releases other effectors, such as *IcsA*, which promotes actin polymerization. *Shigella* strains also produce distinct enterotoxins such as *Shigella* enterotoxin 1 (ShET-1), encoded by the *set* gene, and *Shigella* enterotoxin 2 (ShET-2), encoded by the *sen* gene that could alter the electrolyte and water secretion in the intestine, which is closely related to dehydration apparent in shigellosis. *Shigella* spp. also harbor toxic factors such as *SPATEs* (serine protease autotransporters of *enterobacteriaceae*) which are phylogenetically classified into two main classes: Class 1 comprising *sigA* (the *Shigella* IgA-like protease homologue) and *sat* (secreted autotransporter toxin) are toxic to epithelial cells . *Pic* (protein involved in colonization) and *SepA* (the secreted protein A) belonging to class 2 are non-toxic .

Among different *shigella* spp. *S.flexneri* is relatively more prevalent in developing countries (Kahsay AG *et al* ., 2016 & Qu F *et al* ., 2012) . Antibiotic resistance in *S. flexneri* is also well-documented in numerous studies showing high rates of resistance to at least one of the common antibiotics such as penicillins, tetracycline, and chloramphenicol (Khaghani *et al.,* 2014; Cui *et al.*, 2015). These studies revealed that without access to proper testing facilities or multiple effective antibiotics, patients in developing countries infected by *S. flexneri* have a very low chances of being successfully treated. In Egypt, *Shigella* spp. is considered a major contributor to pediatric diarrhea and comprehensive epidemiological analysis of *Shigella* spp. is essential for the prevention and control of the *shigella* in the region. Moreover, studies attempting to characterize its molecular profile of virulence and antibiotic resistance are limited and needs further investigation.

Materials and Methods

Isolation and identification of the tested isolates

Standard procedures were followed to isolate and identify *Shigella* spp. from stool samples.

Extraction of DNA from bacterial isolates

Using QIAamp DNA Mini Kit (Catalogue no.51304) (USA) according to manufacturer's instructions. Cycling conditions of the different primers during PCR as shown in Table 1:

Table 1

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Detection and typing	95˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
icsA	95˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.		
ipaH	95˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.		
pic *sepA* *sigA*	95˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.		
sat	95˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.		
sen	95˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.		
set1A *set1B* *virA*	95˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	35	72˚C 10 min.
stx1 *stx2*	95˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 72˚C 45 sec.		
blaTEM *blaCTXM*	95˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 72˚C 45 sec.		
qepA	95˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 72˚C 45 sec. 72˚C		
qnrs	95˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 72˚C 45 sec.		
sul1	95˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 72˚C		

				45 sec.		
aadA1	95˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.		

Then the ladder was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded.

Agarose gel electrophoreses (Sambrooket al., 1989) with modification

Electrophoresis grade agarose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, it was heated in microwave to dissolve all granules with agitation, and allowed to cool at 70˚C, then 0.5µg/ml ethedium bromide was added and mixed thoroughly. The warm agarose was poured directly in gel casting apparatus with desired comb in apposition and left at room temperature for polymerization. The comb was then removed, and the electrophoresis tank was filled with TBE buffer. Twenty µl of each PCR product samples, negative control and positive control were loaded to the gel. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to UV cabinet.The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Table 2
Primers used for detection of the tested genes by PCR

Target gene	Primer Sequence (5'-3')	Band size (bp)	Reference
Shigella ipaH1	F GTTACCTGTACTCCCTGCTT	254	Radhika *et al.*, 2014
	R CTAGCCTTCCTTGTGCAA		
S. boydii wzy	F ACCAAGGAGTTGTTCATGA	305	
	R GAAGCCCTGGTAAAGTGC		
S. sonnei wbgZ	F ATGTTGCTAATACCAGTTGG	460	
	R TAGAGAGAACTTCACACGGT		
S. flexneri ipaH	F TGAGAATTTGCCTCCACA	595	
	R CTAGCCTTCCTTGTGCAA		
icsA	F TGATGGACTTTCTCCCTTGG	220	Efremova *et al.*, 2004
	R CCGCTACCACCAAGAATCAT		
IpaH	F GTTCCTTGACCGCCTTTCCGATACCGTC	619	Toma *et al.*, 2003
	R GCCGGTCAGCCACCCTCTGAGAGTAC		
pic	F ACTGGATCTTAAGGCTCAGGAT	572	Boisen *et al.*, 2009
	R GACTTAATGTCACTGTTCAGCG		
sat	F ACTGGCGGACTCATGCTGT	387	Ruiz *et al.*, 2002
	R AACCCTGTAAGAAGACTGAGC		
sen	F ATGTGCCTGCTATTATTTAT	799	Vila *et al.*, 2000

	R CATAATAATAAGCGGTCAGC		
sepA	F GCAGTGGAAATATGATGCGGC	794	Boisen *et al.*, 2009
	R TTGTTCAGATCGGAGAAGAACG		
Set1A	F TCACGCTACCATCAAAGA	209	Vila *et al.,* 2000
	R TATCCCCCTTTGGTGGTA		
Set1B	F GTGAACCTGCTGCCGATATC	147	
	R ATTTGTGGATAAAAATGACG		
sigA	F CCGACTTCTCACTTTCTCCCG	430	Boisen *et al.,* 2009
	R CCATCCAGCTGCATAGTGTTTG		
virA	F CTGCATTCTGGCAATCTCTTCACATC	215	Villalobo and Torres *et al* ., 1998
	R TGATGAGCTAACTTCGTAAGCCCTCC		
Stx1	F GTACGGGGATGCAGATAAATCGC	698	Gray *et al.*, 2014
	R GAAGAAGAGACTGAAGATTCCATCTG		
Stx2	F ACTGTCTGAAACTGCTCCTG	252	
	R GCCAGTTATCTGACATTCTGG		
blaTEM	F ATCAGCAATAAACCAGC	516	Colom *et al.*, 2003
	R CCCCGAAGAACGTTTTC		
BlaCTX-M	F ATG TGC AGY ACC AGT AAR GTK ATG GC	593	Archambault *et al.*, 2006
	R TGG GTR AAR TAR GTS ACC AGA AYC AGC GG		
qepA	F CGTGTTGCTGGAGTTCTTC	403	Cattoir *et al.*, 2008
	R CTGCAGGTACTGCGTCATG		
qnrS	F ACGACATTCGTCAACTGCAA	417	Robicsek *et al.*, 2006
	R TAAATTGGCACCCTGTAGGC		
Sul1	F CGGCGTGGGCTACCTGAACG	4339	Ibekwe *et al.*, 2011
	R GCCGATCGCGTGAAGTTCCG		
Aada1	F TATCAGAGGTAGTTGGCGTCAT	484	Randall *et al,.* 2004
	R GTTCCATAGCGTTAAGGTTTCATT		

F: forward R: reverse

Results

In the present study, the prevalence rate of *Shigella* spp. was 6.7% where 62 *Shigella* isolates were recovered from 920 stool specimens of pediatric patients suffering from various clinical manifestation, including fever n = 28 (45.2 %), abdominal pain n =16 (25.8 %), vomiting n =11 (17.7 %), Bloody diarrhea n =8 (12.9%), mucoid diarrhea n =6 (9.7 %) and watery diarrhea n =53 (85.5 %). Out of 62 *Shigella* spp. n = 35 (56.5 %) and n = 27 (43.5 %) were isolated from male and female patients, respectively. No significant differences in S*higella* infection were observed among male and female patients (P > 0.05). The isolation frequency of the serogroups was 61.3 % (n=38) for S. *flexneri,* 32.3 % (n=20) for *S. sonnei* and 6.5 % (n=4) for *S.boydi ,* interestingly no sample was positive for *S. dysenteriae*. The prevalence of the recovered *Shigella* isolates according to age group is shown in Table (3).

Table 3
Prevalence of identified *Shigella* species among different age groups

Age group (months)	No of collected samples	No (%) of detected *shigella* isolates	Incidence of *shigella* species No (%) of *shigella* isolates		
			S.flexneri	*S.sonnei*	*S.boydii*
< 6	30	2 (3.2%)	2 (3.2%)	0 (0%)	0 (0%)
6 -12	87	3 (4.8%)	2 (3.2%)	1 (1.6%)	0 (0%)
12 -18	180	11 (17.7%)	7 (11.3%)	3 (4.8%)	1 (1.6%)
18 -24	200	14 (22.6%)	8 (12.9%)	4 (6.5%)	2 (3.2%)
24 -30	320	23 (37.1%)	14 (22.6%)	9 (14.5%)	0 (0%)
30 -36	33	4 (6.5%)	3 (4.8%)	0 (0%)	1 (1.6%)
> 36	70	5 ((8.1%)	2 (3.2%)	3 (4.8%)	0 (0%)

Out of 62 *Shigella* isolates 17 isolates were randomly selected involving (10 *S.flexneri* , 5 *S.sonnei* and 2 *S.boydii)* as representative for each tested species . These isolates were subjected to PCR technique for confirmation of the phenotypic identity. The results revealed positive detection of characteristic genes for each species as shown in Fig (1).

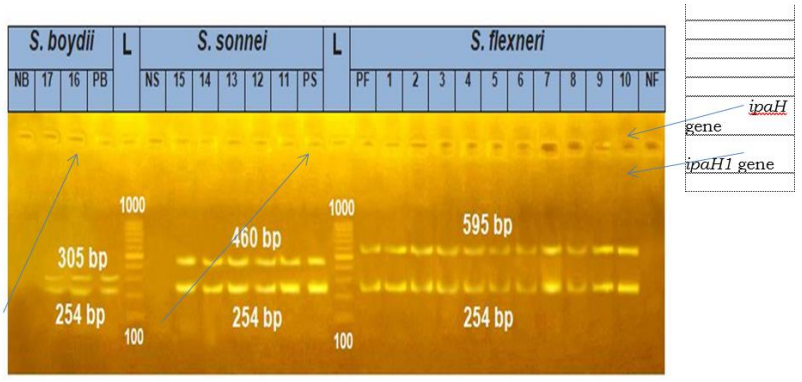

Figure 1. Molecular detection and typing

The selected 17 *shigella* isolates were also tested for different virulence genes using PCR (ipaH *& virA & sigA & icsA & sen* genes were detected in all species while sat *& sepA* were not detected in *S.boydii*. Interestingly, pic and *stx* gene were not detected in the tested species isolates). The obtained data are collected and presented in Table (4). as shown in Fig (2-13)

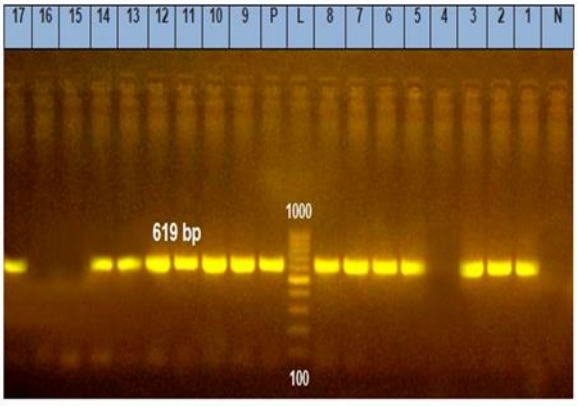

Fig 2. *ipaH* gene

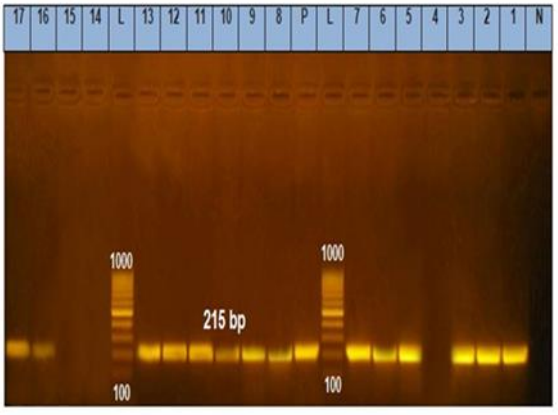

Fig 3. *vir A* gene

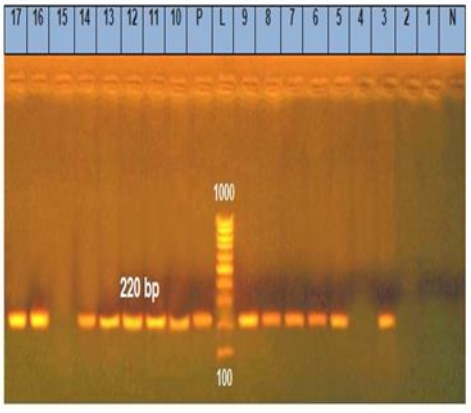

Fig 4. *icsA gene*

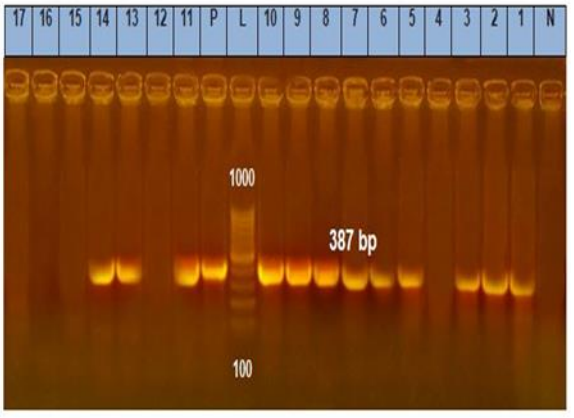

Fig 5. *sat* gene

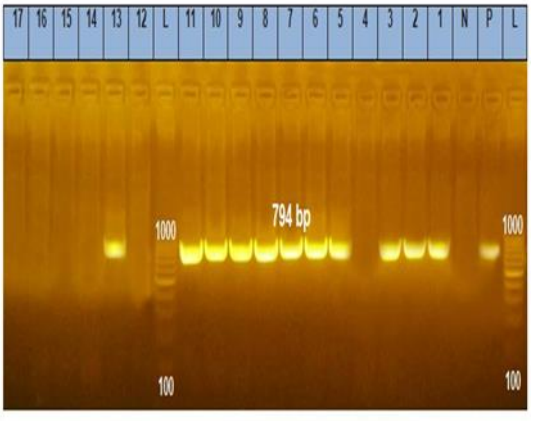

Fig 6. *sepA* gene

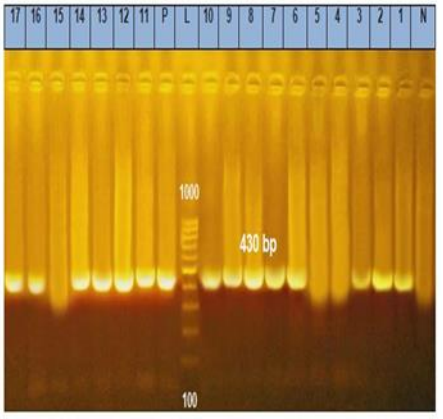

Fig 7. *sigA gene*

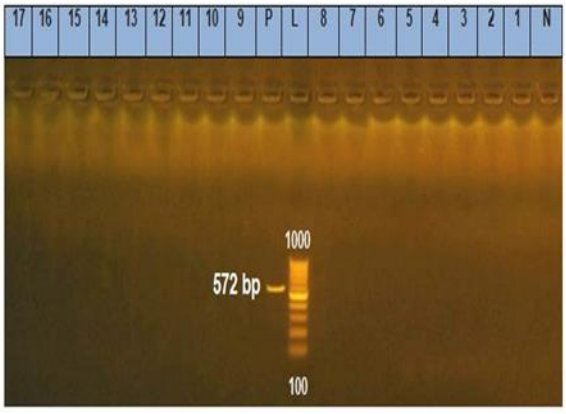

Fig 8. *pic* gene

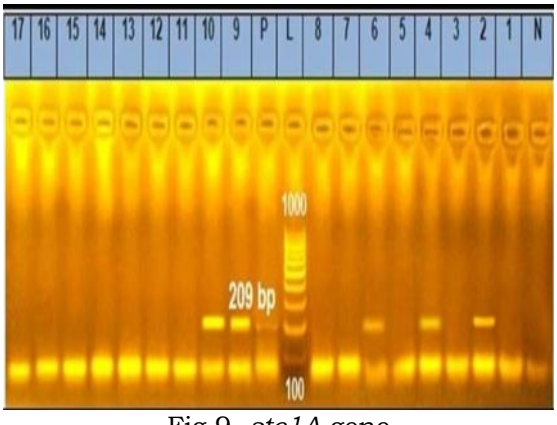

Fig 9. *ste1A* gene

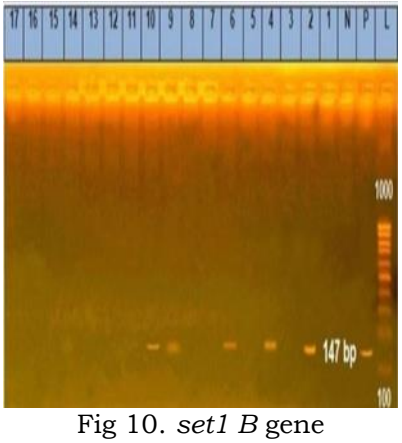

Fig 10. *set1 B* gene

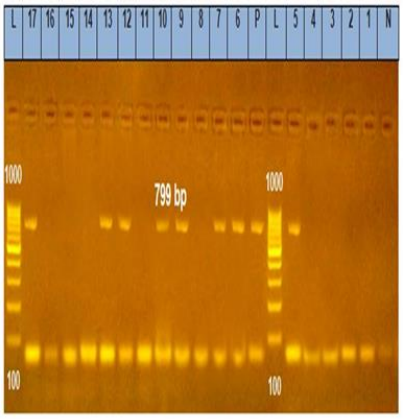

Fig 11. *sen* gene

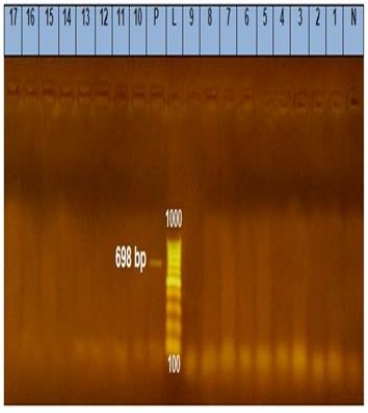

Fig 12. stx *1* gene

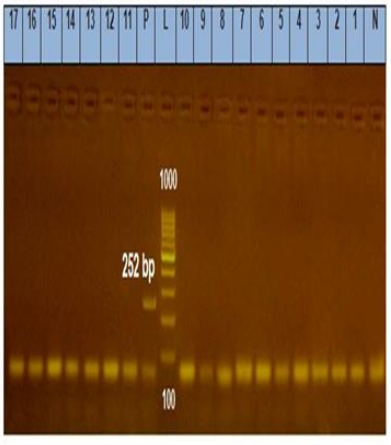

Fig 13. stx2 gene

Table 4
Molecular characterization of different virulence genes among the tested *Shigella* species

Incidence (%) of genes in different *Shigella* spp.

Family	VRG& description	Main function	*S.flexneri (n=10)*	*S.sonnei(n=5)*	*S.boydii(n=2)*
			(%)	*(%)*	*(%)*
T3SS(type 3 secretion system) effectors	*ipaH (invasion plasmid antigen)*	*epithelial cell permeation and cell to cell dissemination*	*n= 9 (90%)*	*n=4 (80%)*	*n=1 50%*
	vir A(virulence A)	*uptake, motility,and intercellular spreading of Shigella within the human host.*	*n=9 90%*	*n= 3 60%*	*n=2 100%*
SPATEs (serin protease autotransporters of enterobacteriaceae)	*sat(secreted autotransporter toxin)*	*Proteolytic cytotoxin*	*n=9 (90%)*	*n=3 60%*	*n=0 0%*
	sigA (shigella IgA-like protease homologue)	*Proteolytic cytotoxin*	*n= 8 80%*	*n= 4 80%*	*n=2 100%*
	Pic(protein involved in intestinal colonization)	*a mucinase that aids in mucosal colonization*	*n=0 0%*	*n=0 0%*	*n=0 0%*
	sepA (shigella extracellular protienA)	*protease activity & invasion*	*n=9 90%*	*n=2 40%*	*n=0 0%*
	icsA (virG) (virulence G)	*promotion of actin polymerization*	*n=7 70%*	*n=4 80%*	*n=2 100%*
Enterotoxins	*Set 1A* *(SHET1) shigella enterotoxins 1*	*alter electrolyte and water transport in the small intestine*	*n=5 50%*	*n=0 0%*	*n=0 0%*
	Set 1B		*n=5 50%*	*n=0 0%*	*n=0 0%*
	Sen (osp D 3) *(ShET2) shigella enterotxins 2*		*n=5 50%*	*n=2 40%*	*n=1 50%*
Exotoxins	*stx 1* *(shiga toxins)*	Cytotoxic & bloody diarrhea	*n=0 0%*	*n=0 0%*	*n=0 0%*
	stx 2				

Table 6
Resistance pattern of the selected 17 *shigella* isolates

Isolate code	Pattern code	Resistance markers
F 12	P-22	AMC — CFR - CAZ – GEN – C
F 86	P-54	TE – DO – AMC- CFR – CTX – CAZ – CPM – GEN – SM – AK –SXT -CIP
F 124	P-55	AMC -CFR – CAZ – C– SXT– GEN – AK
F 236	P- 31	AMC– CFR – CTX – SXT
F 320	P-25	AMC –CTX –CAZ– CFR – GEN– SXT – NOR
F 457	P- 50	LE – AMC - CFR – CTX – CAZ – GEN – SM – AK –

		SXT
F 519	P- 40	LE - AMC — CFR - CTX – CAZ – GEN – SM –AK
F 642	P-30	AMC– CFR – CAZ – SXT– GEN – NOR
F 734	P-46	AMC – CFR – CTX – CAZ- CPM –GEN –SM – AK – SXT -LE
F 862	P-28	TE – DO – AMC- CFR – CTX – CAZ – CPM– GEN – AK - SM –CIP
S 411	P-8	AMC – CFR – CTX – CAZ – C- SM – SXT
S 523	P-9	AMC– CFR – SXT – GEN – SM – AK
S 654	P-19	AMC – CFR – CTX – CAZ – CPM – SM – AK – CIP
S 712	P-38	AMC — CFR– CAZ - CTX – SM
S 879	P-2	AMC – CAZ – SXT –CIP
B 287	P-5	AMC – CFR – SXT– CIP
B 917	P-10	AMC – CFR – CAZ – SXT– SM – CIP

Out of the 17 studied isolates 5 isolates were selected for molecular detection of 6 resistance gens, the results Revealed the isolates harboured all antibiotic resistance genes except blaCTXM *which* was not detected in any of the isolates as shown in Fig (14-16) .

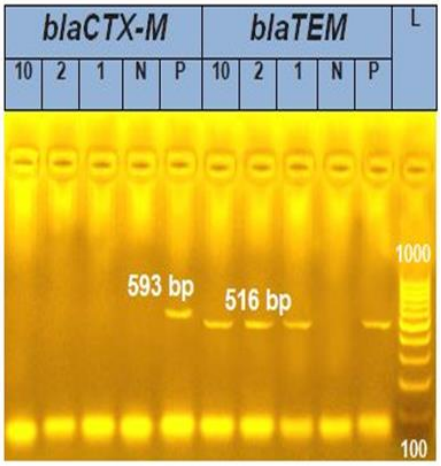

Fig 14. *blaCTX-M & blaTEM* genes

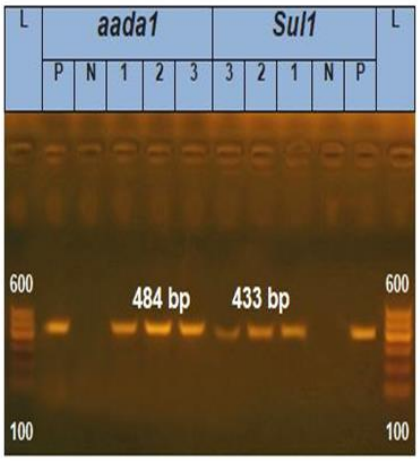

Fig 15. *aadA1 & sul 1 genes*

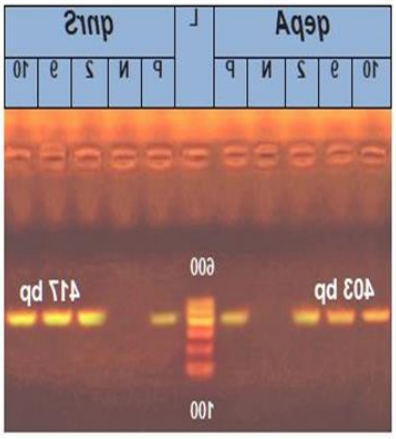

Fig 16. *qepA & qnrS* genes

L: Gene ruler 100 bp DNA ladder (100-1000 bp).
Gel Pilot 100 bp plus ladder (100-1500 b) .

Discussion

During the study period from March 2019 to December 2020, out of 920 stool specimens 62 *shigella* isolates were recovered. The isolation rate of *shigella* species (6.7%) in our study among pediatric cases in Delta area of Egypt. that was comparable to the study done by Sonbol (1992) and Elrefaey (2015) in Tanta (6.1%), (6.5%) respectively. Similar values were also obtained abroad, where shigellosis occurred with a prevalence of 6.6% in Ethiopia (Siraj Hussen et al., 2019) and 7.2% in Iran (Ahmad Farajzadeh Sheikh et al., 2020), on the other hand a lower incidence of *shigella* was reported in Egypt by other studies as in EL-Minia Governorate (4.8%) was reported by Abd-Elmeged (2014) from patients up to 70 years old. However, a markedly higher (49.9%) incidence was reported in

EL-Dakhlia governorate by EL-Sherbiny and Shehata (2014), Moreover (40%) in Banha detected by Elsharkawy (2012).

Our data revealed also that *S. flexneri* was the most commonly isolated species (61.3%) followed by *S.sonnei* then *S.boydii* . this serogroup distribution is nearly congruent with other studies in Egypt like that of Sonbol (1992) and Elrefaey (2015) in Tanta, Abd-Elmeged (2014) in EL-Minia Governorate. on the other hand, EL-Sherbiny and Shehata (2014) in EL-Dakhlia governorate found that *S.sonnei* was the most frequently isolated species .Our findings support the idea that *Shigella* species are geographically stratified based on the level of economic development, being *S. flexneri* the main infectious species in developing countries whereas *S. sonnei* rates increase with economic development (Kotlof *et al.*, 2017 & Kahsay and Muthupandian *et al.,* 2016). In our study it was observed that the vast majority of cases under the age of 5 years which was consistent with previous reports (Karimi-Yazdi *et al.*, 2020; Samane Mohebi *et al.*,2021). this is explained as they in this age are most likely to get shigellosis due to the frequent exposure to the contaminated environment, poor hygiene and less effective immune responses to *Shigella* spp (Orrett *et al*, 2008).

Due to the speed, sensitivity and specificity, molecular methods have offered advantages over the conventional methods. Molecular based detection of *Shigella* at the genus and species level is recommended to be used in the routine diagnostic laboratory. In our study, molecular detection of *ipaH1* (invasion plasmid antigen1) gene which is present in multiple copies on both the plasmid and chromosome of *Shigella* spp is deemed to an appealing target to be a diagnostic tool for the detection of *Shigella* genus. it showed that the *wbgZ* (putative epimerase /dehydratase) gene was conserved for *S. sonnei*, Gene wzy encoding for O-antigen polymerase is highly specific for *S. boydii* while in case of *S. flexneri , ipaH* (invasion plasmid antigen) gene (Radhika *et al.*, 2014) which confirm the phenotypic identity of our isolates .

The pathogenicity of *Shigella* is closely related to certain virulence determinants found in the chromosome or large virulent inv plasmid that result in invasion of the colonic epithelium and cell to cell dissemination (Sethuvel *et al.*, 2019a). Identification of *ipaH* in all *Shigella* species in our study was an expected finding as it is highly conserved in various serotypes. Similar fndings have been revealed in many other studiess (Fan *et al.,* 2017; Wenting Fan *et al.*,2017; Samane Mohebi *et al.*,2021). The *virA* is an essential virulence factor in Shigellosis pathogenesis which is found in a high frequency in all tested *shigella* species. Similar results were reported in a study performed by Cecilia Casabonne et al. (2016).

SPATEs, is a cluster of genes representing another virulence factor in gram negative bacteria have various functions such as protease or mucinase that can be toxic for the intestine cells directly or indirectly (Moosavian *et al.*, 2019). They are subdivided into class 1 and class 2 on the basis of structural and functional properties. class I genes (*sigA* & *sat*) are toxic to epithelial cells while class II genes include *pic & sepA* which are non-toxic. Our study showed that all species harbored at least one *SPATE* gene which is in consistent with other studies (Moosavian *et al.*, 2019; Nave *et al.*, 2016a ; Boisen *et al.*, 2009). In accordance

to our findings, the high distribution of *sigA* gene in various serogroups revealed that *sigA* toxin may play a critical role in the pathogenic process, which supports the previous studies (Nave *et al.*, 2016a; Moosavian *et al.*, 2019; Gu *et al.*, 2019). *Pic* was not found in any tested isolates. This data might suggest the presence of inactive *pic* genes owing to occurrence of internal deletions, sequence alteration or s a different *pic* gene variant is made up (Angela Lluque *et al* 2015). Moreover, *pic* toxin might haven't a significant effect on the pathogenicity.

Sat gene was more commonly detected in *S.flexneri* then *S.sonnei* isolates . In agreement with our study Nave *et al.* (2016a), Eftekhari *et al.* (2013) also indicated a higher rate of *sat* gene in *S. flexneri* strains compared to *S.sonnei* strains. *sepA* and pic genes were not found in the tested *S.boydii* isolates in contrast to Nave et al. (2016a) in Iran reported the existence of the class *2 SPATE* genes not only in *S. flexneri*, but also *S. boydii* serogroup . Our data also revealed the high frequency of *icsA* gene in all tested serogroups which is consistent with Angela Lluque *et al.* (2015). *Shigella* spp. also had the capacity to produce enterotoxins (*set1A/set1B* and *sen*) (Cruz *et al.*, 2014). In this study, *set1A/B* (encodes ShET-1) was found only in S. flexneri isolates (50%), whereas, *sen* (encodes ShET-2) gene which was identified to be responsible for causing bloody diarrhea was detected in all serogroups including *S. sonnei* (40%), *S. flexneri* (50%) and *S. boydii* (50%) in agreement with the studies by Lluque *et al.* (2015), Casabonne *et al* . (2016)and Samane Mohebi *et al* .(2021) .

In this study, *stx* genes encoding shiga toxins not detected in any of the tested isolate. However, Theses toxins were thought to be unique to *S. dysenteriae* serotype 1. Howevre, Miranda D. Gray *et al.* (2014), Katherine Lamba *et al.* (2016) found that other species like as *S.flexneri* and *S.sonnei* respectively may harbor *stx* genes. In general, the *S. flexneri* isolates in accordance to our study tended to harbour the highest number of virulence factors. Comparable results were reported Angela Lluque *et al.* (2015), Yanyan Liu *et al.* (2020) and Samane Mohebi *et al.* (2021). Numerous studies have revealed that there is a link between shigellosis and the possession of virulence genes in the pathogen. In our study, among S.flexneri spp. *blaTEM (*most reported gene related to penicillin resistance) was detected in all (n= 3) (100%) isolates. These isolates were also resistant to penicillins by disk diffusion method. On the other hand. *blaCTX-M* was not detected in any isolates as reported in a study performed by Shalini Anandan *et al.* (2015).

Plasmid-mediated quinolone-resistance regions (PMQRs) namely qnr (*qnrA,qnrB, qnrC, qnrD, qnrS, qep, aac[6']-lb-cr*) genes is the primary reason for quinolones resistance among Shigella isolates . Importantly, qnrS-positive isolates of *Shigella*, especially *S. flexneri* spp., show high-level resistance to fluoroquinolones, and many researchers from different parts of the world suggest that PMQRs gene qnrS plays an essential role in reduced susceptibility of *Shigella* strains to fluoroquinolones (Zhang WX *et al.*,2019; Qin T *et al.*,2017; Pu XY *et al.*,2015). Our data revealed all isolates harbored *qnrS* and *qepA*(for quinolone efflux pump) genes while the PMQR genes, qnrB, qnrS, and aac(6')-Ib-cr, were identified in only 44.4% of *Shigella* isolates reported in a previous egyptian study (Ashraf M. Ahmed , Tadashi Shimamoto . 2015).

Aminoglycoside adenyl transferase gene (*aadA* gene cassettes) are very common in *Enterobacteriaceae,* particularly among *Salmonella* and *Shigella* spp. (McIver CJ *et al.*,2002; Michael GB *et al.*, 2016), many types of *aadA* gene cassettes among *Enterobacteriaceae* have been identified, but types *aadA1* and *aadA2* are detected more frequently among *Shigella* isolates (McIver CJ *et al.*,2002; Chang CY *et al* .,2011 ; Gassama Sow A *et al.*,2010) . Interestingly, all isolates were found positive for *aadA1* gene. As the high level of resistance to trimethoprim–sulfonamide worldwide emerged, these agents are not considered a good choice for empirical therapy of shigellosis (Niyogi SK *et al.*,2005). Sulfonamide resistance is mediated by *sul1, sul2,* and *sul3* genes that are common in *Shigella*. The *sul1* gene is highly frequent among *Shigella* isolates, because it is a part of the 3′conserved region of class 1 integron (Chang CY *et al.*, 2011). our findings revealed that *sul1* gene is present in all studied isolates.

Conclusion

This study presents an in-depth characterization of the molecular basis of virulence and antimicrobial resistance of *Shigella* spp. isolated from children with diarrhea in delta area of Egypt. High prevalence of MDR organisms and heterogeneity of virulence factors leading to various degrees of disease severity within this geographic region was recorded. Both virulence factors and resistance genes were diversely harbored among different species with especial predominance in *S. flexneri* that was the most frequently detected spp. in this study area. Continuous surveillance within each distinct geographic area and characterization of virulence and antimicrobial resistance as well as the development of an effective vaccine or other antibiotic alternatives are highly recommended to maintain effective treatments for shigellosis.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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