Using various techniques to identify antimicrobial susceptibility and phenotypic resistance in a Shigella's spp.

Saja Salim Abdulhassan
Department of Biology, College of Science, University of Babylon, Iraq, sajaalkaraawi@gmail.com

Hassan Fadhil Naji
Department of Biology, College of Science, University of Babylon, Iraq.

Abstract

Shigellosis should be suspected in patients who have a fever and watery diarrhea. Females were found to be infected at a higher rate than males in the overall population samples. The rate of bacterial isolation (17%) was higher than his results (3%), with approximately 53.5% of children infected, approximately 24% in the 11-20 age range, and approximately 22.5% in the early teens and adults (21-30 years). People at nursing homes and psychiatric institutions who had various gastrointestinal symptoms were cured.

Shigella spp. were all resistant (43.6%) to ampicillin plus nalidixic acid (=60%), cefixicin (59.5%), ceftazidime (55%), and ceftriaxone (55%). When antibiotic susceptibility was investigated, Shigella spp. isolates were 76.6% susceptible to ciprofloxacin and 44% susceptible to ceftriaxone, respectively. Almost 100% of the samples tested were insensitive to AMP, 45% to NAL, 55% to ceftriaxone, 15% to CIP, 60% to CHL and cefixime, 65% to ceftazidime, and 40% to gentamicin. S. flexneri isolates were resistant to ampicillin and erythromycin in 100% of cases, while 66.66% were responsive to eftriaxone, ciprofloxacin, gentamicin, and 33.33% were resistant to ceftriaxone, ciprofloxacin, and nalidixic acid. In the case of S. Sonnei, 100% of isolates were resistant to ampicillin and cefixime, while 100% were intermediate to gentamicin and 75.00% were sensitive.

Keywords: *Sigella spp., Antibiotic sensitivity, phenotypic test.*

INTRODUCTION

Shigella is a gastrointestinal bacterium that is highly infectious and sometimes fatal. DuPont et al. (1989) discovered that consuming 10-100 Shigella dysentery can cause illness in healthy persons. Shigella's endotoxin, exotoxin, enterotoxin, and temperature regulation genes can cause fever, bloody, purulent feces, and stomach cramps. Hemolytic uremic syndrome, hypoglycemia, hypoxemia, intestinal perforation, convulsions, dementia, and even death can occur in some people. Some patients, on the other hand, may only experience short-term illness. According to the WHO in 1999, dysentery affects 165 million people worldwide each year, with about 162 million of those people residing in underdeveloped countries. Dysentery was the cause of mortality for 1.1 million people, the majority of whom were children under the age of five (Navia et al.,1999). Shigella spp.

Shigella spp. are Gram-negative bacteria that are rod-shaped, nonspore-forming, facultative anaerobes, and nonflagellated. They induce acute diarrhea, which can proceed to bloody excess mucus diarrhea, a condition known as bacillary dysentery (or

shigellosis) (AL-Musawi et al., 2016 ; Kahsay and Muthupandian, 2016). Shigella was identified as a life-threatening bacteria by the WHO in 1996 due to increasing treatment resistance, morbidity connected to socioeconomic variables, public health issues, lifestyle choices, and epidemic Shigella serotypes. Bacterial dysentery must be controlled by eliminating the source of infection, restricting the transmission vector, and safeguarding vulnerable people as soon as possible. Shigella has recently become more resistant to medicines, making treatment less effective. Shigella patients that have a long illness course are a major source of infection for others, posing considerable clinical issues (Yan et al.,2010).

Materials and Procedures:

Sample assembly:

Two hundred samples of stool were gathered from people of all ages (adults, adolescents, and children), including both men and women. Between November 2020 and May 2021, stool samples were collected from patients with diarrhea who were admitted to general hospitals, teaching hospitals for women and children, and Al-Diwaniyah teaching hospitals.

Identification and isolation:

The specimens were grown on blood agar (Himedia) in a sterile loop for 24 hours at 37 C°. At 4 C°, pure colonies were kept in nutritional broth containing glycerol (Himedia) . Bacteria cultured on MacConkey agar, blood agar, and nutrient agar (Himedia) were investigated for their form, size, texture, and colony organization. A single colony of each isolate was Gram stained and inspected under a 100x oil-emersion light microscope; the isolates were identified using morphological qualities (for cells and colonies) and biochemical tests as reported by MacFaddin (2000), as well as the polymerase chain reaction approach.

Result and discussion:

Demographic characteristics according to the gender and age

Out of 200 patients, only 20 (10.00%) were diagnosed as shigellosis (Table 1). The samples were divided into eighty-five (43%)-males and One hundred and fifteen (57%)-females. The age of patients was divided into three categories. Table .1 showed that the less than 11 years was 107 (53.5%), 11-20 years was 48 (24%), and 21-30 years was 45 (22.5%).

Table (1): Demographic characteristics (Total samples, positive and negative, gender and age).

Characteristic	Category	No. (%)	X^2	P-value
Gender	Male	85(42.5)	9*	0.003
	female	115(57.5)		
	Total	200(100)		
Age	Less than 11	107(53.5)	55.005*	0
	11-20	48(24)		
	21-30	45(22.5)		
	total	200(100)		
Total	Positive	20(10%)	8.5*	0.002
	Negative	180(90%)		
	Total Samples	200(100%)		

However, females were infected at a higher rate than males in the total population samples. Age groups were further divided into three subgroups ranging from 4 to 30 years, with approximately 53.5% of children infected, approximately 24% in the 11-20 age

group, and approximately 22.5% in the early teens and adults (21–30 years). In contrast to S. flexneri serotype variant X (prevalent in urban areas), which equally affects men and women, Jain et al. (2020) discovered that S. flexneri serotype 2 affected women much more than males (predominantly in rural areas). Males were more resistant to disease, and antimicrobial treatments were more successful because men's metabolic systems were biologically faster and more potent than women's. Shigella spp. persisted in the body for longer than usual due to women's more sensitive temperaments, making them less resistant (Ranjbar and Farahani, 2019). Since children's guts are still developing, they receive low dosages of antibiotics to avoid acquiring resistance to the bacteria, which is beneficial to their gut health.

Additionally, broad-spectrum antibiotics eliminate a large number of important intestinal microbes. Similar trends were discovered in adolescents, although they are more likely to be explained by changes in food intake and sanitation than by heredity (Ngoshe et al., 2017). The typical method for examining the cause of dysentery involves doing precise bio-chemical checks to determine the type and class of bacteria through culture, characterized by a tedious and long time. For the detection of shigellosis, DNA-dependent molecular approaches, particularly the PCR method, are now often utilized in scientific and research facilities (Alipour et al., 2012).

Demographic characteristics according to the symptoms:

There were big differences among patients according to the symptoms (diarrhoea conditions) using X2 test. The percentage of the patients with shigellosis were shown in Table (2); all patients suffering from abdominal pain (100 %, 20 ／ 20), fever (51 %, 102 ／ 200), vomiting (69 %, 138 ／ 200), nausea (47 %, 94 ／ 200) and fatigue (16 %, 32 ／ 200).

Table (2): Distribution of specimens according to patients' clinical signs.

Signs	Patient No.	percentage (%)
Abdominal pain	200	100
Fever	102	51
Vomiting	138	69
Nausea	94	47
Fatigue	32	16
X^2	310.14*	
P-value	**0**	

* Significant difference at p≤0.05

The percentage of the patients with shigellosis were as shown in table (3); patients suffering from mucoid bloody (50 %, 10 ／ 20), soft bloody (20 %, 4 ／ 20), liquid bloody (20 %, 4 ／ 20), and liquid mucoid (10 %, 2 ／ 20).

Table (3): Distribution of specimens according to patient's diarrhoea conditions.

Signs	Patient No.	(%)
Mucoid bloody	10	50
Soft bloody	4	20
Liquid bloody	4	20
Liquid mucoid	2	10
X^2	18.33*	
P-value	**0**	

* Significant difference at p≤0.05

The clinical symptoms are indications of various serotypes of Shigella species, which are different in every age group and gender due to the bacterial load and resistivity and how the defense mechanism behaves and counter-attacks the several kinds of species present inside the body during the onset of infection (Maharjan et al., 2017). Ibraheem, (2016) conducted a local investigation in Baghdad that found Shigella spp. to be the next (12%) prevalent bacterium isolated from Ninety- two pediatric cases with diarrhoea. In comparison to the study of Ashkenazi et al. (2003), the rate of bacterial isolation (17%)

was higher than his results (3%). Patients with various symptoms of diarrhea who are lying in nursing homes and psychiatric institutions have been cured (Sheikh et al., 2019). Additionally, homosexual males are more likely to have S. flexneri infections, and persistent, relapsing sickness has been shown to make HIV infection more difficult (Anandan et al., 2017). Shigellosis should be suspected in patients who have fever and watery diarrhea. Clinically, it is impossible to differentiate between diarrhea and a bacterial, viral, or protozoan infection. Infections with enterotoxigenic E. coli and non-typhoidal Salmonella frequently result in nephrotic syndrome and vomiting (Sati et al., 2019). Bloody or mucoid stools, on the other hand, may indicate S. enteritidis, Yersinia enterocolitica, Campylobacter species, or Entamoeba histolytica, all of which should be included in the differential diagnosis. Patients with amebiasis have blood in their stools, albeit it is usually dark brown rather than bright red, as with Shigella infections. Microscopically, amebiasis stool smears should show erythrophagocytic trophozoites in the absence of polymorphonuclear leukocytes (PMNs), but bacillary dysentery stool smears should show sheets of PMN (Halimeh et al., 2020). On sigmoidoscopic examination, patients with shigellosis have a diffusely erythematous mucosal surface with tiny ulcers, whereas those with amebiasis have distinct ulcers without generalized inflammation (Ahmed et al., 2021).

Depending on the Shigella serotype that causes infection, the amount of infection, the host's age, and immunity, the clinical symptoms of Shigella infection might range from mild diarrhea to severe dysentery (Lampel and Maurelli, 2007). Fever, tiredness, and watery diarrhea are early symptoms. In more severe forms of dysentery, patients may have abdominal pain, nausea, and vomiting as well as recurring, unpleasant stools that contain blood and mucus (Niyogi, 2005; Nygren et al., 2013).

Identification of Shigella isolates on different media:

Shigella are small, rod-shaped organisms. About 20 Shigella isolates were identified from the total samples. As shown in figure (1), when Shigella isolates were cultured colonies of non-lactose fermenters that are transparent and pale developed on MacConkey agar. Salmonella-Shigella agar performed worse than Hekton enteric and xylose-lysine-desoxycholate agar, and required more work to do so (Pollock and Dahlgren, 1974). Samples must be plated on MacConkey with one of the following agars: xylose-lysine-deoxycholate, Hektoen enteric, or deoxycholate citrate in order to best detect Shigella in feces.

Shigella does not create H2S, hence its colonies on Hektoen agar were bluish-green rather than the salmon-colored Salmonella colonies' black centers. Shigella is not a lactose or xylose fermenter and exhibits a high level of biochemical inertness (Dekker and Frank, 2015). Shigella most commonly does not create gas, with the exception of some S. flexneri strains, which are outliers and may ferment lactose (Nataro et al., 2007). Kligler iron or triple sugar iron agar were used to further describe the appropriate colonies. Shigella lysine decarboxylase tests usually come out negative. The biochemical properties of Groups A, B, and C are comparable, although S. sonnei has ornithine decarboxylase activity and beta-galactosidase activity, according to Koneman et al. (1997).

Identification of Shigella isolates using biochemical test

The results of the current study of the chemical tests (Indole, catalase and oxidase) showed that all isolates of Shigella were positive for Indole and Catalase. As for the

negative, it was for examining the oxidases in the types identified in table (4).

Table (4): Biochemical tests conducted on the isolated pathogenic bacteria (n=20).

Shigella species	Indole	Catalase	Oxidase
S. flexneri	+	+	-
S. Sonnei	+	+	-
S. dysenteriae	+	+	-
S. boydii	+	+	-

This result is in line with the Bergey's Manual of Determinative Bacteriology (Holt et al., 2012). Regarding the negative biochemical tests, they included urease, lysine decarboxylase, ornithine decarboxylase, and some isolates produce a red ring as a positive result in the indole assay (Saima et al., 2018; Abady et al., 2019). Other study found that Shigella spp. have positive result in Methyl Red, Voges Proskaure, Indole, and Triple Sugar Iron (Chhanda et al., 2019).

Antimicrobial susceptibility test :

Shigella species differed in the presence of resistance genes, and there were statistically significant differences among them as shown in (Table 5). The bacteria were highly resistant to ampicillin and nalidixic acid (=60%), cefixicin (59.5%), ceftazidime (55%), and ceftriaxone (55%), were all Shigella spp. Resistant (43.6 %). Shigella spp. isolates were 76.6 % susceptible to ciprofloxacin and 44 % susceptible to ceftriaxone, respectively, when susceptibility to antibiotics was examined. About 100 % of the samples examined were insensitive to AMP, 45% to NAL, 55% to ceftriaxone, CIP (15%), CHL and cefixime (60%), ceftazidime (65%), and gentamicin (40%).

Regarding to S. flexneri 100% of isolates were resistance to ampicillin and erythromycin, while 66.66% of isolates were sensitive to eftriaxone, ciprofloxacin, gentamicin and 33.33% of isolates were resistance to ceftriaxone, ciprofloxacin and nalidixic acid as shown in (Table 6). Regarding to S. Sonnei 100% of isolates were resistance to ampicillin, and cefixime, while 100% of isolates were intermediate to gentamicin and 75.00 % of isolates were sensitive to ceftriaxone as shown in (Table 7).

Table (5): Antimicrobial susceptibility test (number and percentage).

Antibiotic	**Sensitive isolates**		**Intermediate isolates**		**Resistance isolates**	
	N	%	*N*	%	*n*	%
Ampicillin	0	0	0	0	20	100
Ceftriaxone	6	30	3	15	11	55
Erythromycin	0	0	2	10	18	90
Chloramphenicol	4	20	4	20	12	60
Ceftazidime	3	15	4	20	13	65
Ciprofloxacin	12	60	5	25	3	15
Gentamicin	3	15	9	45	8	40
Cefixime	5	25	3	15	12	60
Nalidixic acid	2	10	9	45	9	45
X2	61.72*					
P-value	0					

Regarding to S. sonnei 100% of isolates were resistance to ampicillin, 69.23 % of isolates were resistance to ceftriaxone and ceftazidime while 53.85% of isolates were sensitive to ciprofloxacin as shown in (Table 8).

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method, as recommended by the clinical and Laboratory Standards Institute (CLSI, 2022). All isolates were tested for antibiotic susceptibility using ceftriaxone (30 g), ampicillin (10 g), chloramphenicol (30 g), nalidixic acid (30 g), trimethoprim-sulfamethoxazole (SXT, 25 g), ceftazidime (30 g), ciprofloxacin (5 g), cefixime (5 g), and gentamicin (10 g) as (Mast Diagnostics Ltd., Merseyside, UK) (Ali et al., 2020).

Table 6: Antimicrobial susceptibility test (number and Percentage) of S. flexneri.

Antibiotic agent	Sensitive isolates		Intermediate isolates		Resistance isolates	
	N	%	*N*	%	*N*	%
Ampicillin	0	0.00	0	0.00	3	100
Ceftriaxone	2	66.66	0	00.00	1	33.33
Erythromycin	0	0	0	00.00	3	100
Chloramphenicol	0	00.00	3	100	0	0
Ceftazidime	0	00.00	0	00.00	2	66.66
Ciprofloxacin	2	66.66	0	00.00	1	33.33
Gentamicin	2	66.66	0	00.00	1	33.33
Cefixime	0	00.00	1	33.33	2	66.66
Nalidixic acid	0	00.00	2	66.66	1	33.33

Table (7): Antimicrobial susceptibility test (number and Percentage) of S. Sonnei.

Antibiotic	Sensitive isolates		Intermediate isolates		Resistance isolates	
	N	%	*N*	%	*N*	%
Ampicillin	0	0.00	0	0.00	4	100
Ceftriaxone	3	75.00	1	25.00	0	0
Erythromycin	0	00.00	1	25.00	3	75.00
Chloramphenicol	0	00.00	2	50,00	2	50.00
Ceftazidime	1	25.00	1	25.00	2	50.00
Ciprofloxacin	0	00.00	2	50.00	2	50.00
Gentamicin	0	00.00	4	100	0	00.00
Cefixime	0	00.00	0	00.00	4	100
Nalidixic acid	0	00.00	1	25.00	3	75.00

Table (8): Antimicrobial susceptibility test (number and Percentage) of S. dysenteriae.

Antibiotic	Sensitive isolates		Intermediate isolates		Resistance isolates	
	N	%	*N*	%	*N*	%
Ampicillin	0	0.00	0	0.00	13	100
Ceftriaxone	2	15.38	2	15.38	9	69.23
Erythromycin	0	0.00	1	7.69	12	92.31
Chloramphenicol	2	15.38	1	7.69	10	76.92
Ceftazidime	2	15.38	2	15.38	9	69.23
Ciprofloxacin	7	53.85	2	15.38	4	30.77

Gentamicin	1	7.69	4	30.77	8	61.54
Cefixime	5	38.46	2	15.38	6	46.15
Nalidixic acid	1	7.69	6	46.15	6	46.15

The susceptibility of Shigella to antibiotics varies according to dosage and formulation. Shigellosis is a disease that mostly affects children in areas of developing countries where it is endemic (Abdulhassan and Naji, 2022). However, Specialists advise the need to treat infected people to prevent transmission and control of infection (Ngoshe et al., 2017). Although Shigella species-related infectious diarrhoea is frequently self-limiting, as a result, empiric antibiotic therapy is frequently used in, as a result, antibiotic treatment is frequently used in children carrying symptoms before the result of stool culture. Most patients will notice an improvement in their symptoms even before the results are available (Alemu et al., 2019). Resistance to nalidixic acid, tetracycline, and trimethoprim-sulfamethoxazole exists in S. sonnei strains. Nalidixic acid-resistant bacteria displayed decreased susceptibility to fluoroquinolones but not complete resistance (Sheikh et al., 2019). In a drug susceptibility test, S. dysenteriae showed resistance to quinolone antibacterial drugs. Streptomycin and spectinomycin are two aminoglycoside antibiotics that bind to ribosomal subunits and limit protein synthesis at intracellular sites (Rahman and Sarker, 2021). A significant ratio of immovability to AMP, TM, and TAC was noted in numerous earlier investigations conducted in Iran and other nations (Jomezadeh et al., 2014). Shigella species and cefotaxime resistance were found to be significantly correlated (p 0.05). S. flexneri strains were primarily resistant to quinolones and chloramphenicol, although overall study revealed that S. sonnei had stronger antibiotic resistance than other species. To ciprofloxacin, all Shigella isolates were sensitive (Sambe-Ba et al., 2013; Hosseini Nave et al., 2016). Ciprofloxacin is beneficial against shigellosis. Multi drug resistant (MDR) Shigella developed due to the medication's overuse and abuse in the treatment of diarrhoea and urinary tract infections (Hussen et al., 2019). Another study from the University of Kolkata discovered that quinolones were 90% resistant to the antibiotics tested (Halimeh et al., 2020).

According to antibiotic susceptibility statistics, ciprofloxacin resistance increased from 57.1% to 100% over five years, while ampicillin resistance ranged between 35.70% and 81.250%. Additionally, nalidixic acid and cotrimoxazole were completely resistant to pressure (Pakbin et al., 2021). Sheikh et al. (2019) discovered identical resistance patterns in 2015-2016, with the same prevalence of indicated resistance (33.3%).

Antibiotic resistance changes as a result of plasmid-borne gene transfer. In their analysis, 43.7% of samples were insensitive to furazolidone, and 33.3% were insensitive to gentamicin (Sati et al., 2019). According to antibiotic susceptibility statistics, ciprofloxacin resistance increased from 57.1 to 100%, while ampicillin resistance ranged between 35.7% and 81.25%. Between 2014 and 2016, nalidixic acid and cotrimoxazole displayed 100% resistance (Anandan et al., 2017). Brander et al. (2017) discovered a similar level of resistant reluctance. About a quarter of the samples were resistant to one of the third generation cephalosporins. S. sonnei strains were 20% resistant to cefotaxime or ceftriaxone, whilst S. flexneri strains were 11.76% resistant. Shigella spp. resistance to cephalosporins was reported to be between 2% and 5.2% in Southeast Asian research (Chung et al., 2016).

According to a large multicenter study conducted in eight Asian nations (Drprabhurajeshwar et al., 2015), resistance to ceftriaxone increased by 5% in Shigella strains

between 2001 and 2004. As a referral center, they accept patients that have been treated partially or completely, which may show the accurate occurrence of Shigella spp. (Jain et al., 2020).

Conclusion:

According to antibiotic susceptibility statistics, Ciprofloxacin resistance increased from 57.1 to 100%, whilst ampicillin resistance ranged between 35.7% and 81.25%. S. sonnei isolates were 100% resistant to ampicillin, 69.23% resistant to ceftriaxone and ceftazidime, and 53.85% susceptible to ciprofloxacin.

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