

## Antibiotic Susceptibility for *Shigella* sp. Isolated from Children under The Age of Five Years

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### Abstract

Shigellosis is caused by different species of *Shigella* and is one of the most common causes of diarrhea in children. This disease is endemic in many developing countries, including Iraq. The aim of this study was to determine the incidence of *Shigella* species and their antimicrobial susceptibility patterns in children with Shigellosis. This prospective cross-sectional study was conducted in a teaching hospital in the continent of Iraq, during the period from January 2024 to October 2024. 50 Stool samples were collected from the children's age group. All isolates were confirmed to be *Shigella* species through biochemical tests. The antibiotic susceptibility pattern of these isolates was studied by disk diffusion method. All stool samples, 18 (36%) showed *Shigella* bacteria. Among the cases, 11 (22%) were male and 7 (14%) were female. The pattern of resistance against various antimicrobials was as follows tetracyclines (TEs) (100%), ampicillin (AM) (66.7%), amoxicillin-clavulanate (AMC) (27.8%). All bacteria isolates were sensitive to sulfamethoxazole / trimethoprim (SXT) (100%) streptomycin (S) (88.9%), and cefotaxime (CTX) (72.2%).

**Keywords:** *Shigella*, Shigellosis, Antimicrobial susceptibility, Bacillary dysentery.

## اختبار الحساسية الدوائية لبكتريا الشيغيلا المعزولة من الأطفال تحت عمر خمس سنوات

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### الخلاصة

داء الشيغيلا يسببه أنواع مختلفة من بكتريا الشيغيلا وهو واحد من أكثر الأمراض شيوعاً عند الأطفال. وهذا المرض متوطن في العديد من البلدان النامية بما فيها العراق. الهدف من هذه الدراسة هو تحديد مدى حدوث أنواع الشيغيلا وأنماط حساسيتها للمضادات الميكروبية لدى الأطفال المصابين بداء الشيغيلا. أجريت هذه الدراسة المقطعية الاستباقية في أحد المستشفيات التعليمية في العراق، خلال الفترة من كانون الثاني/يناير 2024 إلى تشرين الأول/أكتوبر 2024. وتم جمع 50 عينة من البراز من الفئة العمرية للأطفال. تم التأكد من أن جميع العزلات هي الشيغيلا من خلال الاختبارات البيوكيميائية. أظهرت النتائج 18 (36%) تحتوي على بكتيريا الشيغيلا. ومن بين الحالات، 11 (22%) ذكور و 7 (14%) إناث. تمت دراسة نمط حساسية هذه العزلات للمضادات الحيوية بطريقة الانتشار القرصي. كان نمط المقاومة ضد مضادات الميكروبات المختلفة كما يلي التتراسيكلين (100%) (TEs)، الأمبيسلين (66.7%) (AM)، أموكسيسيلين-كلافولانيت (27.8%) (AMC). كانت جميع عزلات البكتيريا حساسة للسلفاميثوكسازول / تريميثوبريم (100%) (SXT) الستربتوميسين (S) (88.9%) والسيفوتاكسيم (72.2%) (CTX).

## 1. Introduction

Gastrointestinal disease ( Shigellosis or Bacillary dysentery) a caused by *Shigella* sp., The genus *Shigella* is classified in the family *Enterobacteriaceae*. It is gram negative bacteria, non-motile, non-spore forming , 0.5- 0.7µm in size and facultative anaerobic bacteria that are closely related to *Escherichia coli*. It is differentiated from *Escherichia coli* on the basis of serology, pathogenesis and physiology.[1] The *Shigella* genus is divided into four species *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella sonnei* (serogroup C) and *Shigella boydii* (serogroup D). *Shigella sonnei* has become the most dominant serotype causing is shigellosis in asian countries in recent years[2, 1].

*Shigella* bacteria renowned as A serious health problem all over the world. It is mostly found in developing countries, due to inappropriate waste management, unsafe drinking water and poor sanitary conditions. This is usually due to travel to non-industrialized countries and consuming of contaminated food in industrialized countries [3]. Diarrheal cases are a major cause of morbidity and mortality in predestined children under five years of age. with an predestined 500 000 deaths annually, rating third in the global disability-regulate life- year burden [4, 5] Globally, mortality and morbidity due to *shigella* sp. (shigellosis) were found to be high rates in children under five years of age [3, 7].The distinguished pathogenic feature of *Shigella* bacteria is its ability to prevade a variety of host intestinal cells, including the macrophages, enterocytes, and dendritic cells, this lead to severe inflammatory responses in intestinal tissue [7].

Consequences of dysentery can include loss of intestinal protein [8]. Symptoms of shigellosis can range from mild watery diarrhea to severe inflammatory dysentery with the transit of mucous and bloody stools. Other clinical appearances include abdominal cramps, nausea, fever, cramps, and vomiting. Other complications of shigellosis include septicemia, joint pain, dehydration, hypoglycemia, and neurological complications. [9, 10]. combination of oral rehydration and antibiotics can lead to the rapid solution of disease. At present, there is no protective vaccines targeting *Shigella* sp, but several vaccine candidates for *Shigella* are stil under development including:( killed, live) bacteria attenuated, ribosomal and conjugate vaccine [11].

## 2. Materials and Methods

### 2.1 Sample collection:

Sample collection: To isolate *Shigella* bacteria, 50 stool samples(25 samples from male and 25 samples from female ) were collected from children under the age of five years from the Martyr Wael Center in Al-Nasr District, Dhi Qar Governorate.

### 2.2 *Shigella* Isolation

Stool samples were cultured in the Microbiology Laboratory at Sumer University/College of Basic Education by taking a full loop of stool and culture it in sterile test tube containing 5 ml of *Shigella* broth. The tubes were incubated at 37°C for 20-24 hours. After the incubation period and observe the growth, a loop completed with the bacterial suspension was cultured on XLD agar. The plates were incubated at 37°C for 20-24 hours. The red colonies were re-cultured on the same medium to obtain pure isolates. On SS agar the pure isolates were cultured

and incubated at 37°C for 19-24 hours. After this period, the suspected *Shigella* colonies were cultured on solid nutrient medium to complete the diagnosis.

### 2.3 Identification of *Shigella*

Pure and typical colonies were selected from Nutrient Agar and were submit to slide preparation, microscopic observation, biochemical tests and Gram staining. Pure and typical colonies were identified by biochemical tests, Indole test, triple sugar iron agar (TSI), Urease test, Motility test, Citrate Utilization and methyl red- Voges-Proskauer Test (MR-VP) (all from Oxoid, UK). . The biochemical tests of *Shigella* sp. are shown in the Table 1.

### 2.4 Antibiotic Susceptibility Test.

Antibiogram potential of the isolates were determined according to the modified Kirby-Bauer disc method using Muller-Hilton agar following Clinical Laboratory Standards Institute guidelines (CLSI Performance Standards for Antimicrobial Susceptibility Testing) [11]. all isolate was distributed on a separate nutrient agar plate, with an antibiotic disc dropped on top and cultured at 37°C for 24 diffusion hours. Antibiotic discs were spread on the medium using a sterile stick, and a dispenser. was used to gently tap each antibiotic disc onto the surface of the agar. ,e double-disc synergy test with cefotaxime and amoxicillin-clavulanate discs was used to screen for extended- spectrum  $\beta$ -lactamases (ESBL). the 6 different antimicrobial agents ampicillin (AMC) (10  $\mu$ g), tetracyclines (TE) (30  $\mu$ g), cefotaxime (CTX) (30  $\mu$ g), sulfamethoxazole/trimethoprim (SXT) (25  $\mu$ g), streptomycin (S), (10  $\mu$ g), amoxicillin-clavulanate (AMC) (30  $\mu$ g), were used, and results were recorded following the guidelines of CLSI [12].

## 3. Result and Discussion

### 3. 1 Study sample

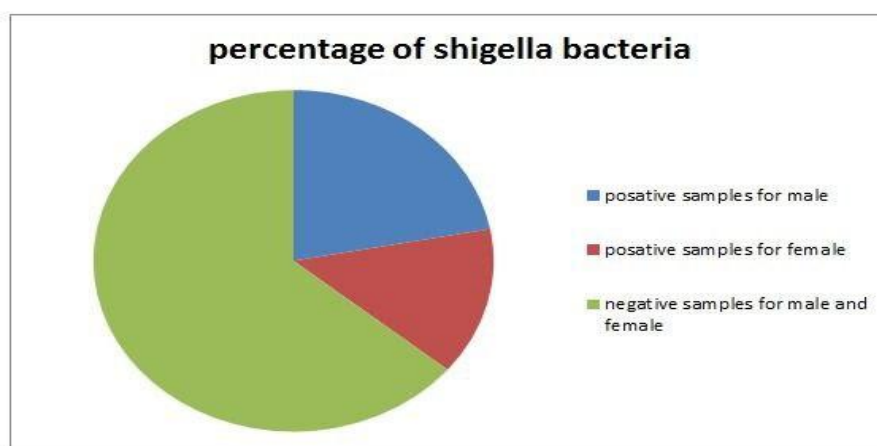
50 stool samples were collected from children suffer from diarrhea caused by isolated *Shigella* bacteria, and the patients' ages ranged from days to 5 years. In this study, 50 samples were examined, 18 (36%) of which were found to be *Shigella* positive. Of these positive samples 11(22%) were isolated from males and 7(14%) were isolated from females as shown in (Figure 1). These results are consistent with a previous study conducted by Gebrekidan et al [13], The worldwide occurrence of shigellosis and bacillary dysentery, In particular in developing countries, is a common phenomenon. The death rate due to this disease is also alarming. Drug-resistant, as well as MDR dysentery-causing pathogens, have been reported all over the world for a few decades. This scenario is alarming as the development of a drug is not an easy task as it is time consuming, laborious, and costly[14]

"*Shigella flexneri* has been the most frequent cause of shigellosis in children in Iran. To evaluate the changes in frequency of serogroups, 302 *Shigella* species were isolated in 2003 from hospitalized children, aged less than 12 years, with acute diarrhoea in Tehran, Iran. The number of collected *S. sonnei*, *S. flexneri*, *S. boydii*, and *S. dysenteriae* isolates was 178 (58.9%), 110 (37.4%), 10 (3.3%), and 4 (1.3%) respectively". [15]. A previous study similar to this study in Abadan, Iran found that of all 705 stool samples, 36 (5.1%) showed *Shigella* sp.. There are 392 (55.6%) were from female and 313 (44.4%) were from male. Most common *Shigella* isolates were *Shigella flexneri* (52.7%), tracked by *Shigella sonnei* (30.5%), *Shigella boydii* (11.1%), and *Shigella Dysenteriae* (5.5%) [16].

### 3.2 Antimicrobial Susceptibility of the Bacterial Isolates

Phenotypic, antibiotic resistance of 18 isolates of *Shigella* is presented in Table 2. The highest resistance percentages against tetracyclines (TEs) (100%), ampicillin (AM) (66.7%), amoxicillin - clavulanate (AMC) (27.8%). All isolates of *Shigella* were sensitive to sulfamethoxazole / trimethoprim (SXT) (100%) streptomycin (S) (88.9%), and cefotaxime (CTX) (72.2%).

"Antibiotics are often used for children with bloody and chronic diarrhea to reduce the duration of the disease. Because shigellosis is very contagious, information about the antimicrobial susceptibility is very important for suitable treatment and management of the disease"[17]. Overall, In this study, a harmful level of *Shigella* spp bacteria was detected. resistance to widely used antibiotics (TE, AM, AMC, CEC) among human and lives tocks ,this resistance might be because of the frequent and improper use of such antibiotics either in animal therapy or as a growth promoter in the veterinary context[18]. In another study majority of *Shigella* isolates were resistant to ampicillin (86.8%), and tetracycline (80.2%), which is similar to the previous study from Iran and other countries[17,19,20]. According to these results, these antibiotics are not suitable for the treatment of shigellosis in these regions[21].



**Figure -1** Percentage of *Shigella* species in cultured samples from diarrhea cases for males and females of children

**Table 1-** Result of Biochemical tests for Identification of *Shigella* species.

<i>Test Media</i>	<i>Result</i>
<i>Urease test</i>	Negative ( No change in colour)
<i>Mannitol motility test</i>	Negative (There is no turbidity and no colour change)
<i>Indole Test</i>	Positive (There is Red ring Development)

<i>Methyl Red Test</i>	Positive (There is Red colour Development)
<i>Voges-Proskauer Test</i>	Negative (There is no pink colour Development)
<i>Citrate Utilization Test</i>	Negative (There is no growth and change in colour)
<i>Gas from glucose (TSI)</i>	Negative
<i>H<sub>2</sub>S from TSI</i>	Negative
<i>Urease test</i>	Negative ( No change in colour)
<i>Mannitol motility test</i>	Negative (There is no turbidity and no colour change)
<i>Indole Test</i>	Positive (There is Red ring Development)
<i>Methyl Red Test</i>	Positive (There is Red colour Development)

**Table 2-** Number and percentge of Shigella species Resistant, Intermediate and Sensitive to Antibiotic.

<i>Antimicrobial class</i>	<i>Antimicrobial</i>	<i>Shigella sp. (n=18)</i>		
		<b>R</b>	<b>I</b>	<b>S</b>
<i>Ampicillin</i>	Am	12(66.7%)	2(11.1%)	4(22.2)
<i>Tetracyclines</i>	TE	18(100%)	0	0
<i>Cefotaxime</i>	CTX	3(16.6%)	2(11.1%)	13(72.2%)
<i>sulfamethoxazole/trimethoprim</i>	SXT	0	0	18(100%)
<i>amoxacillin-clavulanate</i>	AMC	5(27.8%)	3(16.6%)	7(38.9%)
<i>Streptomycin</i>	S	2(11.1%)	0	16(88.9%)

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