Antibiotic Susceptibility Pattern of *Shigella* Isolated from Diarrhea Samples Collected from Medical Facilities in Liberia

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ABSTRACT

Antimicrobial resistance (AMR) is a growing health concern, especially in low- and middle-income countries such as Liberia. No reports exist regarding the antibiotic susceptibility pattern of *Shigella* isolates in Liberia to date. This study aimed to determine the pattern of antibiotic susceptibility of Shigella isolated from diarrhea samples collected from medical facilities in Liberia. A total of 269 diarrheal stool samples were used in this study using Xylose lysine deoxycholate agar (XLD), MacConkey agar, and *Salmonella Shigella* (SS) agar, and plates were incubated at 37 °C for 18 – 24 hours. *Shigella* isolates were identified microscopically, biochemically and by serotyping. The antibiotic susceptibility test was done by the Kirby-Bauer disk diffusion method in Muller-Hinton agar following standard procedures. *Shigella* was isolated from 9% of the total diarrhea samples collected from medical facilities in Liberia. *S. flexneri* and *S. sonnei* were the most isolated *Shigella* species, at 45.80% and 41.60%, respectively. In contrast, *S. boydii* was the least isolated *Shigella* species in this study. The antibiotic sensitivity testing shows that ciprofloxacin (100%) and chloramphenicol (79%) were the most effective antibiotics used in this study. *Shigella* isolates were resistant to tetracycline (100%) and co-trimoxazole (62%). These findings pinpoint the urgent need for AMR surveillance and logical use of antibiotics in Liberia.

Keywords: Antimicrobial resistance, antibiotic susceptibility, ciprofloxacin, diarrhea, Shigella, multidrug resistance.

INTRODUCTION

Shigellosis has become a global public health concern. Annually, *Shigella* is estimated to cause 80 – 165 million cases of disease and 600,000 deaths worldwide (CDC, 2024). According to Aslam *et.al* (2024), Shigellae can be transmitted by the fecal-oral route or by direct person-to-person contact. Shigellae are less susceptible to destruction by gastric acid after ingestion; only a small inoculum, as few as 10 to 100 organisms, is required to cause disease. Although no one is immune to shigellosis, some people are more susceptible than others. Nearly 69% of cases involve children under the age of five. Oral rehydration might be adequate in many cases; in more severe cases, intravenous fluid rehydration with or without hospitalization may be necessary, according to Aslam *et al* (2024). Bengtsson *et. al* (2025) also reported that the severity of shigellosis and potential complications can be managed through supportive care and antimicrobial therapy.

However, antibiotic resistance to *Shigella* and serotypes has emerged throughout the world (Taneja *et al*., 2012). Previously, effective first-line drugs such as sulphonamide-trimethoprim, ampicillin, and nalidixic acid were almost ineffective against the prevalent *Shigella* strains (Kotloff *et al*., 2018). The emergence of bacteria strains that are resistant to widely used antibiotics poses a grave threat to human health. Low- and middle-income countries (LMICs) are disproportionately affected, partly due to poor sanitation, weak infection control, substandard antibiotics, and high a burden of communicable diseases. With the recent rise of flouroquinolone-resistant strains, the choice of drugs for effective antimicrobial therapy has vastly reduced (Kotloff *et al*., 2018; Puzari *et al*., 2018). As an alternative antimicrobial therapy, WHO has recommended three antibiotics, namely, pivmecillinam, ceftriaxone, and azithromycin (WHO, 2005).

However, resistance to these WHO-recommended drugs has been observed as suggested by the findings of various studies. To our knowledge, no reports exist regarding antibiotic susceptibility pattern of *Shigella* isolates in Liberia. Thus, this study was designed to determine the antibiotic susceptibility pattern of *Shigella* isolated from diarrhea samples collected from medical facilities in Liberia.

MATERIALS AND METHODS

Collection of Clinical Samples

A total of 269 diarrheal stool samples were collected in dry, clean, leakproof, and wide-mouth stool containers from medical facilities in the 15 counties and transported in sterile foam boxes with dry ice packs to the Biology Laboratory, Adventist University of West Africa. Samples that were not analyzed the same day were kept in the freezer at -20 °C. Moreover, participants who took antibiotics for the diarrheal attack were excluded from the study. The samples were collected from June 2023 to May 2024.

Isolation and Identification of Bacteria

A loopful of stool sample from each container was inoculated in Xylose lysine deoxycholate agar (XLD), MacConkey agar, and *Salmonella Shigella* (SS) agar. The plates were incubated at 37 °C for 18 – 24 hours. The colony morphology resembling *Shigella* was identified microscopically and biochemically (using KB003 Hi25^{TM} *Enterobacteriaceae* Identification Kit, India). Differential of *Shigella* was done by slide agglutination test using polyvalent antisera (Becton, Dickinson and Company, MD, USA). The appearance of agglutination (distinct clumping) by Group A polyvalent, Group B polyvalent, Group C polyvalent, and Group D polyvalent in the kit were identified as *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, respectively.

Antibiotic Susceptibility Testing

The antibiotic susceptibility test was done by the Kirby-Bauer disk diffusion method in Muller-Hinton agar following standard procedures (Bauer *et al*., 1966). To summarize, a sterile cotton swab was used to wipe the whole surface of Mueller Hinton agar (Oxoid) with a McFarland 0.5 standardized suspension of the bacteria in 0.8% sterile saline. The inoculated surface was subsequently covered with discs (HiMedia, India) that contained single concentrations of each antimicrobial agent. Using a straight-line ruler, the clear zones created by the antimicrobial suppression of bacterial growth were measured in millimeters following an overnight incubation at 37°C and they were interpreted as sensitive, intermediate sensitive, and resistant (CLSI, 2014). The antibiotic susceptibility pattern of six antibiotics, namely Chloramphenicol (CHL, 30 mcg), Co-trimoxazole (COT, 25 mcg), Tetracycline (TET, 10 mcg), Ciprofloxacin (CIP, 10 mcg), Azithromycin (AZM, 30 mcg), Ceftriaxone (CTR, 30 mcg).

Quality Control

Quality control was set up using an *Escherichia coli* strain (ATCC 25922) which was susceptible to all the tested drugs.

Data Analysis

Tables and percentages were used to the describe findings. Data analysis was performed with Microsoft Excel 365.

Ethical Clearence

Ethical clearance was obtained from the Research Ethics Committee of the Adventist University of West Africa. Informed consent was requested from patients. Anonymous forms were used to collect data required for the study to secure confidentiality.

RESULT

Basic Characteristics of *Shigella* Positive Samples

The 24 samples positive for Shigellosis were characterized based on gender and age distribution. The gender with the highest distribution of Shigellosis cases was male (15) and female (9). The age group with the highest cases of Shigellosis recorded was 46 and above (9), followed by 41 – 45 (4), whereas 16 – 20 reported the lowest (0) and 31 – 36 (1).
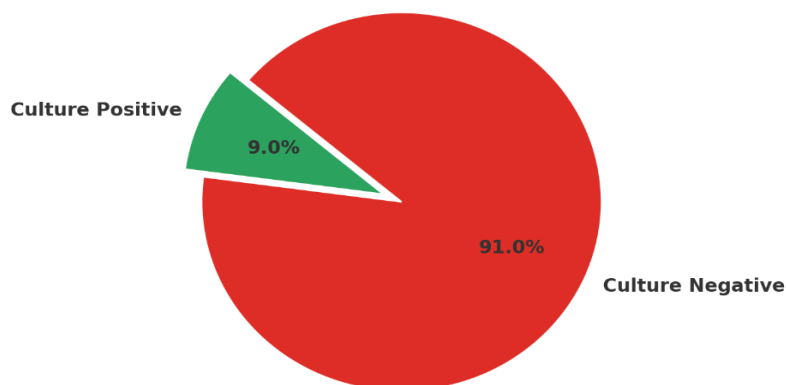
Table 1. Gender and age distribution of Shigellosis samples

Gender			Age Groups		
	Frequency	**Percentage (%)**		**Frequency**	**Percentage (%)**
Male	15	62.5	1 – 15	2	8.3
Female	9	37.5	16 – 20	0	0
			21 – 25	2	8.3
			26 – 30	3	12.5
			31 – 36	1	4.2
			36 – 40	3	12.5
			41 – 45	4	16.7
			46 – above	9	37.5
Total	**24**	**100**	**Total**	**24**	**100**

Prevalence of Shigellosis among diarrhea samples

The total number of diarrhea samples tested in this study was 269 from the 15 counties. Of the total, nine percent (9%) of was positive for Shigellosis.

Figure 1. Prevalence of Shigellosis among diarrheal samples

Proportion of *Shigella* serotypes among *Shigella* positive samples

Shigella flexneri (11) was the most isolated *Shigella* serotypes of the 24 *Shigella* positive samples, followed by *Shigella sonnei* (10). The *Shigella* serotype with the lowest distribution was *Shigella boydii* (1).

Table 2. Proportion of *Shigella* serotypes among *Shigella* positive samples

Shigella serotypes	Frequency	Percentage (%)
Shigella boydii	1	4.16
Shigella dysenteriae	2	8.44
Shigella flexneri	11	45.80
Shigella sonnei	10	41.60
Total	**24**	**100**

Distribution of Shigellosis and *Shigella* serotypes across the counties in Liberia

Nimba County recorded the highest number of *Shigella* positive samples (5), followed by Sinoe and Rivergee (4) each. There was no *Shigella* isolated from samples collected from Grand Gedeh, Lofa, Bomi and Bong.

Table 3. Distribution of Shigellosis and *Shigella* serotypes across the counties in Liberia

Counties	Shigellosis		*S. boydii*	*S. dysentry*	*S. flexneri*	*S. sonnei*
	Frequencies	Percentage (%)				
Gbarpolu	1	4.2	-	-	1	1
Grand Bassa	1	4.2	-	-	-	1
Grand Cape Mount	1	4.2	-	-	1	
Grand Kru	1	4.2	-	-	1	
Margibi	2	8.3	-	1	-	1
Maryland	2	8.3	-	-	1	1
Montserrado	2	8.3	-	1	-	
Nimba	5	20.8	-	-	1	4
Rivergee	4	16.7	-	-	3	1
Rivercess	1	4.2	-	-	-	1
Sinoe	4	16.6	1	-	3	
Total	**24**	**100**	**1**	**2**	**11**	**10**

Antibiotic Susceptibility Pattern of *Shigella* Isolates

The antibiotic susceptibility pattern of *Shigella* isolates were tested against six (6) antibiotics. Of the antibiotics tested, Ciprofloxacin was the most susceptible antibiotics (100%), followed by Ceftriaxone (88%) and Chloramphenicol (79%). All Shigella isolates were resistant to Tetracycline.

Table 4. Antibiotic Susceptibility Pattern of *Shigella* Isolates

Antibiotics	Susceptible		Intermediate		Resistant	
	Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage (%)
Azithromycin	19	79	5	21	0	0
Ceftriaxone	21	88	3	12	0	0
Chloramphenicol	19	79	4	16	1	5
Ciprofloxacin	24	100	0	0	0	0
Co-Trimoxazole	5	21	4	17	15	62
Tetracycline	4	17	1	4	19	79

Figure 2. Antibiotic Susceptibility Pattern of *Shigella* Isolates
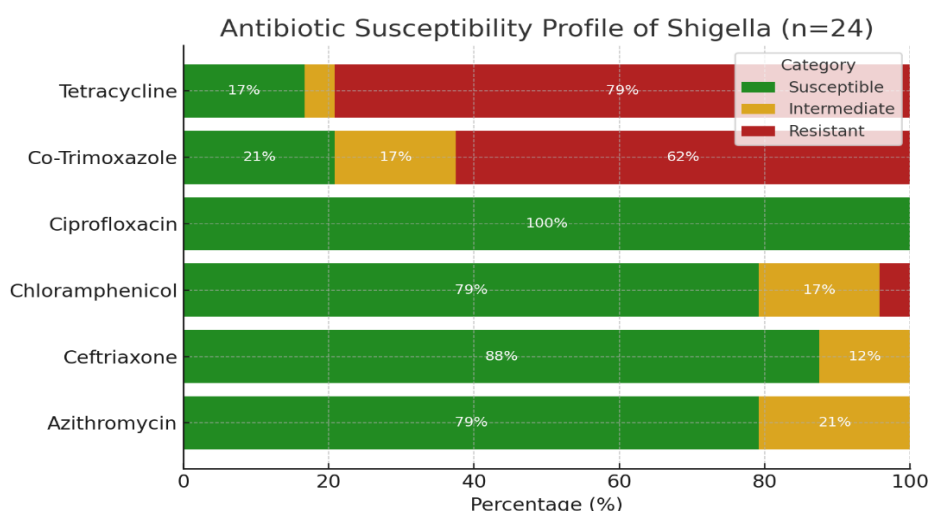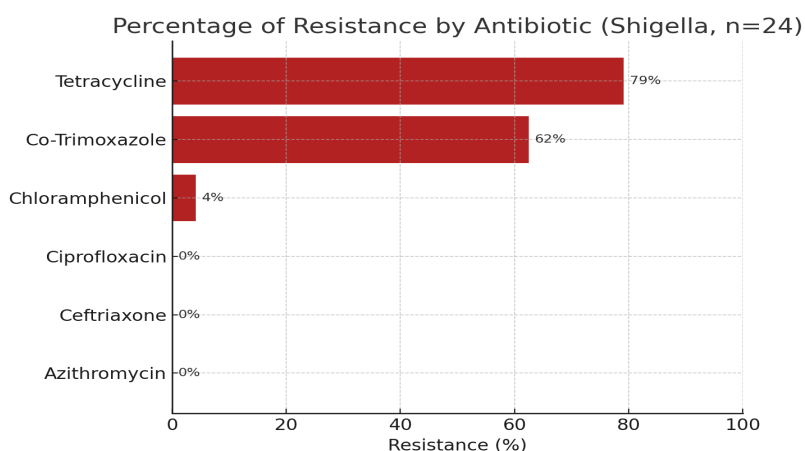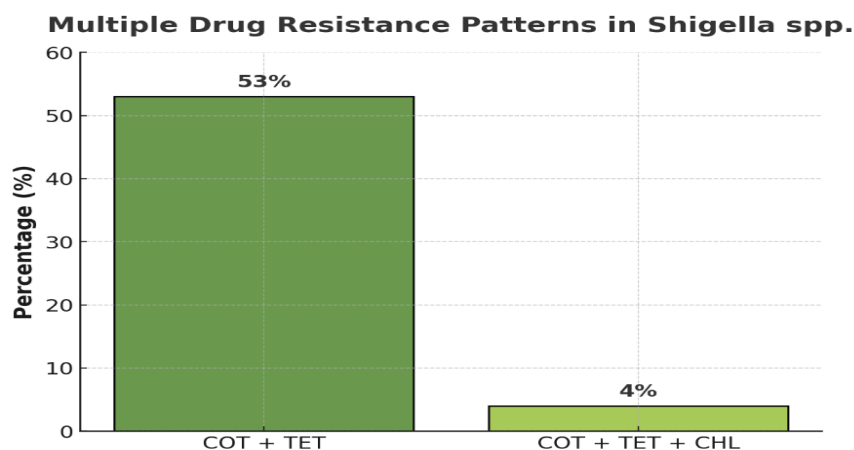

Figure 3. Percentage of Resistant *Shigella* Isolates per Antibiotics

Multiple Drug Resistance Pattern in *Shigella* Isolates

Multiple drug resistance was recorded in more than 50% of *Shigella* isolate. Fifty-three (54%) of *Shigella* isolates were resistant to Co-trimoxazole and Tetracycline and one isolate was resistant to Cotrimoxazole, Tetracycline and Chloramphenicol.

Figure 4. Multiple Drug Resistance Pattern in *Shigella* Isolates

DISCUSSION

Diarrhea is an utmost global health problem, and recent studies have confirmed *Shigella* as a major contributor to this problem. According to WHO, *Shigella* was the second-leading cause of diarrheal mortality in 2016 among all ages and the leading bacterial cause of diarrhea. Literature relating to the antimicrobial resistance pattern of *Shigella* is absent in Liberia. With antimicrobial resistance ranging from region to region, it is crucial that we understand how this AMR is thriving in Liberia.

Our study shows that Shigellosis was higher in males (62.5%) than females (37.5%). This finding correlates with Chen *et al*. (2020), where males accounted for 56.57% of severe Shigellosis compared to 43.43% in females. McCrickard *et al*. (2018) also revealed that male aged 18 – 49 (30%) reported the highest cases of severe Shigellosis compared to their female counterparts (25%).

In our investigation, age group 46 and above reported the highest number of Shigellosis (37.5%), followed by age group 41 – 45 (16.7%). Similar results were reported by Zhao et al. (2021) wherein the age group 25 – 59 years had the highest susceptibility to Shigellosis. They argued that the findings may be related to the tendency of these individuals to take care of others at home. In contrast, several studies have reported higher distribution of Shigellosis in children under five (5), Abebe *et al*. (2018), Mamuye *et al*. (2015), Teshome *et al*. (2019), and Gebrekidan *et al*. (2015).

In this study, *S. flexneri* (45.80%) was the most isolated *Shigella* species, followed by *Shigella sonnei* (41.60%). A systematic review and meta-analysis by Nyarkoh et al. (2024) on 116 studies from 29 African countries involving the examination of 99,510 samples revealed that the most prevalent species of *Shigella* in sub-Saharan Africa is *S. flexneri* (53.6%), followed by *S. sonnei* (11.5%), *S. dysenteriae* (10.1%), and *S. boydii* (7.7%). Numerous studies conducted in Sub-Saharan Africa have familiar results to that of this study. Kasumba *et al*. (2023), found out that *S. flexneri* is predominant among cases (67.6%) of isolates, followed by *S. sonnei* (18.2%). Opintan and Newman (2007) found *S. flexneri* (70.8%) to be the most distributed *Shigella* species in Ghana. Similarly, Iwalokun et al. (2001) found that *S. flexneri* (51.6%) is the most prevalent *Shigella* species in Lagos, Nigeria. According to Taneja and Mewara (2016), *S. flexneri* is the most prevalent *Shigella* species in developing countries.

Our study found out that Nimba County, which is located in Northern Liberia, reported the highest Shigellosis (20.8%), followed by Rivergee County (16.7%), which is located in the Southeast of Liberia. Nimba County reporting the highest Shigellosis could be as a result of the large farming activities in the county, as Lofa and Nimba County are noted for the highest farming activities in the country. A study conducted by the Partnership for Food Safety Education (2021) mentioned that shigellosis may occur from raw fruits consumption, such as fruits and vegetables that may develop in soil.

However, ciprofloxacin was the most defenseless drug (100%) in this study, then chloramphenicol (79%). This finding correlates with several studies in Sub-Saharan Africa. Gebrekidan *et al*. (2015) reported that

ciprofloxacin (93.3%) was the most susceptible drug in Ethiopia. In Ghana, Opitan and Newman (2007) found 100% susceptibility of *Shigella* species isolates to Ciprofloxacin. Several other findings correlate with this study, 100% susceptibility was reported by Debas *et al.* (2011) and Mulatu *et al.* (2014). In contrast, results from other countries showed high resistance to ciprofloxacin, 82% in India (Fauci *et al.*, 2008) and 28.3% in Nepal (Bhattacharya *et al.*, 2005). Tetracycline was the least susceptible antibiotics in this study, as all of the *Shigella* isolates were resistant to it. Afum *et al.* (2022) found *Shigella* isolates to be 100% resistant to tetracycline in Ghana, which correlates with the findings of this study. They argued that this phenomenon might be a result of the extensive use of tetracycline in Ghana over the years. There is an alike pattern of excessive antibiotics use in Liberia which may have resulted to the resistance of *Shigella* to the drug. Our investigation shows that co-trimoxazole (62%) was highly ineffective against *Shigella* isolates. Gebrekidan *et al.* (2015) reported similar findings in Ethiopia (66.7%).

Over 50% of the *Shigella* isolates showed resistance to multiple drugs (Tetracycline and co-trimoxazole). Similarly, Opintan and Newman (2007) reported 96% of *Shigella* were classified as MDR based on their resistance to three or four antibiotics which included tetracycline and co-trimoxazole. Poramathikul *et al.* (2016) reported that 91% of isolates were resistant to multiple drugs including ampicillin, tetracycline, co-trimoxazole, and Nalidixic acid in Cambodia.

CONCLUSION

Shigella was isolated from 9% of the total diarrhea samples collected from medical facilities in Liberia. *S. flexneri* and *S. sonnei* were the most isolated *Shigella* species, at 45.80% and 41.60%, respectively. In contrast, *S. boydii* was the least isolated *Shigella* species in this study. The antibiotic sensitivity testing shows that ciprofloxacin (100%) and chloramphenicol (79%) were the most effective antibiotics used in this study. *Shigella* isolates were resistant to tetracycline (100%) and co-trimoxazole (62%). This study shows that antibiotic resistance is a growing public health concern in Liberia as it is in most developing countries.

ACKNOWLEDGEMENTS

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