Original Research Article

Prevalence and Antimicrobial Susceptibility Patterns of *Salmonella, Shigella and Escherichia coli* among Children suffering from Diarrhoea, Unguja - Zanzibar, Tanzania.

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ABSTRACT

Aims
This study aimed at providing evidence of the prevalence and antimicrobial susceptibility patterns towards *Salmonella*, *Shigella* and *Escherichia coli* among under-five children in Unguja – Zanzibar, Tanzania.
Study design: Cross sectional design was used to collect samples from stool of children suffering from diarrhoea.
Place and Duration of Study: The study was carried out in Zanzibar west urban region between October, 2019 to February, 2020.
Methodology
A cross-sectional study was conducted from October 2019 to February 2020. Random samples were collected to investigate the prevalence of *Salmonella, Shigella and Escherichia coli*. The samples were cultured using Hektoen Enteric (HE) and *Salmonella-Shigella* agar. Antibiotic susceptibility testing was done by Kirby–Bauer disc diffusion method.
Results
A total of 159 stool samples were collected in the study; *Salmonella* was identified 12/159 times (7.5%) of the total samples. *Shigella* and *E. coli* were identified in 7/159 samples (4.4%) and 6/159 (3.7%), respectively. Children between 49 and 60 months showed low prevalence, while a high peak prevalence was reported for children between 7–12 months. All *Salmonella*, *Shigella* and *Escherichia coli* species identified were sufficiently susceptible to chloramphenicol and ceftriaxone, with a varying pattern to azithromycin, ciprofloxacin, ampicillin, nalidixic acid and trimethoprim-sulfamethoxazole.
Conclusion
We found *Salmonella*, *Shigella* and *Escherichia coli* isolates in stools of children ≤ 5years from Unguja, Zanzibar, but all the isolates were susceptible to chloramphenicol and ceftriaxone but partially resistant to other tested antibiotics. Identifying resistant bacteria in this age group should be a concern for the public health authorities and trigger research into finding the cause.

Keywords: Salmonella, Shigella, Escherichia coli, Diarrhoea, Prevalence**,** Antimicrobial Susceptibility and Children under-five years.

1. INTRODUCTION

Diarrhoea is a frequent discharge of a watery stool accompanied by abdominal cramps, nausea and vomiting, sometimes with fever and chills. The World Health Organization (WHO) estimates that about 1.7 billion cases of childhood diarrhoeal diseases are responsible for killing 525,000 children yearly (1,2),(3). Several countries have diarrhoea outbreaks from *Salmonella*, *Shigella* and *Escherichia coli* (*E. coli)* associated with consumption of contaminated food, water or direct faecal contamination through fingers,

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the major source of transmission of diarrhoeagenic disease (4,5). It has been reported that most deaths and hospitalization due to diarrhoea occur in ~~the~~ developing countries, particularly in Africa (1,6). *Salmonella*, *Shigella* and *E. coli* are amongst the species that cause diarrhoea in Africa (4,7,8).

The one health approach is the most recommended method to tackle antimicrobial resistance in human health, animal health, food, and environment sectors. The common antibiotics for treating diarrhoea caused by *Salmonella, Shigella, and E. coli* are ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole but there a~~re~~ is evidence of resistance to these and other antimicrobials commonly used to treat diarrhoea in developing countries (5,9–11). Unfortunately, limited laboratory techniques to test antimicrobial susceptibility ha~~s~~ve resulted in a minimum understanding of the resistance burden and reduced therapeutic efficacy (12–15). Thus, there is a need to understand the country-level epidemiology of these diarrhoegenic bacteria and the efficacy of the available antibiotics to treat the diarrhoea and associated illnesses.

C~~c~~onsumption of contaminated food and water is a common but inadvertent practice in Zanzibar linked to the cultural food system and the environment. For more than 40 years, diarrhoeal diseases have been ~~the~~ a common public health problem presenting with outbreaks in Zanzibar (16). It is hypothesized that *Salmonella, Shigella and E.coli* are responsible for the outbreaks (17). However, there are more microbial organisms causing diarrheoa apart from *Salmonella, Shigella* and *E. coli*. This study aimed to investigate the prevalence and antimicrobial susceptibility patterns of *Salmonella, Shigella and Escherichia coli* among diarrhoea suffering Children in Unguja - Zanzibar. Tanzania.

2. MATERIAL AND METHODS

Study design and site

This was a rapid cross-sectional study, covering public health facilities in the three districts of the west urban region, namely the urban district, west 'A' district, and west 'B' district, from October~~,~~ 2019 to February~~,~~ 2020. A random sampling was used to select nine public health facilities in each district. The twenty-seven selected health facilities were Mnazi Mmoja, Chumbuni, Sebleni, Rahaleo, Kwamtipura, Kidongo Chekundu, Kidutani, Shaurimoyo, Mpendae, Fuoni, Kombeni, Magogoni, Kiembe Samaki, Fuoni Kibondeni, Shakani, Bwefum, Chukwani, Kisauni, Mbweni Matrekta, Mtofaani, Selem, Bubwisudi, Chuini, Kizimbani, Kianga, Beit-el-Ras and Kibweni. These public health facilities are government properties, and treatment of children is free and hence provide~~s~~ health care to most children.

Sample size determination

~~P~~The population proportion formula was employed using the desired characteristics of 12% (18) of diarrhoea cases, as calculated below.

Fisher's formula: $n = Z^2pq/r^2$ (19).

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Several countries have diarrhoea outbreaks from Salmonella XXX, Shigella XXX, and E. coli due to contaminated food, water, or direct faecal contamination, the primary transmission source.

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Where: n = Desired sample size. p = Proportion of the population with a desired characteristic of 12% (18). q = 1-p; z= standard deviation desired degree of accuracy. Where z is 1.96, if the degree of confidence is 95%; r= Degree of error, which was 5%

Therefore: n was found to be 159.

The characteristics of patients whom stool samples were collected from under-five children presenting with diarrhoea frequency > three times per 24 hours, either bloody or watery diarrhoea included suspected cases of cholera. The age and play environment of patients have been shown relationto be related to what has been studied. The samples were collected in rainy and dry seasons with unsuspected and suspected cholera epidemics.

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Sample collection, storage, and transportation

One hundred and fifty-nine stool samples were collected from 27 health facilities using sterile plastic containers. All samples collection was performed from a period of October, 2019 – February, 2020, immediately after samples collection, packed in a cool box at 4°C and sent to the microbiology department of the pathology laboratory at Mnazi Mmoja hospital Zanzibar for further analysis.

Bacterial culture, isolation, and identification

In the laboratory, approximately 1 g of stool specimen was placed overnight into 10 ml Selenite-F enrichment broth (Oxoid UK) in a sterile test tube. A loopful of the suspension of the specimen was streaked onto two different media, namely, Hektoen Enteric (HE) Agar and *Salmonella Shigella* (SS) Agar, both from Oxoid, UK. These plates were incubated under 37 °C for 48 hours. A colorless colony with or without a black centre on SS Agar media and a blue-green colony with or without a black centre on HE Agar were isolated as *Salmonella*-like isolates. A colorless colony on SS and a green, moist, and raised colony on HE Agar were isolated as *Shigella*-like isolates (20). Colonies exhibiting characteristic reactions of *Salmonella*, *Shigella* and *E. coli*-like were further characterized by the pattern of biochemical reactions after inoculation to Triple sugar iron Agar, lysine iron Agar, Simon's citrate Agar and motility test, Indole and Urease production (MIU) test for final identification using the standard procedures (20).

Antimicrobial Susceptibility Testing

Kirby-Bauer's disc diffusion method was used for antibiotic sensitivity tests. One of the easiest and quickest methods that can be used to test the antibiotic sensitivity of a bacterial isolate. Antimicrobial susceptibility testing with discs is a simple, rapid method and provides a reproducible means of testing bacterial sensitivity to various antibiotics and chemotherapeutic agents. Pure overnight cultures of *Salmonella Shigella and E. coli* isolates were mixed with sterile saline and after matching with 0.5 McFarland standards were inoculated in Mueller-Hinton Agar. Antibiotic discs were placed on to the Agar. *Shigella* and *E. coli* isolates were tested against ceftriaxone (CTX, 30 µg), ciprofloxacin (CIP, 5 µg), chloramphenicol (C, 30 µg), ampicillin (AMP, 10 µg), trimethoprim-sulfamethoxazole (SXT, 25 µg) or co-trimoxazole (CO, 25 µg), nalidixic acid (NA, 30 µg), and azithromycin (AZM, 15 µg). Inhibition diameter

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zone readings recorded according to Clinical and Laboratory Standards Institute (21) and results were reported as sensitive (S), intermediate (I) and resistance (R).

Data handling and statistical analysis

Data were initially compiled in an MS excel spreadsheet, and statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (16.0 version). Descriptive statistics were calculated and summarized in frequency and proportions. Prevalence and antimicrobial susceptibility patterns of *Salmonella, Shigella and E. coli* were determined focused on variables potentially magnitude, typical of *Salmonella*, Shigella and *E. coli* isolates and their proportions.

3. RESULTS AND DISCUSION

Results

Socio-demographic characteristics

A total of 159 children under 5-year of age with diarrhoea were included in the study. Of the 159 participants, 73 (45.9%) were females, and 86 (54.08%) were males. The specific age categorization was as follow: 17 (10.69%) of them were between 0 to 6 months, 56 (35.2%) were between 7 to 12 months, 44 (27.67%) were between 13 to 24 months, 24 (15.09%) were between 25 to 36 months, 14 (8.8%) were between 37 to 48 months, and 4 (2.5%) were between 49 to 60 months (Figure 1)

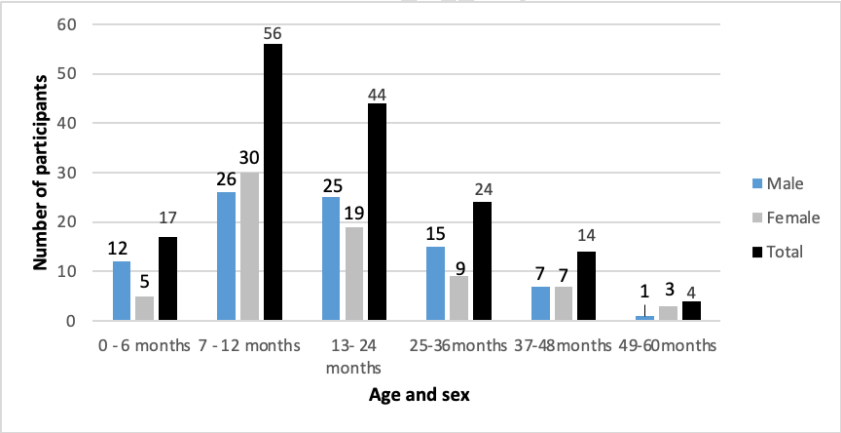

Figure 1: Distribution of Participants by Age and Sex

Prevalence of *Salmonella, Shigella and E. coli*

A total of *Salmonella* 12/159 (7.5%), 7/159 *Shigella* (4.4%) and 6/159 *E. coli* (3.7%) were identified among under-five children with diarrhoea in Unguja - Zanzibar. Tanzania**.** ~~In relation to~~Considering the proportion of isolation, *Salmonella* 4/159 (2.5%) w~~ere~~as the highest among the age group ~~of~~ between 7 to

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12 months, but it was not identified in children above 37 months (Table 1). The *Shigella* 1/159 (0.66%) was identified among 0 to 6 months, 13 to 24 months, 25 to 36 months children, and 37 to 48 months but it was not found in 7 to 12 months and above 49 months children (Table 1). The *E. coli* (1(0.66%)) was identified among 0-6 months, 13-24 months, and 25-36 months children, but not among 7 - 12 months and above 37 months children. Among the 25 culture-positive children, 13 (52%) were female (Table 1).

Table 1: The Distribution of *Salmonella*, *Shigella* and *E. coli* among Children Suffering from Diarrhoea by Age and Sex in Unguja – Zanzibar.

Variables	Patients frequency (%)	*Salmonella* (%)	*Shigella* (%)	*Escherichia coli* (%)
Sex				
Male	86 (54.08%)	6 (3.7)	4 (2.5)	2 (1.3)
Female	73 (45.91%)	6 (3.7)	3 (1.8)	4 (2.5)
Age				
0 – 6months	17 (10.69%)	1 (0.6)	1 (0.6)	1 (0.6)
7 – 12months	56 (35.22%)	4 (2.5)	0(0)	0(0)
13 – 24months	44 (27.67%)	1 (0.6)	1(0.6)	1 (0.6)
25 – 36months	24 (15.09%)	1 (0.6)	1 (0.6)	1 (0.6)
37 – 48months	14(8.8)	0(0)	1(0.6)	0(0)
49 – 60 months	4(2.51)	0(0)	0(0)	0(0)

Antibiotic Susceptibility Test from patients' stool samples

The *Salmonella* isolates displayed different susceptibility rates to the evaluated antibiotics (Table 2). *Salmonella* isolates were highly susceptible to ceftriaxone 12 (100%), chloramphenicol 12 (100%) and trimethoprim-sulfamethoxazole 12 (100%), azithromycin 10 (83.3%), ciprofloxacin 8 (66.6%) and ampicillin 8 (66.6%) while no susceptible to nalidixic acid. They exhibited low resistance to nalidixic acid 5 (41.6%), azithromycin 2 (16. 6%) and ampicillin 2 (16.6%). The results indicate no resistance to Chloramphenicol, Ciprofloxacin, Ceftriaxone and Trimethoprim-sulfamethoxazole.

Table 2. Antibiotic Susceptible Pattern of *Salmonella*, *Shigella* and *E. coli* from Patient

Antimicrobials	Salmonella			Shigella			E coli		
	S	I	R	S	I	R	S	I	R
Ampicillin	8(66.6)	2(16.6)	2(16.6)	2(28.5)	5(71.4)	0(0.0)	2(33.3)	1(33.3	2(33.3)
Ceftriaxone	12(100)	0(0.0)	0(0.0)	7(100)	2(28.5)	0(0.0)	6(100)	0(0.0)	0(0.0)
Chloramphenicol	12(100)	0(0.0)	0(0.0)	7(100)	0(0.0)	0(0.0)	6(100)	0(0.0)	0(0.0)
Nalidixic acid	0(0.0)	7(58.3)	5(41.6)	2(28.5)	3(42.8)	2(28.5)	2(33.3)	2(33.3	2(33.3)
Ciprofloxacin	8(66.6)	4(33.3)	0(0.0)	4(57.1)	0(0.0)	0(0.0)	4(66.6)	2(33.3	0(0.0)
Azithromycin	10(83.3	0(0.0)	2(16.6)	4(57.1)	0(0.0)	3(42.8)	4(66.6)	1(16.6	1(16.6)

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Trimethoprim-sulfamethoxazole	12(100)	0(0.0)	0(0.0)	2(28.5)	0(0.0)	5(71.4)	2(33.3)	0(0.0)	4(66.6)

Abbreviations: ***S*** *- Sensitive,* ***R*** *- Resistant* and ***I*** *- Intermediate*

The *Shigella* isolates were highly susceptible to chloramphenicol 7 (100%) and ceftriaxone 7 (100%) while lowly susceptible to ampicillin 2 (28.5%), nalidixic acid 2 (28.5%) and trimethoprim-sulfamethoxazole 2(28.5%). However, these isolates showed different resistance against trimethoprim-sulfamethoxazole 5 (71.4%), azithromycin 3 (42.8%), and nalidixic acid 2 (28.5%). *Shigella* was found to show no resistance to Chloramphenicol, Ceftriaxone, Ciprofloxacin, and Ampicillin, as shown in Table 2

The *E. coli* isolates were highly susceptible to chloramphenicol 6 (100%) and ceftriaxone 6 (100%) and lowly susceptible to ampicillin 2 (33.3%), nalidixic acid 2 (33.3%) and trimethoprim-sulfamethoxazole 2 (33.3%). *E. coli* isolates showed different resistance against trimethoprim-sulfamethoxazole 4 (66.6%), *azithromycin* 1(16.6%), *ampicillin* 2 (33.3%), and *nalidixic acid* 2 (33.3%). These results indicate that *E. coli* was highly resistant to Trimethoprim-sulfamethoxazole 4 (66.6%), as shown in Table 2

The results of multiple ~~drug~~ drug-resistant (MDR) patterns of *Salmonella*, *Shigella* and *E. coli* isolates exhibited multidrug resistance to five antibiotics azithromycin, nalidixic acid, ampicillin, trimethoprim-sulfamethoxazole and ceftriaxone. *Salmonella* isolates exhibited multidrug resistance to three antibiotics, namely azithromycin, nalidixic acid and ampicillin, while *Shigella* isolates exhibited to four antibiotics; trimethoprim-sulfamethoxazole, azithromycin, nalidixic acid and ceftriaxone. On the other hand, *Escherichia coli* isolates exhibited multidrug resistance to three antibiotics: ampicillin, trimethoprim-sulfamethoxazole and nalidixic acid. These results show that *Salmonella* and *Escherichia coli* were less resistant to drugs than *Shigella*, as shown in Tables 2.

Discussion

This study has provided evidence of prevalence and antimicrobial susceptibility patterns towards *Salmonella*, *Shigella* and *Escherichia coli* among under-five children in Unguja – Zanzibar, Tanzania.

Our results have shown a low prevalence ~~to~~for children between 49 and 60 months and high ~~peak~~ prevalence for children between 7–12 months. The low prevalence of the target pathogens, especially *Shigella*, in the current study could be attributed to improved awareness of the mothers and caretakers of under-five children and community about personal and environmental hygiene from continuous interventions being made by different stakeholders, including Health extension workers through an educational program like Community Based Education. In our study, children within the age range of 1- 3 years were more susceptible to diarrhoea caused by *Salmonella* 12 (7.5%) than *Shigella* 7 (4.4%) and *Escherichia coli* 6(3.7%) These results indicated that there is no statistically significant relationship between age of patients and identification of Salmonella, Shigella and *Escherichia coli* (chi-square with four degree of freedom = 2, p = 0.064). These findings were consistent with several previous studies on the matter (5,8,22). Children in this age group normally like to take contaminated soils, food and water

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into their mouths, eventually leading them to get diseases from the environment easily caused by microbes, including pathogenic *Salmonella*, *Shigella* and *Escherichia coli*.

The results showed that patients' stool had more *Salmonella* isolates, followed by *Shigella* and low isolates of *Escherichia coli*. The *Salmonella* rate of 12 (7.5%) found in this study is higher than the rates found in other studies, including those from retail meat and meat products in China at 3.6% (23), Ethiopia at 6.9% (22), Ethiopia at 1% (5), India at 1% (24) and Qatar at 3.23% (25). Meanwhile, the *Salmonella* rate is still lower compared to the rate found in China at 19.7% (23), China in pork products at 37.3% (23), and China in beef at 16% (23).

On the other side, the isolation rate of *Shigella* 7 (4.4%) was found consistent with the rate in Ethiopia (22), which reported 4.3% of *Shigella* from stools. The high isolates of *Shigella* reported in Tanzania were 16.1% (26) and Ethiopia was 8.3% (5), while low isolates were reported by Jimma 1.1% (27). The isolation rate of *Escherichia coli* 6 (3.7%) was found to be lower than other studies in Tanzania, i.e., 21.6% (26) and 14% in Zanzibar (8).

Looking into treatment options, we found *Salmonella* isolates being highly susceptible to ceftriaxone 100%, chloramphenicol 100%, and trimethoprim-sulfamethoxazole 100%. However, our study reports resistance to nalidixic 41.6%, ampicillin 16.6% and azithromycin 16.6%. Similar studies in Ethiopia reported *Salmonella* susceptible to ceftriaxone 100% (22), while ampicillin had high resistance of 100% (5) in Ethiopia. In addition, several studies reported *Salmonella* resistance to ampicillin at 46% in Zanzibar (8), 45.4% in China (23) and 34% in India (24). The resistance patterns to nalidixic acid 43% were reported in Kolkata, India (24). Other resistance patterns in our study were observed in *Salmonella* to nalidixic acid at 35.8% in China (23). The *Salmonella* isolates in the previous study reported resistance patterns to azithromycin 25% (24), which were inconsistent with our study.

The *Shigella* isolates were found to be highly susceptible to chloramphenicol 100% and ceftriaxone 100%. Nevertheless, our findings reported resistance to trimethoprim-sulfamethoxazole 71.4%, azithromycin 42.8% and nalidixic acid 28.5%. Likewise, a study in Zanzibar reported *Shigella* susceptible to chloramphenicol at 46% (8). Other studies reported *Shigella* resistance of trimethoprim-sulfamethoxazole in Bosnia and Herzegovina 70-86% (28) and 68% in Zanzibar (8).

The *Escherichia coli* isolates are highly susceptible to chloramphenicol 100% and ceftriaxone 100%. However, our study reports resistance to trimethoprim-sulfamethoxazole 66.,6%, ampicillin 33.3% and nalidixic acid 33.3%. A similar study in Zanzibar reported *Escherichia coli* susceptible to chloramphenicol at 77.3%, which was inconsistent with our study (8). Other studies reported *Escherichia coli* resistance to trimethoprim-sulfamethoxazole at 68% (8) in Zanzibar and 40-86% in Bosnia and Herzegovina (28), which is in agreement with our study. These results indicate a reduced efficacy in treating *Salmonella*, *Shigella* and *Escherichia coli* among under-five children with diarrhoea. The species isolates in our study

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exhibited multidrug-resistant patterns to at least five antibiotics, namely Trimethoprim-sulfamethoxazole, azithromycin, nalidixic acid, ceftriaxone and ampicillin.

We reported the prevalence and the resistance patterns of *Salmonella*, *Shigella* and *Escherichia coli* in Zanzibar to guide the first-line and second-line drug choice in the treatment of diarrhoea in the subsequent reviews of the Zanzibar Standard Treatment Guideline (ZSTG) for Diarrhoeal diseases, as needed by Ministry of Health, 2016. Moreover, those medicines are easily available in pharmacies and accessible to anyone. These results are important to the Ministry of Health of Zanzibar in relation to the treatment of diarrhoeal diseases. The Ministry needs to initiate a long-term surveillance program to monitor and identify the changes in the rate of antimicrobial patterns of these bacteria of public health concern. Antibiotics remain the most important therapy for successfully managing diarrhoea infections; however, these inexpensive and widely available antimicrobials can no longer be used empirically. There is a need for appropriate control measures for antimicrobial resistance pathogens in Zanzibar.

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4. CONCLUSION

We found *Salmonella*, *Shigella* and *Escherichia coli* isolates in stools of children ≤ 5years from Unguja, Zanzibar, but all the isolates were susceptible to chloramphenicol and ceftriaxone but partially resistant to other tested antibiotics. Identifying resistant bacteria in this age group should be a concern for the public health authorities and trigger research into finding the cause.

8. CONSENT

All authors declare that written informed consent was obtained among Children suffering from Diarrhoea,

9. ETHICAL APPROVAL

Ethical approval was granted from the Zanzibar medical research ethics committee (Ref. No. ZAHREC/02/DEC/2018/6). Permission to conduct the study was sought from the respective health centres' authorities. The writing informed consent was obtained from mothers or caretakers of children under five years before collecting of information. The patients result of any investigation remained confidential, while identified organisms were referred to attending physicians for treatment. The samples safe discarded and stored in a deep freezer for further investigation.

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