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Abstract

Background: Diarrhoea is an important cause of morbidity and mortality among children in developing countries and resistance of bacteria is a global problem.
Objective: The present study in Unguja Island, Zanzibar, aimed at determining the prevalence and identity of pathogenic enteric bacteria associated with diarrhoea in children under five years and to assess the resistance of these bacteria to antibiotics.
Methodology: A cross sectional quantitative study was conducted, a total of 319 stool samples were collected. The prevalence and identity of pathogenic enteric bacteria were determined through conventional methods and antimicrobial resistance by the Kirby-Bauer antibiotic testing method.
Results: The prevalence isolates were; *Shigella* spp. In 41.5%, *Salmonella spp*.(29.3%), *Vibrio parahaemolyticus*(13.1%) and pathogenic *E. coli* (16.2%). Pathogenic enteric bacteria were commonly more resistant to sulfamethoxazole/trimethoprim, Erythromycin, Tetracycline and Ampicillin but were less resistant to Ciproflaxin, Gentamycin, and Chloramphenicol respectively.
Conclusion: The results indicated that the prevalence of pathogenic enteric bacteria was high and often they were resistant to antibiotics commonly used to treat diarrhoea in children under five years in Zanzibar. We therefore recommend reviewing of guidelines for treatment of childhood diarrhoea, promote health education, scale up vaccination campaign and regulate the use of antimicrobials to prevent further development of antibiotic resistance.

Key words: *prevalence, diarrhoea, bacteria, sensitivity, antibiotics.*

INTRODUCTION

The past thirty years have seen substantial global reduction in overall diarrhoea-specific mortality among children. However, despite this improvement, diarrhoea remains the second leading cause of death due to infections among children under five years of age worldwide[1,2]. In particular, diarrhoea is an important cause of morbidity and mortality among children in developing countries. Every year there are approximately 1.7 billion diarrhoeic episodes worldwide and they are responsible for 4.0 million deaths in children under five years of age[3, 4]

The most common pathogens responsible for acute diarrhoea are *Shigella spp*, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella* spp, *Vibrio cholerae*, *Yersinia enterocolitica* and *Aeromonas* spp. Whereas Enteroparasites include *Giardia* spp, *Cryptosporidium* spp and *Entamoebahistolytica* and *Balatidium coli.* In addition, viruses such as Adenovirus, Norwalk virus and Rotavirus cause potentially serious diarrhoea diseases[2, 5, 6]. Rotavirus is the leading cause of severe diarrhoea, resulting in an estimated 453 000 deaths, most of which occurs in developing countries of sub-Saharan Africa and South-East Asia[6,7,8].Pathogenic enteric bacteria are more studied because they are responsible for most hospital admissions and are easier to identify compared to viruses[4,6,9].

Several studies in developing countries have reported a high prevalence of diarrheic *E. coli* as common pathogenic enteric bacteria in children less than five years of age[5, 10]. In Kenya, diarrhoeic *E.coli* has been reported to be the most dominant bacteria[4]. According to studies in Tanzania, diarrheic *E. coli* strains were the most common enteric pathogenic bacteria isolated from stools in children of less than five years age, with diarrhoea[11, 12].

Like in many developing countries, diarrhoea in children of less than five years is of much concern in Zanzibar, a semi-autonomous island region of Tanzania in East Africa. The studies in Zanzibar, have shown that diarrhoea is the leading course of mortality in children of less than five years. Poor sanitation, sewage and drinking water systems are the major factors that aggravate the problem[13,14]. In Unguja, one of the two major islands forming Zanzibar, children under five years of age are at similar risk of having diarrhoea. However, there is scarce information on the prevalence of diarrhoea among children under-five years and the identity of causal organisms/bacteria.

Zanzibar Ministry of Health has been recommending the use of Trimethoprim/sulfamethoxazole and erythromycin as the first-line antibiotics for the treatment of pathogenic enteric diarrhoea in children under five years of age. Unfortunately, factors that could lead to antimicrobial resistance are common in Zanzibar, but there have been no studies on bacterial antibiotic resistance patterns against the common antibiotics used to treat pathogenic enteric diarrhoea in children less than five years.

Antibiotic resistance is a global problem, and the provision of effective antimicrobial drugs to diarrhoeal patients is still a challenge to health care providers[9, 15, 16]. Resistance of microorganisms to common antibiotics used for treatment of diarrhoea has also been reported in several developing countries. For example, such resistance has been reported in Ethiopia[18] and in Tanzania1[19].

The present study is the first that investigated simultaneously the types of pathogenic enteric bacteria causing diarrhoea among children less than five years old, and their sensitivity to common antibiotics used to treat diarrhoea in Unguja. This information can help in both the preventing and treating diarrhoeal diseases in children in Zanzibar and locations with similar socio-economic and environmental conditions.

MATERIALS AND METHODS

Study area

The study was conducted in Unguja Island, Zanzibar. This island is located about 35km off the coast of Dares Salaam, between 39° degrees longitude and 6° degrees latitude south of the equator. The current population is 900,000. The health care system has three levels. At the central level, the Ministry of Health is responsible for health care in the whole country. At the district level there are hospitals serving an average population of 100,000-150,000. At the ward (Shehia) level, there are health centres serving about 20,000-50,000 people[18].The mean monthly temperature ranges from 21°C to 33°C, with monthly rainfall 25 mm to 434 mm. The long rains and short rains typically occur from March to May, and from October to December, respectively. The heavy rainfall and resulting flooding during the rainy season increases the risk of sewage contaminating the drinking water. In Unguja, the most common toilet facilities are pit latrines where the untreated waste water can overflow or seep through the ground into the drinking water in wells or pipes[12]. Tourism is the major industry, followed by agriculture (growing of spices such as cloves) and fishing. Along the east coast, most villages also rely on seaweed farming[13].

Research design

A cross- sectional quantitative descriptive study was conducted on North, South, and West district of Unguja Island, Zanzibar, from September 2013 to February 2015 to identify pathogenic enteric bacteria associated with diarrhoea in children under five years of age, and to determine the sensitivity of these bacteria to antibiotics commonly used in the health system. Purposive sampling from the three district was carried out to collect stool samples from children under five years of age attending clinic because of diarrhoea.

Specimen collection

A total of 319 freshly passed stool samples from children under five years age were collected in sterile containers, placed immediately in an ice box and transported to the laboratory within six hours after collection. Samples were labelled with date and time and location of collection, sample type and age category.

Prevalence rate

The prevalence rate was calculated by dividing the number of children seen in clinic because of diarrhoea during the study period by the total population of children less than five years in the study area. This approach could undermine or underestimate the actual prevalence, as not all children with diarrhoea receive medical attention.

Bacterial isolation and identification

Isolation and identification of *Salmonella* and *Shigella*, *E.coli* and *Vibrio* species bacteria were done according to the standard microbiological procedures as described by [19,20].The stool specimens were placed in Selenite F enrichment broth (OxoidLtd,Thermo scientific), shaken and incubated at 37°C for 24 hours. Colonies of *Salmonella* and *Shigella* were identified by their characteristic appearance on Xylose LysineDeoxycholate (XLD Ltd, Themo Scientific) agar. For *E. coli* stool samples were inoculated in MacConkey Sorbital agar and incubated at 35-37°C for 18-24 hrs [20,21].For *Vibrio* species *stool* samples were in enriched in Alkaline Peptone Water (APW) 6 to 8 hours, then sub-cultured in Thiosulfate Citrate Bile Salts Sucrose Agar plates and incubated at 37 °C for 18 to 24 hours[21].The isolates also underwent a series of biochemical tests include Motility, Carbohydrate fermentation, MR-VP broth test, Oxidase test, Indole test, Urea test, Citrate utilization test, Hydrogen sulphate test. Their identity was further confirmed by commercial API 20 E test for (BioMerieux France). The analytical profile index (API) 20E strips (BioMerieux) was used as biochemical. These strips were inoculated with bacterial suspension and incubated for 18 to 24 hours at 37^0C. The reading of strips results for identification was obtained by referring to the API20E identification software[19].

Determination of antimicrobial resistance

Antimicrobial susceptibility testing (AST) was performed by using standard antibiotics disc diffusion technique by growing the isolates in the presence of a given antibiotic. Antibiotic impregnated paper disks were placed on the surface of a sensitivity molten Muller- Hilton Agar plate that had been seeded with the isolate being tested. The antibiotic discs used and their concentrations were Ampicillin (AMP, 10 µg), Chloromphenicol (C, 30 µg), Gentamycin (GN, 10 µg), Sulfamethoxazole/ Trimethoprim (SXT, 30 µg), Erythromycin (E, 15µg), Ciproflaxin (CIP, 5µg), Tetracycline (TE, 30 µg). A standard inoculum adjusted to 0.5 McFarland was swabbed onto Muller-Hinton Agar (Oxoid Ltd). The antibiotic discs were dispensed after drying the plate for 3 to 5 minutes and the plates incubated at 35°C for 24 hours. CLSI interpretative criteria for susceptibility and resistance were used[21].

Data collection

The number of samples that were positive/isolated with each type of bacteria were recorded. If an organism is susceptible to an antibiotic tested, its growth is inhibited and a zone of inhibition result around the antibiotic disk. The width of the zone was measured in millimetres (mm) and compared to a standard interpretation chart and the measurements were used to categorize the isolate as susceptible, intermediate susceptible or resistant[22].

Statistical analysis

Data were analysed using Statistical Package for Social Science (SPSS), version 20 software. Descriptive statistics were generated for most variables. One way analysis of variance was done to test whether there were statistical differences between frequencies of occurrence of the different

bacteria among the age groups. Chi-square test was used to compare the relative sensitivity of the different strains of bacteria to antibiotics. P-value of < 0.05 was used as the level of significance.

Ethical clearance

Ethical clearance and permission to conduct this study were obtained from the Health Research Ethical Review Committee of the Zanzibar Ministry of Health.

Limitations of the study

We did not test for *Campylobacter* spp and for *Yersinia* spp, as we lacked a gas system incubator for isolation of the former and a selective media for isolation of the latter.

RESULTS

Prevalence of enteric pathogenic bacteria:

A total of 319 samples were examined for the presence of enteric pathogenic bacteria. One hundred and thirty samples (41%) were positive for enteric pathogenic bacteria. Table 1 presents a summary of the incidence of enteric pathogenic bacteria diarrhoea detected in the 130 stool samples. Table 1 illustrate the incidence of enteric pathogenic bacteria in positive stool samples

Age number	Age groups(months)	*E.coli*	*Salmonella spp*	*Shigellas pp*	*V.parahaemolyticus*	Percentage of isolates in age groups
1	<6	3	3	2	0	8(6.2)
2	7-12	4	11	14	0	29(22.3)
3	13-18	6	9	7	1	23(17.7)
4	19-24	3	5	13	3	24(18.5)
5	31-43	4	6	9	2	21(16.2)
6	44-56	1	1	7	7	16(12.3)
7	57-60	0	3	2	4	9(6.9)
Percentage of isolates		**21(16.2)**	**38(29.3)**	**54(41.5)**	**17(13.1)**	**100**

Salmonella spp were detected in 38(29.2%) followed by *Shigella* spp 54(41.5%) *E.coli* in 21(16.2%) and *Vibrio parahamolyticus* 17(13.1%) respectively of the stool samples. The prevalence isolates based on age groups of under five children from less than 6 to 60 months were

< 6 months was 8(6.2%) , 7-12 was 29(22.3%), 13-18 was 23(17.7%), 19-24 was 24(18.5%), 31-42 was 21(16.2%(and 43-54 was 16(12.3%) and 55-60 was 9(6.9%) *parahaemolyticus* was not detected in samples from under one year old children (Table 1). The incidence of pathogenic bacteria was lowest in the under six-month age group. The difference in frequency of occurrence of the bacteria among the six age groups was highly significant (P<0.01). *Shigella* sp was the most prevalent at 39%followed by *Salmonella* (25%), *Vibrio parahaemolyticus* (19%) and *E. coli* (17%).

Bacterial diarrhoea in children under five years of age was common in children aged from seven to 24 months ($p < 0.05$). *E. coli, Salmonella* and *Shigella* were isolated in all age groups. *Vibrio parahaemolyticus* was isolated in children that were more than 12 months and not found in those below 12 months (Table 1).

Antibiotics Susceptibility Tests

Table 2. Types, concentration and interpretative criteria of sensitive of antimicrobial agents.

Antibiotic	**Code**	**Concentration**	**Inhibition zone**		
			Susceptible	**Intermediate**	**Resistance**
Ampicillin	Amp	10µg	>14	12 – 13	<11
Chloramphenicol	C	30 µg	>18	13 – 17	<12
Erythromycin	ER	15 µg	>18	14 – 17	<13
Gentamycin	GN	10 µg	>15	13 – 14	<13
Tetracycline	TET	30 µg	>19	15 – 18	<14
Trimethoprim/ Sulfamethoxazole	SXT	1.25+23.75 µg	>16	11 – 15	<70
Ciproflaxin	CIP	5 µg	>12	15 – 20	<14

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Antibiotics Susceptibility status of isolated enteric pathogenic bacteria in Zanzibar

Table 2 shows antimicrobial resistance profile of enteric pathogenic isolates from stool samples of under five years children. Most isolated bacteria shown multidrug resistance. All four pathogenic enteric bacteria isolated in children under five years of age showed significant multidrug resistance to the antimicrobials most commonly used to treat diarrhoea infections in Zanzibar.

Isolate	Number of Isolate	Number and percentage of resistant isolate	Percentage of resistance of isolated bacteria to tested antibiotics						
			SXT	ER	TET	AMP	CIP	GN	CH
Salmonella	35	33(28)	7(18)	11(3.3)	5(18)	5(15.2)	3(9)	2(6)	2(6.5)
Shigella	54	49(41)	14(28).	8(16)	11(22.4)	9(18.4)	3(6)	1(2)	2(4)
E.coli	21	20(17)	5(25)	6(30)	4(20)	2(10)	1(5)	2(1)	00
V.parahemo l-yticus	17	16(14)	2(12.5)	5(31)	3(19)	2(12.5)	2(12.5)	1(6.)	1(6.3)
Total	**130**	**118(90.7)**	**28(23)**	**30(2)**	**23(19.5)**	**18(15.3)**	**9(7.6)**	**4(5)**	**5(4.**

Out of the 118 isolates that were antibiotics resistant 33(28%) were *Salmonella spp*, 28(23.7%) *shigella spp* 20(17%) *V. parahaemolyticus* and 16(14%) *E.coli* respectively. The resistance of the isolated bacteria was most common to Erythromycin (30(25.4%), Sulfamethoxazole/Trimthoprime 28(23.7%), Tetracycline 23(19.5%), Ampicillin 18(15.3%), the lowest resistance was observed against Ciproflaxin 9(7.6%), Gentamycin 4(5%) and Chloramphenicol respectively 5(4.2%). Except to *E.coli*, all isolated bacteria shown resistance to all tested antibiotics. Out of 130 isolated bacteria only 12(9%) shown susceptibility to antibiotics.

DISCUSSION

The present study, identified the prevalence and antibiotic sensitivity of four species of bacteria associated with diarrhoea in children under five years of age in Unguja Island, Zanzibar. The findings showed that both prevalence and resistance to commonly used antibiotics were high.
In this study *Shigella spp* were the most prevalent enteric pathogenic bacteria, followed by *Salmonella spp, Vibrio parahaemolyticus and E. coli*. These results are consistent with study in Ifakara, Tanzania[6] and in Kenya[4].

In addition, our results confirm the high incidence of shigellosis reported in children under five years of age in Unguja municipality[13.]The study indicated microbial contamination of street vended food with food borne diseases include shigellosis, typhoid and cholera[6]. The occurrence of *shigellosis* and other diarrhoeas could be associated with improper food handling practices by some street food vendors. Infected food handlers can contaminate food and drinks and could serve as source of infection to public in the community include children via food chain [3], [22-23].

In our study, pathogenic enteric bacteria were isolated in samples from all the age groups, except those from children below six months and between seven to 12 months where *Vibrio parahaemolyticus* were not isolated. This is probably attributable to the fact that children of below six months of age are normally exclusively breastfed, and so were not likely to be infected with *Vibrio parahaemolyticus*, which are mainly transmitted through consumption of contaminated water or food particularly seafood[25,26].

The finding of this study indicated significance association between frequencies of enteric pathogenic bacteria with age among children. The prevalence of pathogenic enteric bacteria was highest in children aged between seven to 24 months and lowest in age below six months and those aged between four and five years (49-60 months). Our findings are in agreement with study in Dar es Salaam, Tanzania[9], found the highest prevalence of enteric pathogenic bacteria in the age group 7-12 months Similar observations have also been reported in several other studies elsewhere[4,26]. The relatively high prevalence of diarrhoea observed at ages 7-24 months can reflect the fact that at the age, of seven month children start to eat weaning foods. Contaminated weaning food has been suggested as a major contributor to diarrhoea in low-income settings, as up to 70% of diarrhoea episodes are caused by water and food contaminated with pathogens[3,25]. In addition, at the age of 12 months children starts to pick and eat various kinds of food items themselves without hygienic precautions such as washing their hands before eating. Furthermore, in developing countries, not all children less than five years of age are vaccinated against common childhood diseases. Parents commonly lack awareness of the importance of providing vaccines to their children and have a negative attitude towards vaccinations[30].

Multidrug resistance in bacteria pathogens is now common in developing countries. This is probably related to the frequent use of over the counter drugs without proper medical supervision[16, 28, 3].The present study assessed the resistance patterns of pathogenic enteric bacterial strains isolated from children under five years with diarrhoea to seven commonly used antibiotics in

Zanzibar. All pathogenic enteric isolates showed high levels of resistance to Ampicillin, Erythromycin, Tetracycline and Trimethoprim/sulfamethoxazole. Studies in other developing have shown similar results[6,26,28.]. The antimicrobial resistance has been attributed to selective pressure of resistance genes because of the indiscriminate usage of antibiotics[29].

In Zanzibar, the high rates of antibiotic resistance could have been caused by indiscriminate and over the counter availability in which the antimicrobial drugs are easily available in pharmacy shop without physicians dosage prescriptions. Furthermore, poor quality antimicrobial drugs imported from other developing countries as well as unskilled personnel dispense such antimicrobial drugs. This might have led to selective pressure among pathogenic enteric bacteria to become resistant. The study reported that, in many developing countries, the use of antimicrobial agents in food-producing animals has important consequences for both human and animal health as it can lead to the development of resistant bacteria[15].

Since the study was limited to enteric pathogenic bacteria the diversity of other diarrhoeal agents such as viruses and protozoa was not investigated. There is a need to study the epidemiology of rotavirus and other agents, since rotavirus is the leading cause of mortality due to diarrhoea in children under five[3,8].
The findings of this study have important implications for future practice. First, the Zanzibar government should improve the sanitation and sewage disposal infrastructure, promote health education and scale up the vaccination campaign against diarrhoeal diseases in children under five years of age. Second, the Zanzibar Ministry of Health should review its Standard Treatment Guidelines for pathogenic enteric diarrhoea in children under five years of age. Trimethoprim/sulfamethoxazole and Erythromycin, which are the first-line antibiotics, should be replaced by other antibiotics including Gentamycin, Chloramphenicol, and Ciproflaxin to which the isolated bacteria showed high levels of susceptibility. Thirdly, the Zanzibar Ministry of Health should regulate the use of antimicrobials to prevent further development of antibiotic resistance.

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