Prevalence of Enterobacteriaceae Isolated from Childhood Diarrhoea in Mukuru Slums, Nairobi- Kenya

Abstract

Diarrhoea in young children continues to be a major public health concern in developing countries, including Kenya. Poor sanitation among other factors can predispose a child to diarrhoea. Therefore, the present study sought to determine the prevalence of enterobacteriaceae isolated from childhood diarrhoea in Mukuru Slums, Nairobi. It employed a cross-sectional design targeting children below 5 years of age. Stool specimens were obtained aseptically and cultured on MacConkey agar and Salmonella-Shigella agar. Biochemical tests were used to identify the isolated bacteria to genus and species using biochemical characterization scheme and the Analytic Profile Index 20E. Drugs sensitivityAntimicrobial susceptibility tests (AST) were done using standard techniques. *Escherichia coli* ATCC 25922 was included as a control strain. Analysis of gender verses diarrhoea revealed that there was no statistical significant association between the gender (male and female) and area of residence in relation to diarrhoea in this study ($p = 0.146 > 0.05$). There was no statistical significant difference between the participant's characteristics and their area of residence ($p= 0.144$). Age of the participants had significant association with the prevalence of diarrhoea ($p=0.00$). The *E. coli* bacteria showed the highest percentage of enteric pathogens isolated (35.2%) from female children at Mukuru kwa Njenga and 29.4% from male children, *Salmonella spp* being second (4.9%) from female at Mukruru kwa Reuben and the least was *Shigella sonnei* (3.2%) from female children at Sinai. Emphasis should therefore be placed on primary preventive measures such as ensuring good sewage management and safe supply of drinking water in the study area and Kenya at large especially in the slums.

Keywords: *Enterobacteriaceae*, Diarrhoea, prevalence, Mukuru slums

Introduction

Diarrhoea is a significant health problem globally, particularly in the developing world where adequate sanitation facilities are lacking [1]. A study by Black et al. [2] reported that globally, diarrhoeal diseases account for almost a fifth of all deaths of children below five years of age, with an estimated 2.2 million deaths annually. Epidemiological studies of diarrhoea have been reported from several African countries including Kenya [3]. In sub-Sahara Africa, an estimated 16% of deaths in children below 5 years of age are diarrhoea related [4]. Human Immunodeficiency Virus (HIV) is also prevalent in Sub-Sahara Africa and diarrhoea can exacerbate HIV related symptoms [5].

Studies have shown that prolonged episodes of diarrhoea in early childhood leads to stunting growth [6]. Poverty, poor sanitation and lack of balanced diet are also risk factors in diarrhoeal diseases [7]. In Kenya, under five year's mortality rate is 74 deaths per 1000 [8]. Sixteen per cent (16%) of children under five are underweight using weight for age index [8]. In Nairobi county, stunting growth in children increased by 4% in 2010 from an earlier survey done in 2003 [8]. Diarrhoea episodes increase with age peaking at six to eleven months at 30% experiencing

diarrhoea because during this age bracket most of the children will have started crawling while others are already walking [8]. The causes of diarrhoea include a wide array of viruses, parasites and bacteria. However, most of the diarrhoeal diseases are caused by the members of the family Enterobacteriaceae [9]. Farmer [10] reported that these pathogens are named as enteric pathogens which belong to the genera that initiate infection by invading the intestinal epithelium. The researcher furthermore explained that the enteric pathogens belonging to the family Enterobacteriaceae are predominantly facultative anaerobic bacterial flora of large intestine of human beings. These are generally non-spore forming, non acid fast and gram negative straight or curved rod.

The enteric disease causing members of family *Enterobacteriaceae are E.coli, Shigella, Salmonella, Proteus, Klebsiella pneumoniae, Citrobacter freundii, Enterobacter aerogenes.* Some enteric organisms, for example, *Escherichia coli* are part of the normal flora and incidentally cause disease while others such as salmonellae and shigellae, are regularly pathogenic to humans [11;12]. The Enterobacteraceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, possess a complete antigenic structure, and produce a variety of toxins and other virulence factors [13]. Enterobacteraceae, enteric gram-negative rods and enteric bacteria may also be called coliforms [10]. Children living in the slums are vulnerable to diarrhoeal diseases mainly due to poor sanitation. Therefore, the present study seeks to study sought to determine the prevalence of Enterobacteriaceae isolated from childhood diarrhoea in Mukuru Slums, Nairobi.

Materials and methods

Study site

The study site was the government hospital located at Mukuru Kwa Njenga slum in Nairobi County. The Hospital serves the residents of Kwa Reuben, Kwa Njenga, Kayaba and Sinai slums along Nairobi River. It is situated within the Industrial area of Nairobi city lying at co-ordinates 1°18'33"S 36°48'12"E. Mukuru Kwa Njenga is a slum in the East of Nairobi, the capital of Kenya. It belongs to Embakasi Constituency. It is one of the largest slums in Nairobi. Among other major slums in Nairobi are Korogocho, Kibera and Mathare. The population of the slum exceeds 100,000. There have been cholera deaths in 2009 [6].

Study design and population

The study employed a cross-sectional laboratory based design [14]. The study population comprised of children who were five years and below, attended to at the government health facility in Mukuru Kwa Njenga with signs and symptoms of diarrheal diseases.

Inclusion criteria

- Children under five years verified by child welfare clinic records.
- Children who had diarrhoea or history of diarrhoea i.e. passage of loose or watery stool more than three times a day (WHO, 1988).
- HIV negative.

- Children whose parents/guardians accepted to sign informed consent form

Sample size determination

The sample size was determined using the formula below according to [14]

$$n = \frac{Z^2 \times P(1-p)}{d^2} \quad \text{..... [1]}$$

Where n is the sample size, z is the confidence interval at 95% and p is the prevalence got from Kenya Demographic health survey (KDHS), 2010, d is the margin of error at 5%

$$n = \frac{1.96^2 \times 0.17\ (0.83)}{0.05^2} \quad \text{..... [2]}$$

Final sample size was 178 stool samples/ anal swabs

N = 178; 190 participants were included in this study.

Sample collection

Stool samples were collected into sterile, wide-mouthed, screw cap containers and preserved in cool boxes. Anal swabs were collected from participants who were unable to produce stool samples and the specimens were labelled and assigned unique code numbers during the time of sample collection. Specimens once collected were taken to the centre for microbiology research laboratory (CMR)-KEMRI and processed within 24hrs.

Specimen processing

Culturing

The specimens were enriched in selenite F media overnight at 37^{o}C. After enrichment, inoculations were done both on MacConkey Agar and Shigella Salmonella Agar (Oxoid, Basingstoke, United Kingdom). Lactose fermenters and non-lactose fermenters that had grown colonies were inoculated onto biochemically impregnated API 20E strips (BioMerieux, Basingstoke, United Kingdom) for identification.

Biochemical tests

Triple sugar iron agar (TSI)

Colonies were selected on plate using a sterile straight wire loop. The centre of the colony was lightly touched and prepared TSI medium were inoculated by stabbing the butt and streaking the slants. These were then incubated at 37^{o}C for 24 hours [15].

Indole test

The bacteria isolated were sub-cultured in nutrient broth and incubated for 24 hours. About 3 drops of Kovac's indole reagent was added and mixed gently [16].

Urease test

Urea agar was inoculated heavily over the entire surface of the slants in bijou bottles, incubated at 37^{o}C for 24 hours.

Citrate utilization test

Simmons citrate slopes were prepared in bijou bottles. The slopes were then stabbed and incubated at 37^{o}C for 48 hours.

Motility test

A sterile straight wire loop was used to inoculate motility indole urea media with bacterial isolate and incubated overnight at 37^{o}C. Motility was shown by diffused turbidity in the medium [16].

N/B: All these tests mentioned above were used for the purpose of identification of Enterobacteriaceae. The results were either positive or negative for a particular entero pathogen.

Ethical Consideration

The study was nested within a bigger study which was funded by The Centre for Disease Control and Prevention in collaboration with the Kenya Medical Research Institute, Opportunistic infection laboratories and the ministry of health central microbiology laboratories. Permission to carry out the study was granted by the investigators of the main study.

Results and Discussion

Participants' characteristics

A total number of 190 children below the age of five years presenting with diarrhoea in the Government health facility in Mukuru kwa Njenga slum participated in this study. The mean age of the participants was 24.21 months with the youngest child being 3 months and the oldest child being 72 months. More children who participated in the study were less than 40 months in age. The children's ages were skewed to the right of the normal curve (Figure 1). The mean age of the children was twice more than the median age with a standard deviation of 17.62. The study recorded a significant association ($p<0.05$) between the age groups and diarrhoea among the participants.

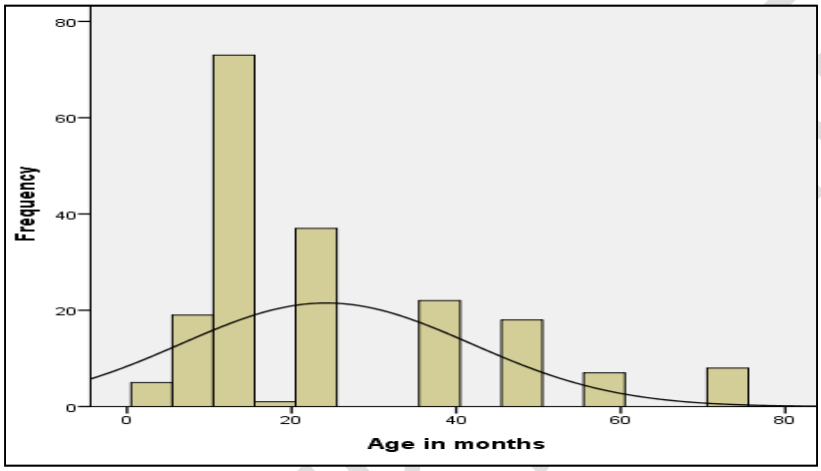

Figure 1: Distribution curve of participants ages

In this study the female children were 105(55.26%) and the males were 85(44.74%). Female children were 3.7 months older than the male children with a standard error difference mean of 0.02 months as shown in Table1 below. These results could be due to the fact that children within this age group are most often than not unaccompanied and cannot differentiate between what to eat and what not to eat; they have not learnt the rules of adherence to aseptic or hygienic practice and they can barely express themselves [13]. Those below the age of twelve months are essentially under their mothers' care, feeding mainly on breast milk thereby reducing their susceptibility to these pathogens.

Table 1: Analysis of age of the participants

Gender	N	Percentage	Mean age	Age Stdev.	Age SE. Mean	SD	*P*-value
Male	85	44.74%	16.414	1.780	16.414	0.02	0.00

Female	105	55.26%	18.449	1.800	18.449

Age can be a predisposing factor to diarrhoea in children below the age of five years (WHO, 2007). Living in the slums is also a predisposing factor to diarrhoeal infections because of the poor hygienic conditions coupled with poor sanitation [6]

Most enteric pathogens stimulate at least partial immunity against repeated infections or illness, which helps to explain the declining incidence of diseases in older children [17].

The analysis of the participants' ages verses gender revealed that there was no significant difference. The $t_{(186)}$ value was 1.458 with probability, $p = 0.146 > 0.05$, the p-value was more than 0.05 therefore there was no association between the gender in relation to diarrhoea in this study. The male participants were 85(45%) while the female were 105(55%) as shown in Figure 2. There was significant association between age and diarrhoea in this study (p=0.01).

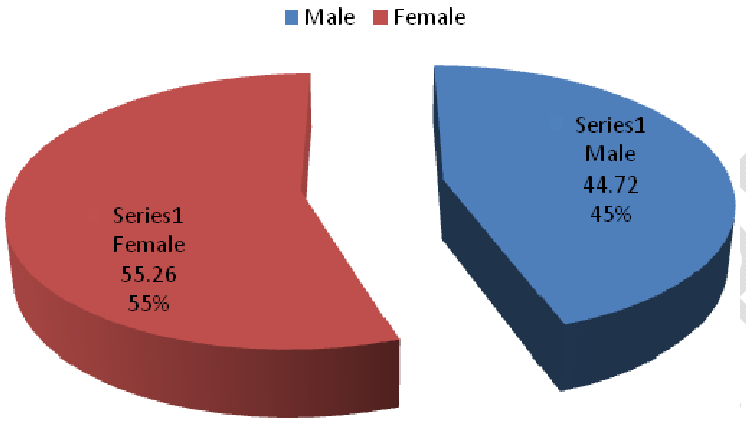

Figure 2: Gender of the participants

The participants attended to at Mukuru Kwa Njenga government health facility were noted to be residents of four neighbouring slums namely; Mukuru Kwa Njenga, Mukuru Kwa Reuben, Mukuru Kayaba and Sinai. The majority of the participants were from Mukuru Kwa Njenga 61(32.6%) followed by Mukuru Kwa Reuben 57(30.5%) then Sinai 35(18.9%) and the least were from Mukuru Kayaba 33(17.9%). Mukuru Kwa Njenga had the highest number of female children (35.2%) while Mukuru Kwa Reuben had the highest number of male children (32.9%). The p-values were greater than 0.05 hence there was no significance difference between the participants from different areas of residence (χ^2 =5.41, p= 0.144) as shown in Figure 3.

The results of other studies concur with the current study. Chitnis et al. [18] in their study observed that patients susceptible to Carbapenem-resistant enterobacteriaceae (CRE) were more likely to be female. The results of the current study concur with a study done by Sule et al. [19] in Kaduna Nigeria where they found the incidence between both sexes showing female children having the highest percentage (26%) compared to males (18%). Abdullahi et al. (2010) reported that male children were more infected (22.33%) than female children (18.33%), although the difference was not statistically significant ($\chi^2 = 0.531$, $p>0.05$) hence contradicting the finding of the current study. Most diarrhoeal episodes occur during the first two years of life due to a combination of factors; declining levels of maternal acquired antibodies, lack of active immunity in the infant, the introduction of food that may be contaminated with enteric bacteria or direct contact with human or animal faeces carrying enteric bacteria when the infant starts to crawl [3].

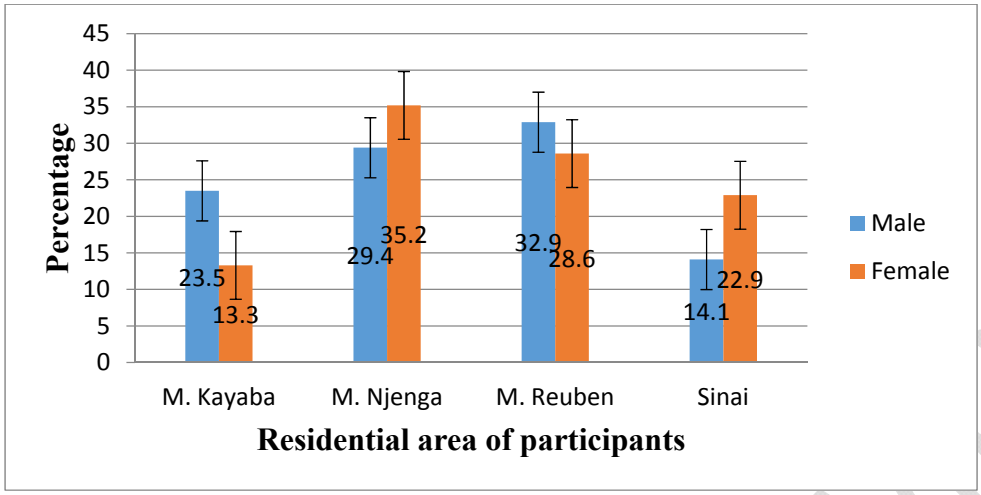

Figure 3: Residence of study participants

Isolation and identification of the bacteria

The prevalence of bacteria isolated from the study were as follows: *Escherichia coli* (87.4%), *Salmonella spp* (4.2%), *Shigella sonnei* (4.2%), *Salmonella typhi* (2.6%), *Shigella dysenteriae* (1.6%) and the prevalence were as follows; *Escherichia coli* (87.4%), as shown in Figure 4.

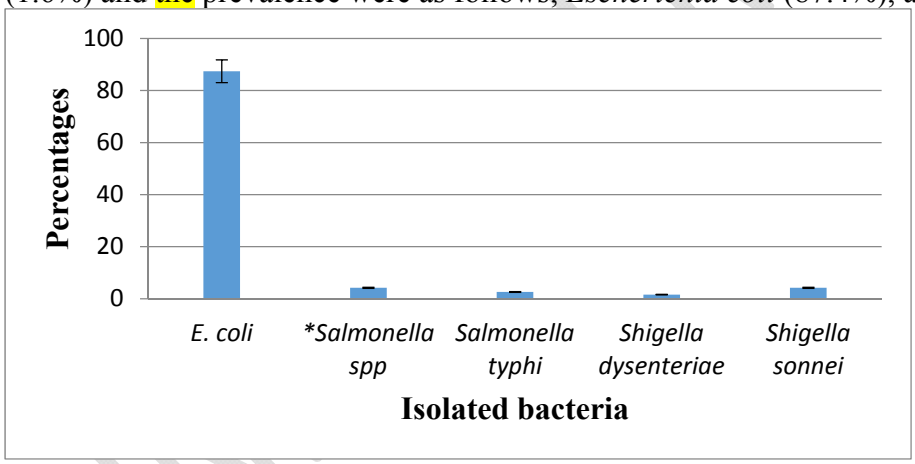

Figure 4: Bacteria species isolated from the stool samples

The total percentage prevalence of bacteria species isolated among the participants by age, gender and residence in the study area were 78% *E. coli*, 4.2% *Salmonella spp* (were not identified to species level), 4.2% *Shigella sonnei*, 2.6% *Salmonella typhi* and 1.6% *Shigella dysenteriae* from all the specimens collected. At Mukuru Kwa Njenga more *E. coli*were isolated from female children (35.2%) than male children (29.4%) the rest of the isolates were uniform in both genders. At Mukuru Kwa Reuben the trend is the same in that more *E. coli* were also isolated from female children (17.5%) than from male children (13.0%). *Salmonella spp* were 4.9% from female children and 0.0% from male children while *S. typhi* were more from male children (3.0%) compared to female children (0.5%). At Sinai the percentage isolates from both

male and female children were almost equal (9.0% and 9.9%, respectively). *Shigella sonnei* were more from (3.2) female than male children (1.0%). The rest were almost the same in both male and female children. At Kayaba *E. coli* isolates were more from female (10.7%) than from male children (7.2%). *S typhi* were 1.6% in females and 0.0% in males while the rest were 0.0%. There was no significant association between the gender and percentage isolates ($p>0.05$). There was also no significant association between the prevalence of the isolates and the area of residence of the children ($\chi^2 = 2.23$, $p=0.693$). The results are as shown in Table 2.

Table 2: Prevalence of bacteria isolated by gender and residence of participants

Residence	Isolated*spp*	Male (% isolates)	Female (% isolates)	χ^2 (p-value)
M. Njenga	*E. coli*	29.4	35.2	2.23 (0.693)
	**Salmonella spp*	1.0	1.1	
	S. typhi	0.0	0.0	
	S. dysenteriae	1.5	1.5	
	Shigella sonnei	1.1	1.0	
M. Reuben	*E. coli*	13.0	17.5	
	**Salmonella spp*	0.0	4.9	
	S. typhi	3.0	0.5	
	S. dysenteriae	1.0	1.1	
	Shigella sonnei	2.9	2.0	
Sinai	*E. coli*	9.0	9.9	
	**Salmonella spp*	2.0	2.2	
	S. typhi	1.6	1.0	
	S. dysenteriae	0.0	1.6	
	Shigella sonnei	1	3.2	
M. Kayaba	*E. coli*	7.2	10.7	
	**Salmonella spp*	0.0	0.0	
	S. typhi	0.0	1.6	
	S. dysenteriae	0.0	0.0	
	Shigella sonnei	0.0	0.0	

**Salmonella spp- other Salmonella isolates which were not identified to species level, Spp - species, χ^2 – Chi square test, p-value- level of significance (0.05)*

Acute diarrhoea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Kenya especially in the slums [20]. Identification of the Enteropathogens causing diarrhoeal diseases in the country is an essential step towards the implementation of effective primary health care activities against the disease [21]. Poor sanitation in the study area could have also contributed to the high prevalence of bacteria isolated. The residents live in congested environments with their domesticated animals which could have contributed to the high prevalence of isolated enteric bacteria. According to a study done by Kariuki et al. [21], a significantly higher proportion of younger children (< 3 years of age) and those from the slums presented with invasive non- typhoidal *Salmonella spp* compared to older children and those from upper socio-economic groups ($p < 0.001$).

In terms of gender and area of residence, Mukuru kwa Njenga, had more *E. coli* isolated from female children (35.2%) compared to male children (29.4%) the rest of the isolates were uniform in both genders. In Mukuru kwa Reuben the trend was the same in that more *E. coli* were also isolated from female children (17.5%) than from male children (13.0%). *Salmonella spp* were 4.9% from female children and 0.0% from male children while *S. typhi* were more from male children (3.0%) compared to female children (0.5%). At Sinai the percentages of the isolates from both male and female children were almost equal (9.0% and 9.9%, respectively). *Shigella sonnei* were more from (3.2) female than male children (1.0%). The rest were almost the same in both male and female children. At Mukuru Kayaba *E. coli* isolates were more from female (10.7%) than male children (7.2%). *S typhi* were 1.6% in females and 0.0% in males while the rest were 0.0%. There was no significant association between the gender and percentage isolates ($p>0.05$). There was also no significant association between the prevalence of the isolates and the area of residence of the children ($\chi^{2=}2.23$, $p=0.693$). The results of this study do not concur with what Sang et al. [6] found in their studies on the prevalence of bacteria in four provinces in Kenya where they had recruited 651 participants and isolated pathogenic bacteria in (17.7%) of the participants. Among the isolated bacteria were; pathogenic *E. coli* (11.2%), *Salmonella* (3.5%) and *Shigella* (2.3%) [3]. The reason for the different results could be because the study area was basically a slum hence the high prevalence of bacteria isolated especially the *E. coli.*

A similar study was done by Ifeanyi et al. [22] in Abuja Nigeria among cases of diarrhoea with potential bacterial pathogens detected being 65.8% of all patients screened. This was in contrast to a report of the prevalence of 83.1% from similar study in Abakaliki, south –eastern Nigeria [23]. Another study reported a prevalence of 63.3%-71.83% isolation of enteric bacteria in ifakara Tanzania. The variation in prevalence between the two Nigerian cities might be attributed to differences in infrastructural and socioeconomic [23]. In a different study, the prevalence of bacterial aetiology of diarrhoea was 44% which follows the same trend with the research conducted in Kano State which was found to be 40.67%. In Gabon prevalence of diarrhoea with bacterial aetiology was 38% [17]. In Tanzania it was 36%. The study showed that *Shigella spp* appears to be the predominant bacteria causing diarrhoea followed by *E. coli*, and *Salmonella* in that order. A total of 56% of the hundred diarrhoea cases investigated had no bacterial pathogen suggesting viral, protozoan or nonpathogenic factors [24].

Salmonella spp isolated in Mukuru slums could be non- typhoidal salmonella which is a zoonotic strain. The children could have been contaminated with faecal matter of the domesticated animals hence the acquisition of the bacteria. Occurrence of diarrhoeagenic bacteria in the current study showed that gram negative bacteria (*Shigella spp, Salmonella spp, Escherichiacoli*) are the main cause of bacterial diarrhoea. Sule et al. [19] in Kaduna Nigeria conducted a similar

study and found similar results. Generally, the aetiology of diarrhoea in young children could be attributed to a wide range of factors, but one of the main causes of diarrhoea is related to bacteria [24]. Results from the current study shows that, though there are a number of causative agents of diarrhoeal diseases, bacteria still remain one of the major causes with *Shigella, Salmonella* and *Escherichia coli* being the most important pathogens among paediatric patients presenting with diarrhoea in Mukuru kwa Njenga Government health facility. Judicious use of antibiotic therapy requires education of health workers and patients, adequate laboratory diagnostic capabilities and government regulations.

Conclusion

In this study the female participants were more than the males. There was no statistical significant difference between the participants' characteristics and their area of residence. Age of the participants had significant association with the prevalence of diarrhoea. The total prevalence of isolated bacteria among the participants was very high. The *E. coli* bacteria showed the highest percentage of enteric pathogens isolated from female children from the slum.

Recommendation

Further studies should investigate social demographic characteristics of children, parents and their households in order to understand more the causes and predisposing factors of diarrhoea in the slums.

Conflict of Interest

The authors declare no conflict of interest

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