

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

3
4 **Abstract**

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

25
26 **Keywords: Enterobacteriaceae, Diarrhoea, prevalence, Mukuru slums**

27
28 **Introduction**

29
30 Diarrhoea is a significant health problem globally, particularly in the developing world where
31 adequate sanitation facilities are lacking [1]. A study by Black et al. [2] reported that globally,
32 diarrhoeal diseases account for almost a fifth of all deaths of children below five years of age,
33 with an estimated 2.2 million deaths annually. Epidemiological studies of diarrhoea have been
34 reported from several African countries including Kenya [3]. In sub-Saharan Africa, an estimated
35 16% of deaths in children below 5 years of age are diarrhoea related [4]. Human
36 Immunodeficiency Virus (HIV) is also prevalent in Sub-Saharan Africa and diarrhoea can
37 exacerbate HIV related symptoms [5].

38 Studies have shown that prolonged episodes of diarrhoea in early childhood leads to stunting
39 growth [6]. Poverty, poor sanitation and lack of balanced diet are also risk factors in diarrhoeal
40 diseases [7]. In Kenya, under five year's mortality rate is 74 deaths per 1000 [8]. Sixteen per
41 cent (16%) of children under five are underweight using weight for age index [8]. In Nairobi
42 county, stunting growth in children increased by 4% in 2010 from an earlier survey done in 2003
43 [8]. Diarrhoea episodes increase with age peaking at six to eleven months at 30% experiencing

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

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

64

65 **Materials and methods**

66 **Study site**

67 The study site was the government hospital located at Mukuru Kwa Njenga slum in Nairobi
68 County. The Hospital serves the residents of Kwa Reuben, Kwa Njenga, Kayaba and Sinai slums
69 along Nairobi River. It is situated within the Industrial area of Nairobi city lying at co-ordinates
70 1°18'33"S 36°48'12"E. Mukuru Kwa Njenga is a slum in the East of Nairobi, the capital of
71 Kenya. It belongs to Embakasi Constituency. It is one of the largest slums in Nairobi. Among
72 other major slums in Nairobi are Korogocho, Kibera and Mathare. The population of the slum
73 exceeds 100,000. There have been cholera deaths in 2009 [6].

74 **Study design and population**

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

78 **Inclusion criteria**

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

83 • **Children** whose parents/guardians accepted to sign informed consent form

84 **Sample size determination**

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

86
$$n = \frac{Z^2 \times P(1-p)}{d^2} \dots\dots\dots [1]$$

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

89
$$n = \frac{1.96^2 \times 0.17(0.83)}{0.05^2} \dots\dots\dots [2]$$

90 Final sample size was 178 stool samples/ anal swabs
91 N = 178; 190 participants were included in this study.

92 **Sample collection**

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

98 **Specimen processing**

99 **Culturing**

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

105 **Biochemical tests**

106 **Triple sugar iron agar (TSI)**

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

110 **Indole test**

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

113 **Urease test**

114 Urea agar was inoculated heavily over the entire surface of the slants in bijou bottles, incubated
115 at 37°C for 24 hours.

116 **Citrate utilization test**

117 Simmons citrate slopes were prepared in bijou bottles. The slopes were then stabbed and
118 incubated at 37°C for 48 hours.

119 **Motility test**

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

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

124 **Ethical Consideration**

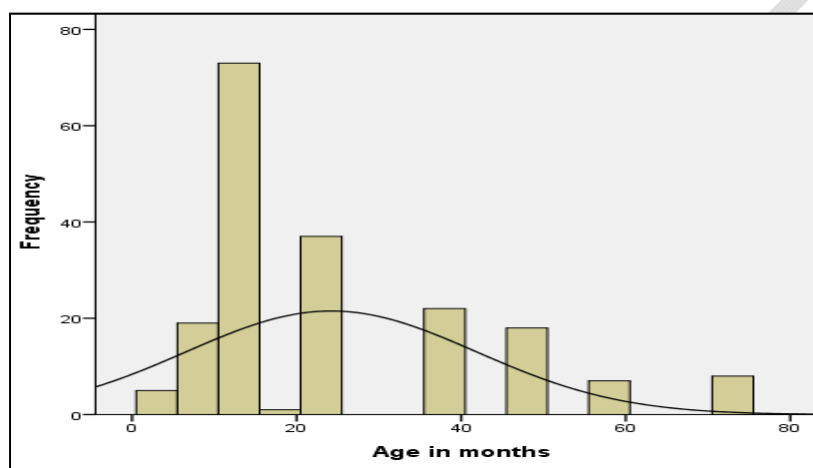
125 The study was nested within a bigger study which was funded by The Centre for Disease Control
 126 and Prevention in collaboration with the Kenya Medical Research Institute, Opportunistic
 127 infection laboratories and the ministry of health central microbiology laboratories. Permission to
 128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

129 **Results and Discussion**

130 **Participants' characteristics**

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

139



140

141 **Figure 1: Distribution curve of participants ages**

142 In this study the female children were 105(55.26%) and the males were 85(44.74%). Female
 143 children were 3.7 months older than the male children with a standard error difference mean of
 144 0.02 months as shown in Table1 below. These results could be due to the fact that children
 145 within this age group are most often than not unaccompanied and cannot differentiate between
 146 what to eat and what not to eat; they have not learnt the rules of adherence to aseptic or hygienic
 147 practice and they can barely express themselves [13]. Those below the age of twelve months are
 148 essentially under their mothers' care, feeding mainly on breast milk thereby reducing their
 149 susceptibility to these pathogens.

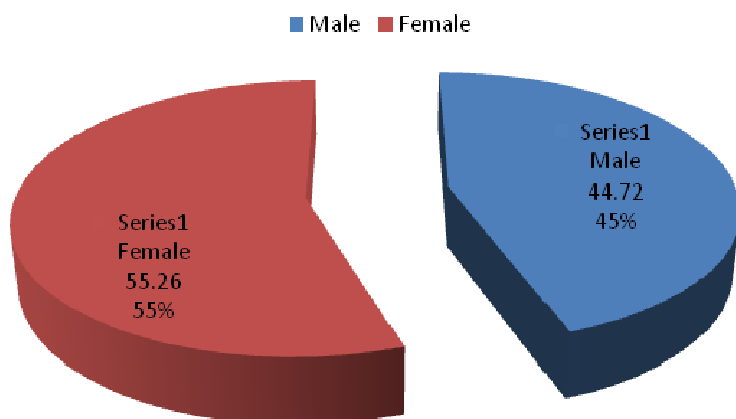
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151 **Table 1: Analysis of age of the participants**

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

152 Age can be a predisposing factor to diarrhoea in children below the age of five years (WHO,
 153 2007). Living in the slums is also a predisposing factor to diarrhoeal infections because of the
 154 poor hygienic conditions coupled with poor sanitation [6]
 155 Most enteric pathogens stimulate at least partial immunity against repeated infections or illness,
 156 which helps to explain the declining incidence of diseases in older children [17].
 157 The analysis of the participants' ages versus gender revealed that there was no significant
 158 difference. The $t_{(186)}$ value was 1.458 with probability, $p = 0.146 > 0.05$, the p-value was more
 159 than 0.05 therefore there was no association between the gender in relation to diarrhoea in this
 160 study. The male participants were 85(45%) while the female were 105(55%) as shown in Figure
 161 2. There was significant association between age and diarrhoea in this study ($p=0.01$).

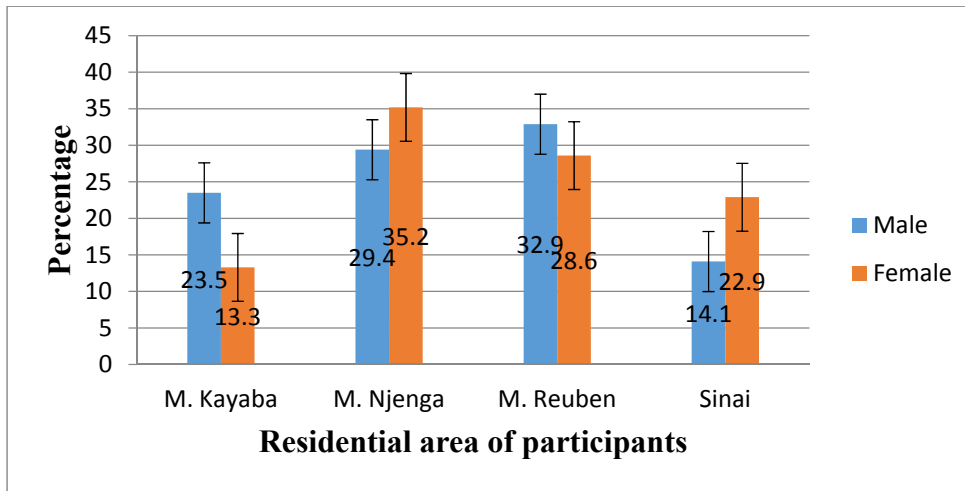


162

163 **Figure 2: Gender of the participants**

164 The participants attended to at Mukuru Kwa Njenga government health facility were noted to be
 165 residents of four neighbouring slums namely; Mukuru Kwa Njenga, Mukuru Kwa Reuben,
 166 Mukuru Kayaba and Sinai. The majority of the participants were from Mukuru Kwa Njenga
 167 61(32.6%) followed by Mukuru Kwa Reuben 57(30.5%) then Sinai 35(18.9%) and the least were
 168 from Mukuru Kayaba 33(17.9%). Mukuru Kwa Njenga had the highest number of female
 169 children (35.2%) while Mukuru Kwa Reuben had the highest number of male children (32.9%).
 170 The p-values were greater than 0.05 hence there was no significance difference between the
 171 participants from different areas of residence ($\chi^2 = 5.41$, $p = 0.144$) as shown in Figure 3.

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

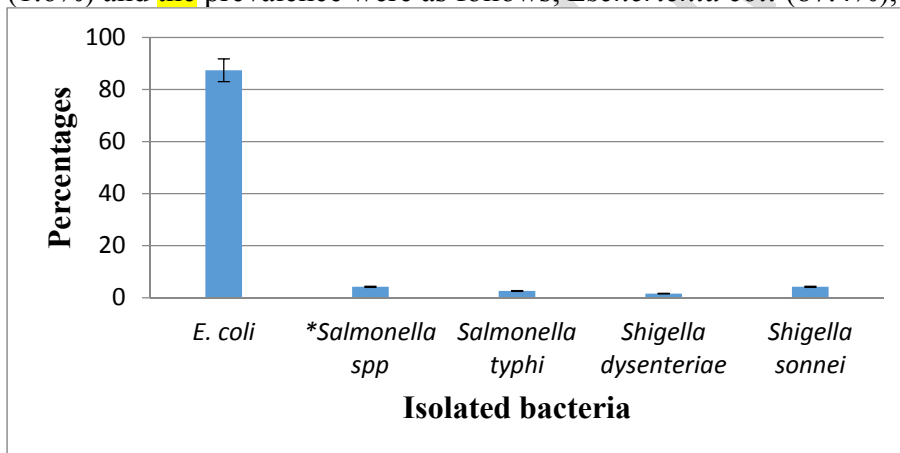


184

185 **Figure 3: Residence of study participants**

186 **Isolation and identification of the bacteria**

187 The prevalence of bacteria isolated from the study were as follows: *Escherichia coli* (87.4%),
 188 *Salmonella spp* (4.2%), *Shigella sonnei* (4.2%), *Salmonella typhi* (2.6%), *Shigella dysenteriae*
 189 (1.6%) and the prevalence were as follows; *Escherichia coli* (87.4%), as shown in Figure 4.



190

191 **Figure 4: Bacteria species isolated from the stool samples**

192

193 The total percentage prevalence of bacteria species isolated among the participants by age,
 194 gender and residence in the study area were 78% *E. coli*, 4.2% *Salmonella spp* (were not
 195 identified to species level), 4.2% *Shigella sonnei*, 2.6% *Salmonella typhi* and 1.6% *Shigella*
 196 *dysenteriae* from all the specimens collected. At Mukuru Kwa Njenga more *E. coli* were isolated
 197 from female children (35.2%) than male children (29.4%) the rest of the isolates were uniform in
 198 both genders. At Mukuru Kwa Reuben the trend is the same in that more *E. coli* were also
 199 isolated from female children (17.5%) than from male children (13.0%). *Salmonella spp* were
 200 4.9% from female children and 0.0% from male children while *S. typhi* were more from male
 201 children (3.0%) compared to female children (0.5%). At Sinai the percentage isolates from both

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

209 **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	<i>E. coli</i>	29.4	35.2	2.23 (0.693)
	* <i>Salmonella spp</i>	1.0	1.1	
	<i>S. typhi</i>	0.0	0.0	
	<i>S. dysenteriae</i>	1.5	1.5	
	<i>Shigella sonnei</i>	1.1	1.0	
M. Reuben	<i>E. coli</i>	13.0	17.5	
	* <i>Salmonella spp</i>	0.0	4.9	
	<i>S. typhi</i>	3.0	0.5	
	<i>S. dysenteriae</i>	1.0	1.1	
	<i>Shigella sonnei</i>	2.9	2.0	
Sinai	<i>E. coli</i>	9.0	9.9	
	* <i>Salmonella spp</i>	2.0	2.2	
	<i>S. typhi</i>	1.6	1.0	
	<i>S. dysenteriae</i>	0.0	1.6	
	<i>Shigella sonnei</i>	1	3.2	
M. Kayaba	<i>E. coli</i>	7.2	10.7	
	* <i>Salmonella spp</i>	0.0	0.0	
	<i>S. typhi</i>	0.0	1.6	
	<i>S. dysenteriae</i>	0.0	0.0	
	<i>Shigella sonnei</i>	0.0	0.0	

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

212

213 Acute diarrhoea due to bacterial infections is an important cause of morbidity and mortality in
214 infants and young children in most developing countries including Kenya especially in the slums
215 [20]. Identification of the **Enteropathogens** causing diarrhoeal diseases in the country is an
216 essential step towards the implementation of effective primary health care activities against the
217 disease [21]. Poor sanitation in the study area could have also contributed to the high prevalence
218 of bacteria isolated. The residents live in congested environments with their domesticated
219 animals which could have contributed to the high prevalence of isolated enteric bacteria.
220 According to a study done by Kariuki et al. [21], a significantly higher proportion of younger
221 children (< 3 years of age) and those from the slums presented with invasive non- typhoidal
222 *Salmonella spp* compared to older children and those from upper socio-economic groups ($p <$
223 0.001).

224 In terms of gender and area of residence, Mukuru kwa Njenga, had more *E. coli* isolated from
225 female children (35.2%) compared to male children (29.4%) the rest of the isolates were
226 uniform in both genders. In Mukuru kwa Reuben the trend was the same in that more *E. coli*
227 were also isolated from female children (17.5%) than from male children (13.0%). *Salmonella*
228 *spp* were 4.9% from female children and 0.0% from male children while *S. typhi* were more from
229 male children (3.0%) compared to female children (0.5%). At Sinai the percentages of the
230 isolates from both male and female children were almost equal (9.0% and 9.9%, respectively).
231 *Shigella sonnei* were more from (3.2) female than male children (1.0%). The rest were almost the
232 same in both male and female children. At Mukuru Kayaba *E. coli* isolates were more from
233 female (10.7%) than male children (7.2%). *S typhi* were 1.6% in females and 0.0% in males
234 while the rest were 0.0%. There was no significant association between the gender and
235 percentage isolates ($p > 0.05$). There was also no significant association between the prevalence of
236 the isolates and the area of residence of the children ($\chi^2 = 2.23$, $p = 0.693$). The results of this study
237 do not concur with what Sang et al. [6] found in their studies on the prevalence of bacteria in
238 four provinces in Kenya where they had recruited 651 participants and isolated pathogenic
239 bacteria in (17.7%) of the participants. Among the isolated bacteria were; pathogenic *E. coli*
240 (11.2%), *Salmonella* (3.5%), *Shigella* (2.3%) and *Vibrio cholera* (0.6%) [3]. The reason for the
241 different results could be because the study area was basically a slum hence the high prevalence
242 of bacteria isolated especially the *E. coli*.

243 A similar study was done by Ifeanyi et al. [22] in Abuja Nigeria among cases of diarrhoea with
244 potential bacterial pathogens detected being 65.8% of all patients screened. This was in contrast
245 to a report of the prevalence of 83.1% from similar study in Abakaliki, south –eastern Nigeria
246 [23]. Another study reported a prevalence of 63.3%-71.83% isolation of enteric bacteria in
247 ifakara Tanzania. The variation in prevalence between the two Nigerian cities might be attributed
248 to differences in infrastructural and socioeconomic [23]. In a different study, the prevalence of
249 bacterial aetiology of diarrhoea was 44% which follows the same trend with the research
250 conducted in Kano State which was found to be 40.67%. In Gabon prevalence of diarrhoea with
251 bacterial aetiology was 38% [17]. In Tanzania it was 36%. The study showed that *Shigella spp*
252 appears to be the predominant bacteria causing diarrhoea followed by *E. coli*, and *Salmonella* in
253 that order. A total of 56% of the hundred diarrhoea cases investigated had no bacterial pathogen
254 suggesting viral, protozoan or nonpathogenic factors [24].

255 *Salmonella spp* isolated in Mukuru slums could be non- typhoidal salmonella which is a zoonotic
256 strain. The children could have been contaminated with faecal matter of the domesticated
257 animals hence the acquisition of the bacteria. Occurrence of diarrhoeagenic bacteria in the
258 current study showed that gram negative bacteria (*Shigella spp*, *Salmonella spp*, *Escherichiacoli*)

259 are the main cause of bacterial diarrhoea. Sule et al. [19] in Kaduna Nigeria conducted a similar
260 study and found similar results. Generally, the aetiology of diarrhoea in young children could be
261 attributed to a wide range of factors, but one of the main causes of diarrhoea is related to bacteria
262 (such as *Salmonella spp*, *Shigella spp*, *Vibrio*, *Escherichia coli*, *Aeromonas* and *Pseudomonas*
263 [24]. Results from the current study shows that, though there are a number of causative agents of
264 diarrhoeal diseases, bacteria still remain one of the major causes with *Shigella*, *Salmonella* and
265 *Escherichia coli* being the most important pathogens among paediatric patients presenting with
266 diarrhoea in Mukuru kwa Njenga Government health facility. Judicious use of antibiotic therapy
267 requires education of health workers and patients, adequate laboratory diagnostic capabilities and
268 government regulations.

269 **Conclusion**

270 In this study the female participants were more than the males. Mukuru Kwa Njenga had the
271 highest (35.2%) number of female children while Mukuru Kwa Ruben had the highest (32.9)
272 number of male children. There was no statistical significant difference between the participants
273 characteristics and their area of residence ($p= 0.144$). Age of the participants had significant
274 association with the prevalence of diarrhoea ($p=0.00$). The total prevalence of isolated bacteria
275 among the participants was very high (90.6%). The *E. coli* bacteria showed the highest
276 percentage of enteric pathogens isolated (35.2%) from female children at Mukuru Kwa Njenga
277 and 29.4% from male children, *Salmonella spp* being second (4.9%) from female at Mukuru
278 Kwa Reuben and the least was *Shigella sonnei* (3.2%) from female children at Sinai.

279 **Recommendation**

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

283 **Conflict of Interest**

284 The authors declare no conflict of interest

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