# The Burden and Antibiotic Sensitivity of Salmonella Non-Typhi and Shigella Related Bloody Diarrhea in Children

Abir Rashid Al Sinani, Tawfiq Taki Al Lawati\*, Hajar Musabah Al Saadi and Aamera Al Majrafi

Department of Pediatrics, Rustaq Hospital, Rustaq, Oman

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### ABSTRACT

Objectives: We sought to report the frequency of non-typhoidal Salmonella (SNT) and Shigella spp. diarrhea and the antibiotic sensitivity in children. Methods: We conducted a retrospective study of children with bloody diarrhea seen at Rustaq Hospital between 1 June 2019 and 31 June 2023. We collected data related to demographic characteristics, symptoms, blood investigations, stool bacterial culture, and antimicrobial sensitivity. Stool samples were tested for Salmonella and Shigella growth. Results: Out of 1160 children with diarrhea, 153 (13.2%) had bloody diarrhea of which 129 (84.3%) were under five. Ninety-two (60.1%) children were positive for either Salmonella or Shigella. Among the positive cultures, 58 (63.0%) children had SNT, while 34 (37.0%) had Shigella infection. Three children had bacteremia, all under one year old. SNT demonstrated high sensitivity primarily to ceftriaxone (n = 41; 70.7%), ampicillin (n = 53; 91.4%), and ciprofloxacin (n = 54; 93.1%). In contrast, Shigella showed high resistance to ceftriaxone and only 15 (46.9%) patients showed sensitivity. Additionally, 29 children had Entamoeba histolytica trophozoites co-infection with Salmonella on stool microscopy. Conclusions: Salmonella is more prevalent than Shigella in children under five years, while Shigella is more common in children over five. Salmonella is sensitive to both ceftriaxone and ampicillin. Shigella demonstrates resistance to multiple antibiotics, including ciprofloxacin. It is recommended that children under the age of one be admitted and treated empirically with either ceftriaxone or ampicillin. In older children, antibiotic therapy should be guided by stool culture results. Ciprofloxacin is not a good empirical choice for Shigella in our population due to its high resistance and is contraindicated in patients with glucose-6-phosphate dehydrogenase.

iarrheal diseases in children under the age of five are the second most common cause of death, accounting for 525 000 deaths annually. Globally, the incidence of bacterial origins of diarrhea has been documented with varying degrees of occurrence. As an illustration, in South Asian nations, the occurrence has been estimated to be approximately 10%, whereas in Jordan, it reaches 24% among children. <sup>2,3</sup>

In a multicentered study, *Shigella* was the most common organism found in bloody diarrhea in children under five in Sub-Saharan Africa and South Asian countries.<sup>4</sup> In Oman, the reported prevalence of acute bloody diarrheal illness in a single study from A'Dhahira was 9.1% with no mortalities.<sup>5</sup> In 2022, a total of 65 391 diarrheal cases were reported in Oman in under five-year-olds, an increase of 35

episodes per 1000 children compared to 2021. No mortalities were reported.<sup>6</sup>

Acute febrile bloody diarrhea can be caused by multiple pathogens. *Shigella* is the second most common cause of diarrhea mortality and morbidity, accounting for 60 000 deaths in children under five annually. A recent study in Africa and Asia showed the most common organisms for acute diarrhea were rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli* (*E. coli*), and *Shigella*. In a regional study from Dammam, Saudi Arabia, the commonest organism of diarrhea in children under five years was rotavirus followed by *Salmonella*, *Shigella*, *Campylobacter jejuni*, enteropathogenic *E. coli*, and non-agglutinating vibrios.

The detection of the diarrhea-causing organism employs various methods. Polymerase chain reaction

is primarily utilized for viruses and specific bacteria. Microscopy serves as a valuable tool for identifying parasites. Meanwhile, stool culture stands as the gold standard for enterobacteria detection, encompassing organisms like *Shigella*.<sup>10</sup>

Stool culture, although being the gold standard for bacterial identification and determination of antimicrobial sensitivity, has low sensitivity and poses many technical difficulties.<sup>11</sup>

Recognizing bacterial causes, especially *Shigella*, holds significance in averting mortality and minimizing morbidity as emphasized by the World Health Organization (WHO).<sup>12</sup> Data suggest that there is increased mortality in children given antibiotics for bloody or non-bloody diarrhea, and the use of antibiotics was proposed to be limited to patients with other comorbidities or children younger than three months.<sup>13</sup>

The South Al Batinah governorate of Oman has about 476 008 population, mainly low- and middle-income people. <sup>14</sup> While bacterial diarrhea is known to occur in low-income areas around the world, there is no data on the prevalence or etiology of bloody diarrhea in the South Al Batinah. <sup>15</sup>

Our study aimed to determine the occurrence rate and clinical characteristics of children experiencing acute bloody diarrhea associated with *Salmonella* and *Shigella* at Rustaq Hospital. Additionally, it sought to outline the antimicrobial sensitivity profile of the stool isolates.

# **METHODS**

We conducted a retrospective study of all children under the age of 13 years with bloody diarrhea from 1 June 2019 to 31 June 2023. All children who presented with acute fever and bloody diarrhea seen or admitted in Rustaq Hospital were included in the study. Children > 13 years, known to have other etiological causes of diarrhea, and those who did not fit the definition of acute bloody diarrhea were excluded from the study. The data were retrieved from the Al Shifa electronic database. Demographic data (name, age, sex, nationality, and residence), clinical symptoms (fever, vomiting, abdominal pain, mucus stool, and dehydration status), and laboratory details (C-reactive protein, hemoglobin, leukocytosis, stool red and white blood cell counts, stool culture, and blood culture) were collected. The degree of dehydration was categorized into mild,

moderate, or severe. Mild dehydration was defined as when the child had only thirst but normal moist mucous membranes, normal pulse, and normal capillary refill with good urine output. Moderate dehydration was defined as when the child had two of the following signs: restlessness and irritability, reduced tears, deep-set eyes, thirst, and slow return of skin pinch. Severe dehydration was defined as when the child had two of the following signs: lethargy or unconsciousness, sunken eyes, inability to drink or drink poorly, and very slow return of skin pinch. <sup>16</sup>

Stool samples were collected in a wide-necked sterile container for bacterial culture and microscopy for parasites before commencing antibiotic treatment. MacConkey agar was used in the lab to identify Salmonella-Shigella agar and thiosulfatecitrate-bile salts-sucrose agar. After overnight incubation at 37 °C, the plates were observed for Salmonella and Shigella colonies. For stool bacterial sensitivity, the following antibiotics were tested using the disc diffusion method: ampicillin, ceftriaxone, chloramphenicol, and ciprofloxacin. At times, not all antibiotic discs were available for testing. Only Salmonella and Shigella were cultured due to their management impact in the case of Salmonella typhi and the need for treatment for Shigella. Other bacterial causes such as E. coli and Campylobacter are self-limited diseases that require only supportive care.

The data were analyzed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Numeric values underwent a normality test before analysis. Results were expressed as mean and SD or median with interquartile range. Statistical associations were calculated using the Student's *t*-test for continuous data and the chi-square test for categorical data. A *p*-value < 0.05 was considered statistically significant.

Ethical approval was obtained from the Research Centre in South Al Batinah with research code no. 01062023.

# RESULTS

A total of 1160 children were admitted with gastroenteritis at Rustaq Hospital during the study period. Out of the total number, 154 (13.3%) presented with acute bloody diarrhea. One child had missing data. Among these patients, 151 (98.7%) were Omanis, with a male-to-female ratio of 1.3:1.

Table 1 demonstrates the demographic data of the population. Most of the patients came from Rustaq, followed by Al Musannah.

The most frequent complaint of the children was watery stools, followed by fever. Table 2 demonstrates the clinical signs, symptoms, and basic blood labs of the patients.

Of the 153 children, 92 (60.1%) had positive stool cultures and 61 (39.9%) had negative stool cultures for either *Salmonella* or *Shigella*. Of the children with positive stool culture, 58 (63.0%) had non-typhoidal *Salmonella* (SNT), and 34 (37.0%) had *Shigella* infection. No further identification of the *Salmonella* or *Shigella* type was required for clinical management purposes.

On a sub-analysis of the population age-wise, in children younger than one year with bloody diarrhea, 41 (78.8%) had fever and only 35 (67.3%) had stool culture positive for bacteria. Thirty-two (61.5%) children had *Salmonella* growth and three (5.8%) had *Shigella* in the stool. No significant statistical association was noted between fever and stool bacterial growth (p = 0.676) in children under the age of one year. Only 39 (25.5%) patients were not given antibiotics while the rest of the patients did receive antibiotics.

Bacteremia with blood culture positive for the same organism as isolated from the stool was evident in only three children. All of these children were under the age of one year (10, seven, and eight months), and all had *Salmonella* growth. The *Salmonella*-causing bacteremia was sensitive to ceftriaxone, ampicillin, and trimethoprim. All children had a normal leukocyte count. None of the children had further complications from *Salmonella* bacteremia, and none of them had sickle cell disease.

For children under five years, 55 (43.0%) children had *Salmonella* isolated and 26 (20.3%) had *Shigella* isolated. Forty-six (35.9%) children had no growth in stool cultures. In contrast, among children > five years, only three (12.0%) had *Salmonella* and eight (32.0%) had *Shigella*.

Regarding antibiotic sensitivity, some patients were not tested for the whole panel of antibiotics sensitivity based on test availability. However, the lowest number of patients tested for antibiotic sensitivity was for ceftriaxone at 41 (70.7%) patients. For the rest of the patients, > 90% of the isolates were tested for the whole panel of antibiotics, except trimethoprim for *Shigella*, where 29 (85.3%)

Table 1: Demographic data of the study group.

Variable	Frequency	Percentage
Sex		
Male	87	56.9
Female	66	43.1
Nationality		
Omani	151	98.7
Non-Omani	2	1.3
Age, years		
Median	2	IQR = 1-5
≤ 5	128	83.7
≤ 1	52	34.0
>5	25	16.3
Median weight, kg	13.0	IQR = 10.0 - 18.0
Residence		
Rustaq	65	42.5
Al Mussanah	34	22.2
Barka	21	13.7
As Suwaiq	12	7.8
Al Awabi	10	6.5
Nakhal	8	5.2
Wadi Maawil	3	2.0

IQR: interquartile range.

**Table 2:** Clinical characteristics of patients with bloody diarrhea.

Symptoms	Frequency	Percentage
Watery stools	136	88.9
Fever	122	79.7
Vomiting	98	64.1
Mucoid stools	54	35.3
Bloody stools	50	32.7
Abdominal pain	30	19.6
Degree of dehydration		
None	59	38.6
Mild	57	37.3
Moderate	21	13.7
Severe	16	10.5
Type of dehydration		
Eunatremia	140	91.5
Hypernatremia	2	1.3
Hyponatremia	7	4.6
Blood results	Value	IQR
Median hemoglobin, gm/dL	13.0	11.4-12.3
Median C-reactive protein	45.5	20.0-126.0
White blood cell $\times 10^9/$ mL	9.5	6.6-13.0
Median stool white blood cells/HPF	5.5	3.0-20.0

HPF: high power field; IQR: interquartile range.



Table 3: Antibiotic sensitivity of isolated Salmonella and Shigella.

Sensitivity	Ceftriaxone n (%)	Ampi n (%)	Cipro n (%)	TMP n (%)
Salmonella, n = 58	41 (70.7)	53 (91.4)	54 (93.1)	54 (93.1)
Sensitive	38 (92.7)	43 (81.1)	43 (79.6)	50 (92.6)
Not sensitive	3 (7.3)	10 (18.9)	11 (20.4)	4 (7.4)
Shigella, $n = 34$	31 (91.2)	31 (91.2)	32 (94.1)	29 (85.3)
Sensitive	4 (12.9)	5 (16.1)	15 (46.9)	5 (17.2)
Not sensitive	27 (87.0)	26 (83.9)	17 (53.1)	24 (82.7)

Cipro: ciprofloxacin; Ampi: ampicillin; TMP: trimethoprim.

children were tested. Table 3 demonstrates the pattern of antibiotic sensitivity of isolated *Shigella* and *Salmonella*.

Thirty-eight (92.7%) children with *Salmonella* were sensitive to ceftriaxone and 43 (79.6%) were sensitive to ciprofloxacin. The three (7.3%) children with *Salmonella* resistant to ceftriaxone were all sensitive to ciprofloxacin.

Regarding *Shigella*, out of the 31 children tested, only four (12.9%) children were sensitive to ceftriaxone. Regarding ciprofloxacin, out of the 32 children tested, only 15 (46.9%) were sensitive while 17 (53.1%) were resistant to ciprofloxacin. Additionally, none of the 17 children with *Shigella* resistant to ciprofloxacin were sensitive to ceftriaxone.

Overall, 108 (70.6%) children received empirical antibiotics, while 39 (25.5%) did not receive antibiotics. The most common prescription pattern was combined ceftriaxone and metronidazole (n = 43; 28.1%), followed by ceftriaxone or cefotaxime alone. Table 4 demonstrates the pattern of empirical antibiotics used for the patients.

Using analysis of variance, no association was detected with fever, abdominal pain, leukocytosis, CRP, and stool culture positivity for *Shigella* or *Salmonella*. However, there was an association noted between stool leukocyte count and stool culture positivity (p = 0.026)

Ten children had a co-infection of *Salmonella* and *Entamoeba histolytica* trophozoites, while four had *Shigella* and amoebic trophozoites. Table 5 demonstrates the isolated parasites detected on stool microscopy.

Regarding the seasonal variation of the incidence of bloody diarrhea, the months from January to July had the most recorded cases, while there was a sharp decline immediately after July until the end of December. This phenomenon was consistent over the years of the study [Figure 1].

**Table 4:** Frequency and type of antibiotics used in children with bloody diarrhea upon presentation.

Antibiotics prescribed	n (%)
Ceftriaxone and metronidazole	43 (28.1)
No antibiotics	39 (25.5)
Ceftriaxone or cefotaxime	29 (19.0)
Metronidazole	28 (18.3)
Amoxy-clavulanic acid	8 (5.2)
Missing	6 (3.9)
Total	147 (96.1)

**Table 5:** Detection of parasites on stool microscopy from the children with bloody diarrhea.

Parasites	n (%)
E. histolyica cyst	63 (41.2)
E. histolyica cyst trophozoite	29 (19.0)
Giardia lamblia	2 (1.3)
Hymenolepis nana	1 (0.7)
Taenia species	1 (0.7)
Nil	56 (36.6)
Missing	1 (0.7)
Total	153 (100)

# **DISCUSSION**

This study reports a prevalence of 13.2% for bloody diarrhea among children admitted to a regional area with low resources and acute diarrhea. While *Shigella* has been reported as the most common bacterial pathogen in multiple studies,<sup>2-4</sup> in our study, *Salmonella* was the most common (63.0%) among the whole group particularly in children < five years. Our findings are similar to a study from Brazil on 260 children, which showed that *Shigella* occurred mostly in children > five years, while *Salmonella* was more common in younger children.<sup>17</sup>



**Figure 1:** The frequency of bloody diarrhea in Rustaq Hospital during the years of the study.

The use of antibiotics is recommended mainly for *Shigella* as it leads to improvement of symptoms within 48 hours and prevents the prolongation of the disease over many weeks. <sup>18</sup> Treatment of SNT is mainly supportive except for infants < six months or immunocompromised patients. <sup>19</sup> Unlike *Shigella*, *Salmonella* is also a self-limiting disease. <sup>20</sup>

Regarding antibiotic sensitivity, our study demonstrated that 92.7% of the *Salmonella* isolates were sensitive to ceftriaxone, and nearly 80.0% were sensitive to ampicillin and ciprofloxacin. The low resistance to ceftriaxone is similar to the prevalence reported in the Eastern Mediterranean study, with only about 10% resistance.<sup>21</sup> Our study was also in line with the reported resistance to ciprofloxacin for *Shigella* in the Southeast Asian Region, which rose from 4% to 76% in 2008, close to our *Shigella* resistance to ciprofloxacin of 53.1%.<sup>21</sup>

Antibiotic use in cases of *Salmonella*-related diarrhea is not routinely recommended. *Salmonella* enterocolitis is a self-limited disease.<sup>22</sup> Moreover, antibiotic use in *Salmonella* infection might trigger hemolytic uremic syndrome and possibly prolong the disease duration.<sup>23,24</sup> In our setup, as *Salmonella* was the most common pathogen in children under the age of one year and few children had bacteremia, it was reasonable to start children under the age of one with either ampicillin or ceftriaxone until stool culture results are available.

In contrast, the situation is reversed with *Shigella*, where > 83.0% of the isolates were resistant to ceftriaxone and ampicillin, and > 50.0% are resistant to ciprofloxacin. Hence, it is not effective to use any of these medications for *Shigella* enterocolitis. The WHO recommends ciprofloxacin as an oral medication for ambulatory care for bloody diarrhea in children related to *Shigella* mainly. However, glucose 6-phosphate dehydrogenase deficiency (G6PD) represents an extra obstacle to tackle in our

population, as G6PD deficiency reaches 26% of the general population.<sup>25</sup> Therefore, in children with G6PD deficiency or of unknown status, macrolides like clarithromycin might be more appropriate<sup>26</sup> and needs to be tested in our population.

The children who were stool culture negative (n = 61, 39.9%), even though they had fever and bloody diarrhea, were likely to have another pathogen that was not tested, mainly *E. coli* and *Campylobacter*. Acute bloody diarrhea is not commonly attributed to amoebic diarrhea. Amoebic colitis is usually an insidious process and does not present with acute febrile diarrhea. Moreover, it is important to note that *Entamoeba histolytica* can be easily confused with the normal gut flora of *Entamoeba dispar* and the low-virulence *Entamoeba moshkovskii*.<sup>27,28</sup>

It is interesting that 70.6% of the population received antibiotics regardless of age or clinical condition of the child. Moreover, nearly 50.0% of the patients received metronidazole in combination with cephalosporin or in isolation. The use of metronidazole in not indicated in acute febrile diarrhea. The overuse of antibiotics in general and using inappropriate antibiotics or bloody diarrhea raise concerns on the medial awareness amongst physicians in the institution.

Of interest is the seasonal distribution of bloody diarrhea, as it is almost confined to the first half of the year with no reported cases during the second half. Infective diarrhea is not commonly associated with seasonal changes, and this phenomenon needs to be studied further.

The limitations of the study mainly include the absence of any data on the growth of *E. coli* or *Campylobacter* due to the unavailability of testing means in a resource-limited secondary hospital like AGH, particularly as the management is not affected by these organisms. Additionally, there was no testing done for macrolide sensitivity for either *Salmonella* or *Shigella* organisms.

# CONCLUSION

Acute bloody diarrhea is a significant problem in our hospital. Fever and mucoid loose stools are the most common symptoms associated with bacterial pathogens. SNT is more common than *Shigella* in children under the age of five and is sensitive to a wide range of antibiotics. *Shigella*, on the other hand, is less common but is resistant to multiple antibiotics,



including ciprofloxacin. There is significant overuse of antibiotics in children with bloody diarrhea. Our study advocates for starting antibiotics only after verification of the organism by stool culture, except for children under the age of one year or those who are immunocompromised. G6PD is of concern in our population, and macrolides might be a safer option than fluoroquinolones for the treatment of *Shigella*.

#### Disclosure

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