Prevalence of intestinal parasite, *Shigella* and *Salmonella* species among diarrheal children in Jimma health center, Jimma southwest Ethiopia: a cross sectional study

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**Abstract**

**Background:** Diarrheal disease continues to be an important cause of morbidity and mortality among young children in developing countries including Ethiopia. Globally, intestinal parasite, *Shigella* and *Salmonella* species remain major contributors to acute enteric infections. The study was aimed at determining the frequency of intestinal parasite, *Shigella* and *Salmonella* species identified from diarrheic children at Jimma Health Centre, Jimma south west Ethiopia.

**Methods:** A health institution based cross sectional study was conducted from March to November 2012. A structured questionnaire was used for collection of data on socio-demographic characteristics. Parasite and bacteria identification as well as susceptibility testing was done using standard parasitological and bacteriological procedures.

**Results:** A total of 260 diarrheal children were included in the study. A total of 129 (49.6%) samples were positive for intestinal parasite, *Shigella* and *Salmonella* species. Of these, 107 (41.1%), 6 (2.3%) and 16 (6.2%) samples were positive for intestinal parasite, *Shigella* and *Salmonella* species respectively. The dominant isolated parasite was *G. lamblia* with prevalence of 13.5% followed by *A. lumbricoides* (11.5%). The least identified parasites were *Schistosoma mansoni* and *Taenia species* accounting 0.4% each. Multiple parasitic infections were observed in 19 (7.3%) patients. *Shigella* species showed hundred percent resistances to ampicillin, amoxacillin, and cotrimoxazole. All *Salmonella* isolates were resistant against amoxicillin. All *Shigella* and *Salmonella* species were susceptible to ceftriaxone, ciprofloxacin and gentamycin.

**Conclusion:** The presence of reasonably high amount of intestinal parasite and *Salmonella* and *Shigella* species that are drug resistance to the commonly prescribed drugs is a treat to the children and community at large. Therefore, measures including health education, improvement of safe water supply, sanitation facilities and continuous monitoring of microbiological and antimicrobial surveillance is crucial.

**Keywords:** Intestinal parasite, *Shigella*, *Salmonella*, Susceptibility test, Jimma, Ethiopia

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Introduction
Diarrheal disease continues to be an important cause of morbidity and mortality among young children in developing countries [1]. Children and young adults are the most affected, particularly in regions with limited resources and where hygienic measures are inadequate. Causes of diarrhea in endemic areas include a wide variety of bacteria, viruses, and parasites [2].

Gastrointestinal parasitic infections are amongst the most common infections worldwide. These cases are attributed to three common intestinal parasites: *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*. The global prevalence of parasitic diseases is estimated to be 478 million children for *A. lumbricoides*; 280 million for hookworms and 347 million children for *T. trichiura* [3]. In Ethiopia, intestinal parasitic infections are of serious public health concern [3]. According to a report by the Ministry of Health, helminthiasis is the third leading cause of outpatient visits in health institutions in 2005-2006 [4].

Recently, it was observed that the prevalence of intestinal parasitic infections in Jimma, Ethiopia [5] was as high as 86.2%; whereas in Tigray, the overall prevalence was 48.1% [6]. According to the study findings conducted in Angolela, Ethiopia, on school children by Nguyen et al (2012), one-third of the children were infected with protozoan while 7.1% were found to have helminthic infections [7].

Among the diarrheal pathogens, *Shigella* continues to play a major role in etiology of inflammatory diarrhoea and dysentery, thus presenting a serious challenge to public-health authorities worldwide [8]. The few studies conducted on shigellosis in Ethiopia revealed that, Shigellosis and the emergence of antimicrobial resistant *Shigella* species is a major health problem [9-14]. Recent data different health institutions in Ethiopia have indicated that salmonellosis is a common problem and also showed the presence of a number of serogroups/serotypes in humans, animals, food animals, food products animal origins and other food stuff [15-19].

Infections by most species of *Shigella* and *Salmonella* can be asymptomatic, or can be treated with rehydration solutions except for infection by invasive strains. The use of antibiotics might shorten the duration of diarrhea and limit the shedding of the organisms which otherwise might continue to spread among people and in to the environment and further pose a risk of transmission of infections. However, antimicrobial resistance is an over-growing problem, and there is a need to monitor the susceptibility of common bacterial isolates to drugs used in the community to provide guidelines for the empirical treatment of bacterial infections.

In the present study, a prospective cross sectional study was conducted to determine the prevalence of intestine parasite, *Shigella* and *Salmonella* species among diarrhoeic children visiting Jimma Health Center from March to November 2012, south West Ethiopia. We determined the prevalence of intestinal parasites, *Shigella* and *Salmonella* species in diarrhoeal stools of children including also the susceptibility to antimicrobial agents of the investigated pathogens.

Material and methods
Study design, area and period
A prospective cross sectional study was conducted to determine the prevalence of intestinal parasite, *Shigella* and *Salmonella* species among diarrheic children visiting Jimma Health Center, Jimma south West Ethiopia from March to November 2012.

Sample size and sampling technique
A total of 260 children were participated as a study subject. The sample size was determined based on the prevalence rate of the study done by Mache on children at Jimma [15] and calculated with the formula recommended by Daniel W [20].

Demographic data collection
Histories were taken from each child and informed consent was obtained from the parents or guardians before sample collection was attempted by the attending pediatrician. All relevant demographic, clinical and laboratory data were recorded and transferred to the questionnaire prepared for this study.

Specimen collection and identification of pathogens
Freshly passed stool and rectal swab was collected, placed immediately in Cary Blair transport medium (Oxoid Ltd, Basingstoke, UK) and transported to the laboratory within six hours of collection. For identification of *Shigella* and *Salmonella* species, specimens were placed in Selenite F enrichment broth (Oxoid) and incubated at 37°C for 24 hours, then subcultured onto deoxycholate agar (DCA) and xylene lysine deoxycholate agar (XLD) (Oxoid) agar at 37°C for 18-24 hours. The growth of *Salmonella* and *Shigella* species was detected by their characteristic appearance on XLD agar (*Shigella*: red colonies, *Salmonella* red with a black centre) and DCA (*Shigella*: pale colonies, *Salmonella* black centre pale colonies). The suspected colonies were further tested through a series of biochemical tests to identify *Shigella* and *Salmonella* species [21].

Parasitological examination of stool
Stool specimens were obtained from all participants and examined for the presence of intestinal parasite cysts, eggs, trophozoites and larvae. In the laboratory, slides were prepared directly for wet mount in saline as well as in iodine and then were microscopically examined.
initially under low power (10X10 magnifications) bright field then under high-power (40X40 magnification) bright field. Finally the sample was concentrated using the procedure of formalin ethyl acetate technique4 and iodine stained slides were prepared and examined microscopically.

Susceptibility testing
Antimicrobial drug susceptibility testing was carried out using disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guide lines [22]. The antibiotic discs used and their concentrations were: ceftriaxone (CRO, 30 μg), chloramphenicol (C, 30 μg), ciprofloxacin (CIP, 5 μg), gentamicin (GM, 10 μg), nalidixic acid (NA, 30 μg), trimethoprim-sulfamethoxazole/cotrimoxazole (SXT, 25 μg) ampicillin (AMP 10 μg) and amoxicillin (AML, 20 μg). All antibiotic were obtained from Oxoid Limited, Basingstoke Hampshire, UK. A standard inoculum adjusted to 0.5 McFarland was swabbed on to Muller- Hinton agar (Oxoid Ltd. Bashingstore Hampaire, UK); antibiotic disc was dispensed after drying the plate for 3-5 min and incubated at 37°C for 24 hours. The reference strains used as control were E.coli ATCC 25922.

Data were entered and analyzed using SPSS version 16.0 computer software. Ethical clearance was secured from Ethical Clearance Committee of College of Public Health and Medical Sciences Jimma University. Permission was obtained from Health center officials.

Results
Stool specimens of 260 children were collected and examined for the presence of intestinal parasites and cultured for Salmonella and Shigella species. Out of the total 260 study participants 114 (43.8%) were males and 146 (56.2%) were females showing an overall male to female ratio 1:0.8. The age of the studied children ranges from one month of age to 15 years with mean age of five year (+SD 4.1): the majority (60.3%) of the study subjects were between 1-5 years of age and list frequency (8.4%) was observed for children less than one years old (Figure 1).

Out of the 260 stool samples, 129 (49.6%) samples were positive for intestinal parasite, Shigella and Salmonella species. Of these, 107 (41.1%), 6 (2.3%) and 16 (6.2%) samples were positive for intestinal parasite, Shigella and Salmonella species respectively. The dominant isolated parasite was Giardia. lamblia with prevalence of 13.5% followed by A. lumbricoides (11.5%). The least identified parasites were Schistosoma mansoni and Taenia species accounting 0.4% each (Table 1).

The distribution of enteropathogens according to the different age groups is listed in Table 2. The majority (58.1%) of enteropathogens were found in children aged 1–5 years. Whereas, 26 (20.2%), 21 (16.3%) and seven (5.4%) pathogens were observed in children within age groups of 6 – 10, 11 – 15 and less than one years, respectively. Multiple parasitic infections were observed in 19 patients (Table 3). Triple infection was observed in five children (1.5%) and double parasitic infections were observed among 14 children. The commonest parasites in multiple infections were A. lumbricoides + G. lamblia. The commonest double infections were T. trichuria + G. lamblia + G. lamblia + E. histolytic. The commonest triple infections were A. lumbricoides + H. nana + G. lamblia (15.8%). There was no confection of bacteria (Salmonella and Shigella species) and parasite among the children (Table 3).

Shigella species showed hundred percent resistances to ampicillin, amoxacillin, and cotrimoxazole while all (100%) isolates were susceptible to ceftriaxone, ciprofloxacin and gentamicin (Table 4). Resistance to ampicillin,
cotrimoxazole, chloramphenicol and nalidixic acid was observed in 62.5, 31.3, 18.8% and 12.5 of Salmonella isolate respectively. All Salmonella isolates were resistant against Amoxicillin and susceptible to ceftriaxone, ciprofloxacin and gentamicin (Table 4).

Over all three resistance patterns were seen among Shigella isolates. All Shigella species were multi-drug resistant (resistant to three or more antimicrobial drugs). About 66.6% of Shigella species were resistant to three (ampicillin, amoxicillin, cotrimoxazole) antibiotics. On the other hand 62.5% of Salmonella species were multi-drug resistant, ranging from 2 to 4 drugs (Table 5).

**Table 1 Frequency of intestinal parasite and bacteria isolated from 260 diarrheic children**

| Etiological pathogens | Patient (n = 260) no. (%) |
|-----------------------|--------------------------|
| **Parasite**          |                          |
| A. lumbricoides       | 30 (11.5)                |
| T. trichiura          | 8 (3.1)                  |
| Hook worm             | 4 (0.4)                  |
| H. nana               | 20 (7.5)                 |
| T. mansoni            | 1 (0.4)                  |
| G. lamblia            | 35 (13.5)                |
| E. histolytica        | 8 (3.1)                  |
| **Total**             | 107 (41.1)               |
| **Bacteria**          |                          |
| Shigella species      | 6 (2.3)                  |
| Salmonella species    | 16 (6.2)                 |
| **Total**             | 22 (8.5)                 |
| **Enteropahogens total** | 129 (49.6)              |

**Table 2 Age distribution of intestinal parasite, Shigella and Salmonella species among the study participants**

| Age group in years | No. (%) |
|--------------------|---------|
|                    | < 1     | 1–5    | 6–10   | 11–15  |
| **Parasite**       |         |        |        |        |
| A. lumbricoides    | 13 (10.1)| 10 (7.8) | 7 (5.4) |
| T. trichiura       | 5 (3.9)  | 1 (0.8)  | 2 (1.6) |
| Hook worm          | 1 (0.8)  | 1 (0.8)  | 2 (1.6) |
| H. nana            | 14 (10.9)| 4 (3.1)  | 2 (1.6) |
| T. mansoni         | -        | 1 (0.8)  | -       |
| G. lamblia         | 28 (21.7)| 3 (2.3)  | 4 (3.1) |
| E. histolytica     | 1 (0.8)  | 3 (2.3)  | 1 (0.8) |
| **Bacteria**       |         |        |        |        |
| Shigella species   | 1 (0.8)  | 4 (3.1)  | 1 (0.8) | -       |
| Salmonella species | 5 (3.9)  | 7 (5.4)  | 3 (2.3) | 1 (0.8) |
| **Total**          | 7 (5.4)  | 75 (58.1)| 26 (20.2)| 21 (16.3)|

**Table 3 Frequency of multiple (double and triple) parasitic infections of children**

| Parasite combinations | No % |
|-----------------------|------|
| As, Hy, GL            | 3 (15.8)|
| As, Tt, GL            | 1 (5.3) |
| As, Tt, EH            | 1 (5.3) |
| As, HW                | 2 (10.5)|
| As, Tt                | 2 (10.5)|
| As, GL                | 2 (10.5)|
| Hy, GL                | 2 (10.5)|
| Tt, GL                | 3 (15.8)|
| GL, Eh                | 3 (15.8)|
| **Total**             | 19 (100.0)|

Keys: - Al- Ascaris lumbricoide, Hy: Hyminolopis nana, GL: Giardia lamblia, Tt: Trichuris trichiuria, Eh: Entamoeba histolytica, HW: Hook worm.

**Discussion**

The study results indicated, of the total 260 study participants, 107 (41.1%) of the symptomatic children were infected with one or more intestinal parasites. It is comparable with study done by Unasho in southern Ethiopia, where 170 (41.9%) children were found to have single and double intestinal parasitic infections [23], but higher than study conducted in Gondar where the observed prevalence of intestinal parasites was 34.2% [24] and the study conducted in Gamo area where 342 (39.9%) study subjects were found positive for at least one intestinal parasite [25]. Our study prevalence result was lower compared with reports of other similar studies, 72.9% in Gondar, Azezo [25], 83% in Jimma [5] and 83.8% in South East of Lake Langano [26]. These variations in prevalence might be due to differences in climatic conditions, environmental sanitation, economic and educational status of parents and study subjects, and previous control efforts. The low prevalence of intestinal parasite in this study compared to

**Table 4 Resistance pattern of Shigella and Salmonella species**

| Antimicrobial | Shigella species (n = 6) | Salmonella species (n = 16) |
|--------------|--------------------------|-----------------------------|
| Ampicillin   | 6 (100)                  | 10 (62.5)                   |
| Amoxicillin  | 6 (100)                  | 16 (100)                    |
| Cotrimethoxazole | 6 (100)            | 5 (31.3)                    |
| Nalidixic acid | 1 (16.7)              | 2 (12.5)                    |
| Ceftriaxone  | -                        | -                           |
| Ciprofloxacin | -                      | -                           |
| Chloramphenicol | 1 (16.7)             | 3 (18.8)                    |
| Gentamicin   | -                        | -                           |
the other previous studies in Jimma (5) and elsewhere in the country (7) could be due to increased awareness of the community about personal and environmental hygiene from the continuous awareness creation and interventions made by the health science students from Jimma University during their practical training conducted in the field as well as in different health institutions.

Among helminthiasis, *Ascaris lumbricoides* (11.5%) was the most prevalent parasite and followed by *H. nana* and *T. trichiura*, whereas giardiasis was the leading infection among protozoan infections. Though the rate of prevalence is different the dominancy of *A. lumbricoides* is in line with the study result by Abayne in south Ethiopia [23] and with previous studies done in Assedabo (Jimma, Ethiopia), where *Ascaris lumbricoides* was the leading (56.4%) [5]. But different with study conducted in Gondar, where multiple infections (polyparasitism) occurred in 14 individuals or 4.6% of the total examined subjects and 13.5% of those who had intestinal parasites [32]. The difference could be due to geographical location or variation in study subject sample size.

Even though, the study was conducted in different age groups, our prevalence rate of 2.3% lowers than that a report by Ashenafi, 1983 (9%) [33] and 11.7% isolation rate reported by Asrat et al. 1999 at Tikur Anbessa, Ethio-Swedish children’s hospital [34], a report by Ayala (6.7%) in Harar [35] and a report 15.6% by Hiruy in Gondar [36]. The low isolation of *Shigella* in this study compared to the previous study in Jimma (14) could be due to increased awareness of the community about personal and environmental hygiene from the continuous interventions made by the health science students from Jimma University during their filed practice.

Epidemiological investigation of salmonellosis in developing countries like Ethiopia is difficult because of the very limited scope of the studies and lack of coordinated surveillance systems. The overall prevalence of *Salmonella* in this study was 6.2%. This is comparable with studies conducted in Ethiopia at different times, 4.5% in Addis Ababa [37], 6.4% in Addis Ababa [16], 4.5% in Addis Ababa [33] and higher than the findings reported by Asrat et al. 1999 (3.8%) in Addis Ababa [34] but lower than reported in Jimma (15%) [15]. Antibiotic susceptibility data to *Shigella* isolates showed that all isolates were resistance to ampicillin, amoxicillin, and cotrimoxazole. Similarly a study conducted in Awassa showed that all isolates were resistant to amoxicillin and ampicillin (12).

Table 5. Antibiogram of *Shigella* and *Salmonella* isolates

| Resistance pattern | Resistant isolates no. (%) |
|--------------------|----------------------------|
|                    | *Shigella* species (N = 6) | *Salmonella* species (N = 16) |
| Amp                | 1 (6.3)                    |                               |
| Ampx               |                           | 5 (31.3)                      |
| Amp, Amx           |                           | 1 (6.3)                       |
| Amp, Amx, C        |                           | 1 (6.3)                       |
| Amp, Amx, Sxt      | 4 (66.6)                   | 3 (18.8)                      |
| Amp, Amx, NA       |                           | 2 (12.5)                      |
| Amp, Amx, Sxt, C   | 1 (16.7)                   | 2 (12.5)                      |
| Amp, Amx, Sxt, NA  | 1 (16.7)                   |                               |

| Keys: Amp: Ampicillin, Ampx: Amoxicillin, C: Chloramphenicol, NA: Nalidixic acid, Sxt: Cotrimethoxazole. |
antibiotic resistance was documented against ampicillin (79.9%), tetracycline (86%), and cotrimoxazole (73.4%) by Yismaw et al. [38]. As reported isolation of Shigella species with high resistance to erythromycin (100%), Tetracycline (97.3%), and ampicillin (78.7%) in Addis Ababa [39]. High resistance against amoxicillin (100%) and ampicillin (100%) was also reported by Reda et al. in Harar [35]. Our Shigella isolates were highly susceptible to ceftriaxone, ciprofloxacin and gentamicin. Comparatively high rate of resistance to ciprofloxacin (8.3%) was reported in Gondar. In parallel to our result lower (2%) resistance rate was reported from Gondar [38]. Comparable to the study conducted by Daniel [39] in Addis Ababa where all Shigella isolates were susceptible to gentamicin. The low resistant rate of isolates to chloramphenicol (18.8%), could be that physicians stopped to prescribe the drug before long time a go and once again the strains started to become sensitive. All Shigella isolates were MDR (resistant to two or more drugs). Similar findings were seen in other studies in Ethiopia [12,38].

Infection with non-typhoidal Salmonella in infants and children commonly produces self-limited diarrhoea. Studies have indicated that antimicrobial treatment for uncomplicated gastroenteritis does not shorten the duration and severity of symptoms; in contrast, it may prolong fecal excretion, increase the risk of relapse, and result in the emergence of antibiotic resistance [40]. Nevertheless, if extra-intestinal complications occur, effective antimicrobial treatment is essential. Multidrug resistant phenotypes have been increasingly described among Salmonella species worldwide, according to the infectious disease report released by the WHO in 2000 [41].

In this study, Salmonella isolates showed high resistance to ampicillin and amoxicillin which is comparable with previous study done in Harar, Ethiopia where the highest level of resistance was detected to ampicillin (100%) and amoxicillin (100%) [35]. A relatively similar pattern of resistance (74 to 97.3%) was reported from other parts of the country [42] and outside [43]. Our study findings showed that all Salmonella species were susceptible to ceftriaxone and ciprofloxacin, which is inline with recently study results conducted in Nigeria where all isolates were susceptible to ciprofloxacin, and ceftriaxone [44]. Un like most previous study findings, in this study Shigella and Salmonella species showed low resistance level to chloramphenicol, this could be due abandoning of prescribing the drug by the responsible health personnel before a long time ago.

Conclusion

This study indicated that intestinal parasite and some enteric bacteria such as Salmonella and Shigella species are responsible for the majority cases of diarrhoea in children. The results of the present study suggested that antibiotics which were more commonly prescribed in the study area previously, like ampicillin, amoxicillin and cotrimethoxazole developed resistance to Shigella and Salmonella species and should not be used as empirical treatment of diarrhoea in children at least in the study area. Therefore, measures including health education, improvement of safe water supply, sanitation facilities and continuous monitoring of microbiological and antimicrobial surveillance is crucial.

Competing interests

The authors declared that they have no competing interests.

Authors’ contribution

GB: Participated from inception of the research question to design, analysis, interpretation and preparation of the manuscript. HT: - Participated in proposal development, analyzed the data, edit and wrote the manuscript for publication. Both authors have given final approval of the version to be published.

Acknowledgements

We would like to acknowledge study participants or their guardians who were volunteers to participate in the study. We are grateful to acknowledge Dr. Rene S. Hendriksen from Technical University of Denmark, National Food Institute, for giving constructive comment during preparation of the manuscript. We would like to acknowledge Jimma University for funding the research work and the Ethiopian Health and Nutrition Research Institute (EHNRI) for providing us control organisms.

Received: 2 December 2013 Accepted: 1 February 2014
Published: 5 February 2014

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