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Original Research Article

Laboratory confirmed shigellosis in Malagasy children

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ABSTRACT

Background: Diarrheal diseases are a major public health problem in developing countries with high mortality and morbidity rates, especially among children. *Shigella* species is the leading cause of paediatric bacterial diarrhea and shigellosis data are very scarce in Madagascar.
Methods: A 3 year retrospective study from January 2018 to December 2020, at the University Hospital Mother and Child Tsaralalàna laboratory was performed to assess the bacteriological and epidemiological characteristics of laboratory confirmed shigellosis cases.
Results: During the study period, 298 stool samples were examined, of which 48 (16.1%) were positive for *Shigella sp*. The mean age of infected children was 29.7 months, predominantly in the 24-59 months age group. The infection was found mainly in male children (56.2%). Most isolates of *Shigella* sp showed resistance to co-trimoxazole and amoxicillin. All the strains were susceptible to third-generation cephalosporins. Of the isolated *Shigella* sp, 14 strains were tested for species identification and serotyping, twelve of which were *Shigella flexneri* and two were *Shigella sonnei*. The most frequent serotypes were *S. flexneri* 1b and 2a.
Conclusions: This study found a *Shigella* sp positivity rate of 16.1%. This pathogen frequently infects infants age group. Bacteriology laboratory surveillance and a multicenter survey are essential to control the spread of drug-resistant *Shigella* and to monitor circulating strains and the burden of this disease. Awareness of water, hygiene and sanitation (WASH) and community water supply is also necessary to reduce this infection.

Keywords: Antibiotic, Laboratory, Paediatry, Serotype, Shigella

INTRODUCTION

Shigellosis or bacillary dysentery is a bacterial diarrhea due to bacteria of the genus *Shigella* sp.[1] The relatively high morbidity affects mostly children under 5 years of age.[2] The clinical symptoms are related to the invasive nature of *Shigella* sp. on the intestinal epithelium associated with a strong inflammatory reaction in the lamina propria. Additionally, to this invasive mechanism, toxin secretion leads to intestinal hypersecretion. These physiopathological mechanisms result clinically in a dysenteric syndrome with bloody stools, abdominal pain, tenesma and fever.[3,4] In older children and young people, the disease can be mildly symptomatic.[5,6] The diagnosis of shigellosis is based on the laboratory identification of *Shigella* by bacteriological examination. Coproculture remains the gold standard in the laboratory. Rarely, a blood culture is requested to diagnose shigellosis. Other biological tests like molecular biology are currently available for the detection of *Shigella* sp but they do not allow antibiotic susceptibility testing. The *Shigella* genus contains 4 species: *S. flexneri*, *S. boydii*, *S. dysenteriae*

and *S. sonnei*, which are classified into several serotypes according to the biochemical and antigenic characteristics of the bacteria.[7] *Shigella* sp is the second leading cause of death from infectious diarrhea in children after rotavirus and is the leading cause of bacterial diarrhea.[8] Furthermore, shigellosis in the past was very different from the current situation. In the past, *S. dysenteriae* was the commonest species responsible for severe disease and it has been replaced by *S. flexneri* in many countries.[9,10] The treatment is based on antibiotic therapy.[11]

Also the emergence of multidrug-resistant bacteria is threatening worldwide due to the irrational use of antibiotics both in developed and developing countries, so that the WHO established a global antimicrobial resistance surveillance to tackle these new problems and *Shigella* sp is one of its target pathogens.[12] Data on shigellosis in Madagascar are very scarce. This study aimed to describe the epidemiological and bacteriological profile of *Shigella* sp strains circulating in Antananarivo Madagascar.

METHODS

It was a 3 year descriptive retrospective study from January 2018 to December 2020, performed at the Mother and Child University Hospital of Tsaralalana (CHUMET) bacteriology laboratory. This 82-bed public pediatric referral hospital in the capital of Madagascar offers care primarily to local patients under 15 years of age, although there were some patients from elsewhere in the country.

All hospitalized and outpatient children who performed a stool culture at the CHUMET laboratory with a positive result for *Shigella* sp with all available results were included. Coprocultures positive for other pathogens were excluded. The laboratory logbook and patient records were used to collect data and the analysis was performed with EPI info v7.0. The studied variables were socio-demographic characteristics (age, gender, origin of the sample), bacteriological results (species and serotypes of *Shigella* sp isolated, susceptibility profile to antibiotics routinely used in practice).

Stool samples were plated on a selective culture medium Hektoen agar (Biokar Diagnostics, Allonne, France). A phenotypic method by the appearance of colonies was used for identification. The appearance of suspect colonies (lactose negative) on this medium was followed by the isolation of three isolated colonies on another Hektoen agar and simultaneously on a chromogenic agar Uriselect (BioRad, Californie, Etats-Unis). Lactose-negative colonies on Hektoen agar with a small white appearance on chromogenic agar were further tested by biochemical identification using the API 20E gallery (BioMérieux, Marcy l'Etoile, France). Antibiotics susceptibility testing of *Shigella* sp. were performed by Kirby Bauer disc diffusion method according to the current CASFM/EUCAST standard. The antibiotics tested were amoxicillin, amoxicillin/clavulanic acid, ceftriaxon, imipenem, gentamicin, amikacin, ciprofloxacin and sulfamethoxazole/trimethoprim. All the *Shigella* sp isolated strains were stored in a 20% glycerol-brain infusion (BHI) storage medium at -80°C. A total of 14 *Shigella* sp strains were tested for serological identification or serotype by slide agglutination according to the manufacturer's instructions (*Shigella* anti-sera, Eurobio scientific®, France) which enabled identification of *S. dysenteriae*, *S. flexneri*, *S. sonnei* or *S. bodyii* species and serotypes of each (Figure 1). The test kit included ready-to-use polyvalent and monovalent sera for the determination of serotypes belonging to each group.

RESULTS

During the study period, 298 stool specimens from patients under the age of 15 years were studied, of which 48 (16.1%) were positive for *Shigella* sp (Figure 1). The ages of the *Shigella* patients range from 8 to 96 months with a mean age of 29.7 months. The peak frequency was in the age range 24-60 months (Table 1). The sex ratio was 1.2. Twenty-eight (n=28, 58.3%) stool cultures were from hospitalized patients. Shigellosis cases in this study were all community acquired infections. The Kirby-Bauer disc diffusion method showed marked drug resistance of the *Shigella* sp strains to cotrimoxazole (93.7%), aminopenicillin (87.5%), amoxicillin-clavulanic acid (54.1%) (Table 2).

Table 1: Characteristics of patients with shigellosis.

Parameters	Number (N)	Percentage (%)
Age group (month)		
0-12	5	10.4
12-24	10	20.8
24-60	31	64.5
≥60	2	4.1
Gender		
Male	27	56.2
Female	22	45.8
Origin		
Hospitalized	28	58.3
Outpatient	20	41.6

Table 2: Susceptibility of *Shigella* sp to antibiotics.

Antibiotics	Susceptible	Resistant
	N (%)	N (%)
Amoxicillin	6 (12.5)	42 (87.5)
Amoxicillin/clavulanic acid	22 (45.8)	26 (54.1)
Ceftriaxon	48 (100)	0
Imipenem	48 (100)	0
Gentamicin	46 (95.8)	2 (4.1)
Amikacin	48 (100)	0
Sulfamethoxazole/ trimethoprim	3 (6.2)	45 (93.7)

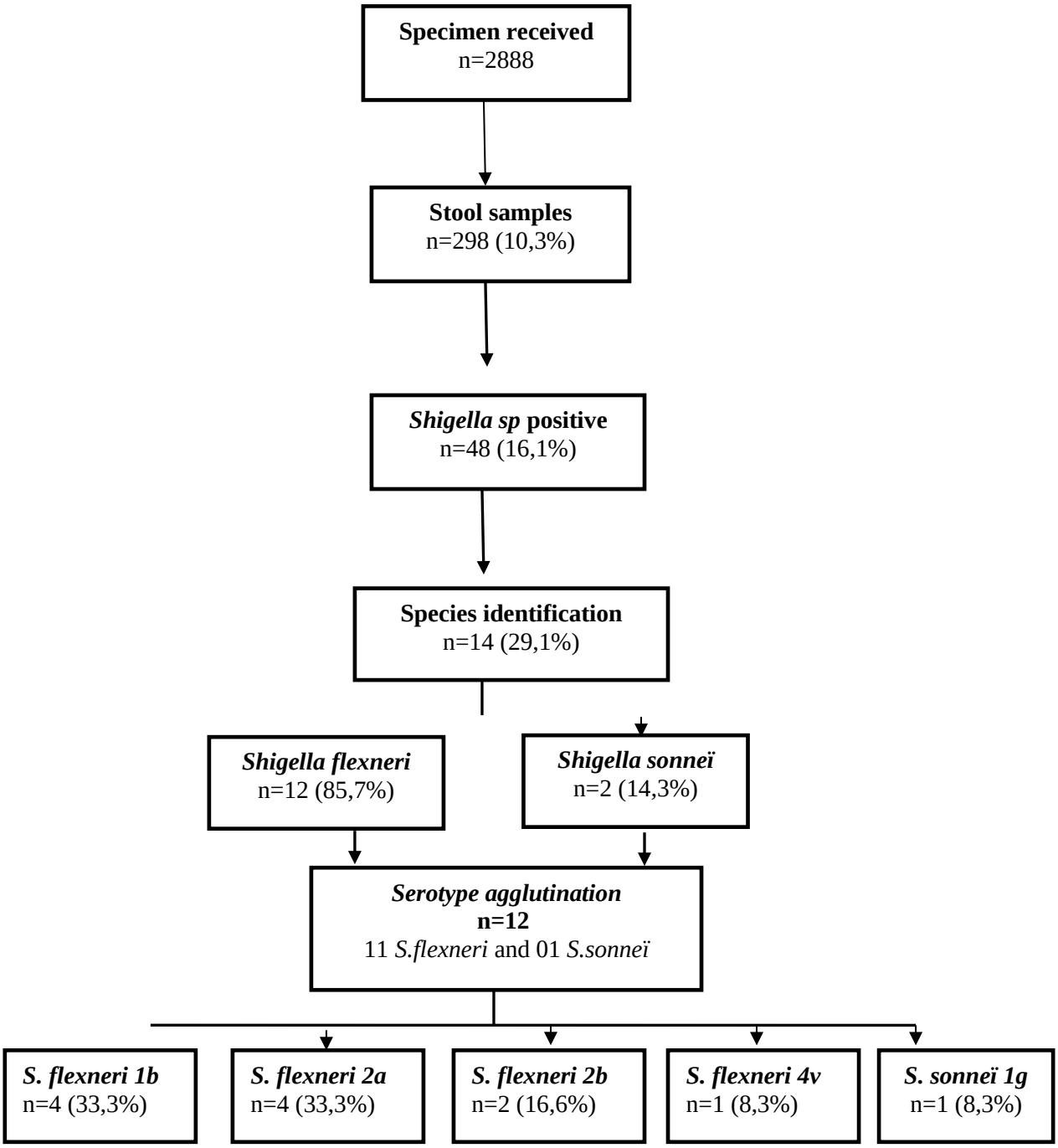

Figure 1: Shigellosis positivity rate, species distribution and serotypes of the studied strains.

No extended-spectrum beta-lactamase (ESBL)-producing strains were found. Fourteen *Shigella* sp strains were identified by species in this study, with a predominance of *S. flexneri* (n=12, 85.7%) and *S. sonnei* (n=2, 14.3%) (Figure 1).

DISCUSSION

Shigellosis or bacillary dysentery is a fecal peril disease caused by *Enterobacteriaceae* of the genus *Shigella* sp. This disease had been responsible for important epidemics in wartime. They currently persisted in endemic form in tropical countries, where they occurred frequently, particularly during the hot, wet season of the year.[13] During this study, two hundred and ninety-eight coprocultures (n=298) were performed in the bacteriology laboratory of CHUMET, which represented 10.1% of all samples received in the laboratory. Forty-five (n=45) were positive for *Shigella* sp, giving a positivity rate of 16.1%. This finding was lower compared with other studies in resource-limited countries like Nepal and Togo where the positivity rate was 52.2%, 47% respectively.[14-16]

This rate may be certainly underestimated, since coproculture was not routinely performed in all dysenteric syndromes, due mainly to the accessibility of this test in public hospitals, as well as to the high cost of the assay and to the lack of good laboratory capacity. The conventional bacterial culture remained the gold standard for the biological diagnosis of shigellosis. It also allowed for antibiotic susceptibility testing, which was particularly important in this era of antimicrobial resistance. The mean age of the children in our study was 29.7 months, with range of 8 and 96 months. Most of the children were less than 60 months of age (95.8%), with high frequency of positivity in the 24-59 months age range. Our results were consistent with other studies from 2017 and 2018 that reported a high frequency of shigellosis in this age group.[16,17] The vulnerability of children under 5 years of age to shigellosis might be due to their dietary behavior as well as a problem of sanitation and accessibility to safe water. According to the literature, children under 5 years of age are the principal targets of shigellosis and they were rarely infected before the age of 6 months if they were breast-fed.[12,18]

We found the predominance of males. This result was similar to other published studies reporting a male predominance.[15,16,19] Genetics factors could explain this male over female infectious predominance.

Shigellosis is a highly infectious disease.[20,21] The most common symptom of shigellosis was the dysenteric syndrome, which was manifested by afecal, frequent, glairy, bloody and sometimes mucopurulent stools, abdominal pain, epithelial discharge, tenesmus with false needs. The majority of the patients with shigellosis were hospitalized which was also found by other authors.[14,22]

The clinical manifestations of shigellosis may vary in different degree, they can be well tolerated by patients but they can lead to hospitalization because of complications which can be immediate or delayed, like dehydration with hydroelectric losses, neurological damage (convulsions, consciousness disorders), hemolytic uremic syndrome (HUS) and severe malnutrition.[12,18]

In contrast to other diarrhoeal diseases, the therapy for shigellosis cannot be treated by rehydration alone. The first-line treatment was based on antibiotics, which generally allowed a rapid recovery without sequelae.[11]

The classical therapy for Shigellosis consisted of the use of aminopenicillin-amoxicillin or cotrimoxazole, but since several years, a spread of drug-resistant strains had been reported.[12,23] Monitoring of disease incidence and the antimicrobial susceptibility of the strains were important for appropriate curative treatment and patient management. Acquired resistance was noted in this study with a high proportion of resistant strains to sulfamethoxazole/trimethoprim, amoxicillin and amoxicillin and clavulanic acid. On the other hand, all strains were susceptible to third-generation cephalosporin and imipenem, 95.7% and 91.3% to gentamicin and ciprofloxacin, respectively. Multiple drug resistance was observed in more than two-thirds of *Shigella* isolates. Lango-Yaya et al in 2017 from the Central African Republic reported a high level of amino penicillin and co-trimoxazole resistance of about 100%.[24] *Shigella* sp was group 0 in the beta-lactam susceptibility phenotypic classification. They were naturally susceptible to all beta-lactams and antibiotics used in practice. During the last half century, an alarming increase in antimicrobial resistance has been reported, especially in developing countries, where use of these medications is relatively limited. In fact, the extraordinary ability of *Shigella* to acquire plasmid-encoded resistance to antimicrobial drugs previously considered as first-line treatments had been demonstrated.[25] The spread of bacterial resistance was due to the irrational overuse of antibiotics in veterinary medicine. The use of azithromycin was currently recommended for the treatment of shigellosis 28 but this antibiotic was not tested in our study due to the non-availability of the disc from local suppliers.

The frequency of the different *Shigella* species varied in different parts of the world.[27,28] People living in resource limited countries had natural immunity to *S. sonnei* from exposure to feces contaminated water containing *Pleisomonas shigelloid* which had a similar O type antigen as this strain.[17,29,30] Also, *S. sonnei* was phagocytized by the *Acanthamoeba castellanii*, ubiquitous amoeba that phagocytizes *S. sonnei* in nature and provided an intracellular environment immune to the use of chlorination and other forms of sanitation processes. In contrast, *S. flexneri* was lethal to the amoeba *A. castellanii* and cannot have this protection.[31,32] Despite the numbers studied strains, *Shigella* serotypes isolated were *S. flexneri* 1b and *S. flexneri* 2a. *S. sonnei* serotype g. Knowledge of the serotype had no immediately impact on the management of the patient. However, it is critical for the monitoring of *Shigella* virulence, for epidemiologic surveillance and for vaccine development.

The limitation of our study was that it was carried out in a single pediatric site and therefore could not be generalised to the entire country. A perspective of a multicenter study would allow a continuous monitoring with representative data. All *Shigella* sp strains isolated from the stool culture were not tested for species identification. Some of the strains were no longer viable for further identification.

CONCLUSION

Shigellosis or bacillary dysentery is a public health problem with a high morbidity in the developing countries. Despite the limits of the number of sites and the number of tested strains in this study, it showed a picture of *Shigella* species and serotypes circulating and

the antibiotic susceptibility for treatment in Madagascar. Bacteriology laboratory has a crucial role in diagnosis and treatment of shigellosis cases as well as in epidemiological surveillance which needs to be ongoing and extend to other parts of the country.

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