International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 5, No. 6, p. 1-6, 2017

Open Access RESEARCH PAPER

First report of *Salmonella meningitis* in children during 2011-2015 meningitis surveillance in Niger

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Keywords: CERMES, Meningitis, Niger, *Salmonella,* Surveillance

Publication date: June 30, 2017

Abstract

Meningitis due to *salmonella* is a rare infection poorly or not reported from NIGER. The infection is a complication of *Salmonella* infection that occurs in infants. *Salmonella* is the fourth cause of meningitis in Niger children, after *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae b.* This retrospective study aimed to describe cases of *salmonella* meningitis in Niger infants. From January 2011 to December 2015, 6630 CSF collected nationwide were sent through an effective samples transport circuit already in place to allow the rapid delivery of thé CSF in CERMES, the National Reference laboratory-based meningitis surveillance for analysis. All turbid CSF from under 5 years patients were respectively cultured on Hektoen medium, Trypticase soy agar plate containing 5% sheep blood; and on chocolate agar plate. Gram negative bacilli were identified using API20 system. A total of 11/6630 (0.2%) *Salmonella* strains were isolated during the study. Most of the patients (72.7%) were less than 2 years old. The average age of children was 2.63 years with an extreme of 1 month to 14 years and the sex ratio M/F was 0.83. Of the eleven *salmonella* species detected, we found 7(63.6%) *Salmonella typhi*, 2 (16.7%) *Salmonella* spp, 1(8. 3%) *Salmonella paratyphi* A and 1 (8.3%) *Salmonella paratyphi* B. This study showed that *Salmonella* meningitis is a rare public health problem in Niger, despite the limitation on clinical symptoms data from patient case forms. Though, there was a risk which deserves to take *salmonella* in account in case of child meningitis.

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Introduction

Salmonella is a common causative agent, responsible for acute infections known as typhoid and paratyphoid fever. Salmonellas is a widespread food born disease encountered frequently in countries with sanitation problem. Transmission is through food contaminated with feces(Anon 2009). Salmonellas can also be transmitted through various types of vegetables and animal products (E.1982).

Some epidemiological reports indicate that poultry and pork are the main reservoir of *salmonella*. The disease is highly associated with septicemia in children and adult with gastroenteritis and cause diarrhea, fever, vomiting, abdominal cramps and intestinal inflammation. The infection may spread from intestine to blood stream causing bacteremia (Altun *et al*., 2014). Salmonellas is a public health problem. According to World Health Organization (WHO) each year, 550 million people are affected, including 220 million children under the age of five years.

The disease has a worldwide distribution. Only sporadic cases of typhoid occur where standards of hygiene are high although, occasionally localized outbreaks may develop from the contamination of water or milk supplies. Where hygiene condition is however low, vulnerable people such as infants, small children, and the elderly can be at risk. *Salmonella* infections can become very serious, leading to complications such as brain inflammation or meningitis (Khoshbakht *et al*., 2013).

However, meningitis due to *salmonella* is relatively uncommon but it is of importance because of high mortality rate (Watson, 1957). Ghon reported the first documented case of *Salmonella* meningitis in 1907. It is now recognized that meningitis can be a rare complication of *Salmonella* infection that occurs almost exclusively in infants (Hardy *et al*., 1984).

The disease is known to be the fourth cause of meningitis in children, after *Neisseria meningitidis* (Nm), *Streptococcus pneumoniae* (S.pn), and *Haemophilus influenzae* type b (H.i b). Though, there is limited data from Africa (Owusu-Ofori & Scheld, 2003), a meta-analysis of published African studies suggests that *Salmonella* bacteremia accounts for 21.4% of all bacteremia (Keddy *et al*., 2015). In NIGER, *Salmonella* meningitis was not reported because this bacterium is neglected or not considered by clinician as cause of meningitis. This may provide relevant information in considering *salmonella* as the fourth cause of meningitis and to laboratory technician not neglected *salmonella* in their diagnosis whenever Gram negative bacilli were found. This study aimed to describe cases of *salmonella* meningitis in infant from Niger.

Material and methods

This retrospective study was conducted in Center for medical and Health research (CERMES), the National Reference Laboratory for meningitis in Niger. Cerebrospinal spinal fluids (CSF) were collected nationwide from suspected cases by medical personnel and sent to CERMES through an effective samples transport network already in place for diseases surveillance. The CSF were collected in cryotubes (container) and/or in Trans-isolate transport medium (Ti) by health professionals across the country. CSF collected in cryotubes was transported in vaccine carrier boxes at low temperature (4°C to 10°C) and Ti were sent at room temperature. Each CSF collected was sent together with its notification form. All freshly collected CSF and Ti were subject to culture by standard bacteriological method for isolation and characterization of bacterial meningitis etiologies (culture, Gram, latex, antimicrobial sensibility). Refrigerated or frozen CSF was directly tested by polymerase chain reaction (PCR) method that was set to detect only Neisseria meningitidis, S.pn and Hib. Socio-demographic information and history of the infection were also collected and analyzed in the study.

Culture

All turbid CSF from under 5 years patients were respectively cultured on Hektoen medium, trypticase soy agar (TSA) plate containing 5%sheep blood; and on chocolate agar plate supplemented with hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD; V factor). Blood and chocolate agar plates were incubated in candle jar for 18-24 hours at 35-37°C in 5%-10% CO_2. Hektoen medium plates were aerobically incubated at 35-37°C also for 18-24 hours. All culture media were previously tested for growth promotion and sterility with reference strains as part of internal quality control.

Identification of Salmonella

Gramstain

Gram stain examination of grown colonies a rapid, was performed to have a rapid, presumptive identification of the causative bacterium. All Bacteria that appeared Gram Negative rod shaped bacilli after staining, were considered as suspect of *salmonella* and purified on nutrient agar plate after 18 to 24 hours period of incubation at 37°C. Suspected *salmonella* colonies were confirmed by biochemical reactions (indole production, lysine decarboxylase, carbohydrate fermentation) using 20E API kit (Biomérieux, Marcy l'étoile, France) and serotyped by slide agglutination with *Salmonella* antigen antis era (Mahalakshmi, 2013). The Analytical Profile Index (API 20E) identification test of *salmonella* species was performed according to user manual instructions given by the company. Then, agglutination test was carried out using a panel of *salmonella* antisera according to Kauffmann and White scheme (Difco™).

Result

A total of 6630 Cerebro-spinal fluids (CSF) were analyzed and identified through national laboratory based surveillance at CERMES. All the patients presented some of the presumption symptoms of meningitis such as fever, vomiting, rounded fontanel or convulsion depending on their age.

The prevalence varied among strains isolated and year of study. A Gram stain film of CSF showed many Gram negative bacilli. Culture resulted in the isolation and identification of *Salmonella* group. Eleven (11) *Salmonella* strains were isolated from CSF at CERMES from January, 2011 to December, 2015. The number of *salmonella* isolated was 1 (0.09%) in 2011, 2 (0.6%) in 2012, 1 (0.3%) in 2013, 6 (1.5%) in 2014 and 1 (0.02%) in 2015. These accounted for 0. 2% of the overall CSF received at CERMES during the 5 years of study. Of the eleven *salmonella* species detected, we found 7(63. 6 %) *Salmonella typhi*, 2 (16.6%) *Salmonella* spp, 1(8.3%) A and 1 (8.3%) *Salmonella paratyphi* B. Most of the patients (72.7%) were less than 2 years old. The average age of children was 2.63 years with an extreme of 1 month to 14 years and the sex ratio M/F was 0.83. The Table 2 above gave the distribution of *salmonella* with respect to age. Among the eight districts of patients' provenance, Maradi had the highest rate of *salmonella* (6/11, 54.5%), followed by Tillabery (3/11, 27.3%) then Zinder and Niamey the capital of Niger with 1 case (9.1%).

Table 1. Etiology of bacterial meningitis

Type of bacteria	Year of study					Frequency
	Numbers of Strains identified	2011	2012	2013	2014	2015	%
Nm	1910	408	29	11	26	1436	28.80
Sp	306	68	33	35	43	127	4.61
Hi	26	1	3	4	7	11	0.39
Sal	11	1	2	1	6	1	0.16
Negative	4377	550	224	248	314	3041	66.01
Total	6630	1028	291	299	396	4616	100

Table 2. Distribution of *Salmonella* with respect to age of patient

Organisms	Age groups			Total
	0-11months	12-59months	5 years and above	
S. typhi	4	1	2	7
S. paratyphi A	0	0	1	1
S. paratyphi B	1	0	0	1
Salmonella spp	1	1	0	2

The serotype prevalence was given by year and by region (Fig. 1). None patient was notified to be vaccinated against Salmonellosis and/or meningitis. The estimation of fatality rate may be difficult since most patients were not present in hospital during all the period of treatment.

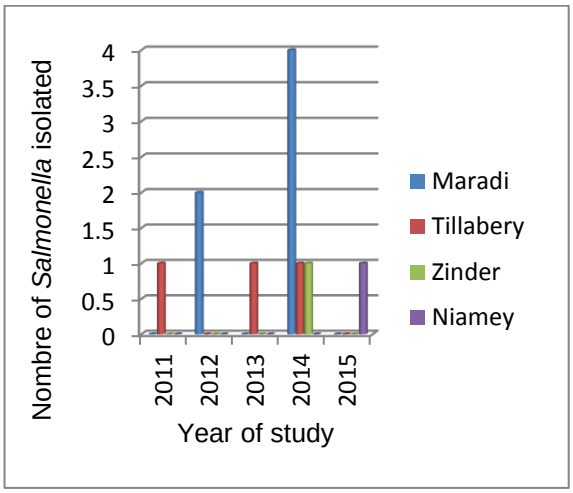

Fig. 1. Salmonella distribution per year and per region**.**

Discussion

This study revealed for the first time the implication of *salmonella* as cause of meningitis in Niger*. Salmonella* meningitis was often under notified or neglected during diagnosis by bacteriologist. This may probably due to the fact that *Salmonella* was mostly recognized as cause of typhoid fever; In any case of fever or headache, diagnosis was oriented towards malaria and typhoid fever by using Rapid and widal tests. Another reason may be the lethality rate. In the African meningitis belt, *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Heamophilus influenzae* were mainly known infectious agents responsible for meningitis (Kanchanapongkul, 1995). From this study, it was found only eleven (11) *salmonella* strains were isolated in routine meningitis surveillance during the five-year.

This account for (0.2%) of the overall isolates. The prevalence was very low comparing to the main microorganisms such as *Neisseria, Streptococcus* or *Haemophilus* known to cause meningitis (Christensen & Frederiksen, 1988). This confirmed that the disease was relatively rare as determined by other finding from Nigeria and Chad (Williamson & Murti 1990; Tabo *et al*. 2015). The highest prevalence (6.8%) of *salmonella* isolated in the course of study, was notified in 2014, followed by (2.9%) in 2012, (1.9%) in 2013 while the less prevalence was reported in 2011 (0.2%), and 2015 (0.06%). This relative prevalence show that the distribution of *salmonella* meningitis is all years round since the disease is a complication of endemic Salmonellosis in Niger.

In this study, *Salmonella typhi* has the highest prevalence 7/11 (63. 6 %); these finding were different from those found by, Djim- Adjim *et al* (2015) and L. Sangaré *et al* (2007) respectively from Chad and Burkina Faso; this may be due to the unavailability of typhoid fever vaccine in Niger since it's not free. The age incidence shown that infants less than 1 year (54.5%) were likely affected, which is in concordance with data from Nigeria. The reason for this age distribution was not clearly explained since study on *Salmonella* meningitis was yet completely understood (Watson, 1957; Singhal *et al*., 2012; Totan 2001). However, the role of breast milk in transmission of *Salmonella* is discussed (Cooke *et al*., 2009). Pregnant woman who develops *salmonella* infection may be a cause of meningitis for her baby (Unhanand *et al*., 1993). The immaturity of cellular immunity may also be evoked as responsible for the highest prevalence in this age group (Srifuengfung *et al*., 2005). The distribution of *salmonella* strain by region was not depended on the population rate.

However, Maradi came after Zinder in terms of population and the region had the highest malnutrition rate in Niger (Anon, 2015). So the prevalence of *salmonella* (6/11) may be due to mother carrier, sanitation, hygiene and alimentation problem. Vaccination against typhoid fever is not practiced in Niger, in routine preventive vaccination of infant less than 5 years, and vaccination against meningitis was performed only in case of epidemic according to the affected area.

Conclusion

Salmonella meningitis is a public health problem in Niger even though the disease is rare. This study showed that there was a risk which deserves to take *salmonella* in account in case of child meningitis. These results provided relevant information to clinicians, in considering *salmonella* as the fourth cause of meningitis and to laboratory technician not neglected *salmonella* in their diagnosis whenever Gram negative bacilli were found.

Acknowledgements

The author would like to express his appreciation to Dr. Y. Chaibou and ML. Moustapha for their contributions in the review of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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