

Salmonella Carriage among Patients in Fako Division, Cameroon: a cross-sectional study of its Prevalence and Associated Risk Factors

Abstract

Introduction: This study was aimed at evaluating the prevalence and the risk factors of Salmonellosis in patients who were consulted in some medical facilities in Fako Division of Cameroon.

Methods: A prospective cross-sectional study was carried out from November 2017 to November 2018 in three hospitals in Fako division of Cameroon; Tiko District Hospital, Mutengene Medical Center and Buea Regional Hospital. We enrolled 510 individuals presenting with symptoms of Salmonellosis, to whom a comprehensive was administered. *Salmonella enterica* strains were cultured from stool and identified using API 20E. Data was entered into Excel and imported into STATA v.12 for Windows, for statistical analysis. Odd ratios were calculated to determine the risk factors associated with Salmonellosis.

Results: Fifty *Salmonella enterica* strains were isolated giving a prevalence of 9.8%. Univariate analysis showed the following risk factors for Salmonellosis: area of residence; suburban $p=0.037$, OR=5.7 95% CI (1.1-30.03) and rural $p=0.077$, OR=2.3 95% CI (0.91-5.76), overcrowding (2 persons in a room) $p=0.047$, OR=2.3 95% CI (1.01-5.41); drinking tap-water, $p=0.032$ OR=0.38(.16-.092); auto-medication by buying drugs from the pharmacy, $p=0.079$ OR= 0.35(0.11-1.13) as being relatively significant risk factors.

Conclusion: The prevalence was found to be higher among the very young and older people greater than 45years. The risk factors identified in this study are: age, area of residence; overcrowding; consuming locally prepared yoghurt or Kosam; eating out or auto-medication by taking leftover drugs. These findings highlight the need of reinforcement of hygiene promotion especially in infants and overpopulated communities, educate on proper prescription and usage of drugs, in addition to the intensification of environmental interventions

Key words: *Salmonella*, carriage, risk factors, prevalence, Fako

Introduction

Salmonellosis continues to be a health problem worldwide causing 16 million illnesses globally and 600000 deaths [1, 4, 5]. It is primarily found in developing countries where sanitary conditions are poor [7, 8]. Globalization, international travel, and trade among countries facilitate the rapid global spread and transmission of food borne pathogens. This disease is now uncommon in developed countries where most occurrences are either acquired abroad or imported by emigrants [9].

The primary *Salmonella*-induced diseases in humans are gastroenteritis (caused by non-typhoidal *Salmonella*; NTS) and typhoid fever (caused by *S. Typhi* and the various *S. Paratyphi* pathovars). Infections with *S. Typhi* are responsible for approximately 21 million new cases of typhoid each year, globally [2, 3]. Annual mortality from typhoid is estimated to be >190,000 and has increased by 39% between 1990 and 2010 [1,3]. Although rarely encountered in western countries, typhoid is not a conquered disease; a recent analysis of global mortality data revealed that, in highly endemic regions such as Southeast Asia [10-11] and sub-Saharan Africa[11–13], the relative years of life lost to typhoid ranked similarly to those lost to breast cancer, prostate cancer, and leukemia in North America [3].

Despite this marked public health burden, little is known about the carriage, transmission of Salmonellosis or its risk factors in most parts of Cameroon. The vast majority of investigations which are on the susceptibility of *Salmonella enterica* species in this setting have been based on the animal sources of contamination [12, 13]. Other studies have been focused on bringing out the diagnostic possibilities of typhoid fever in concordance with the diagnosis of Malaria [14-16]. Studies which investigate on the sources of contamination of *Salmonella enterica* in humans and the risk factors involved are inexistent in this setting.

To direct public health interventions, we conducted a study to identify the carriage or disease burden and risk factors for developing a *Salmonella enterica* infection in patients in Fako division of Cameroon.

METHODS

Setting

Fako is a division of Southwest Region in Cameroon. The division covers an area of 2,093 km² and as of 2005 had a total population of 466,412 inhabitants and a density of 216 persons per square km. For the purpose of this study, participants were drawn from three hospitals found in the Division; Buea Regional Hospital, Tiko District hospital and Mutengene Medical Unit chosen because their geographical accessibility [17]. These hospitals

68 receive patients of various socio-economic statuses and are very diverse in the type of
69 services they render.

70 **Sampling**

71 We sampled following Lorrentz formula and with a prevalence of **8.7%** [20]. Based on these,
72 our minimum sample size was **122 patients** per site.

73 **Design**

74 We conducted a consecutive prospective based hospital study in which participants were
75 recruited for convenience. We identified cases from among patients who had been requested
76 to do a typhoid test from the symptoms they presented. We included patients of all ages who
77 presented with abdominal disturbances, nausea, vomiting, and fever. Stool and blood samples
78 were collected for culture at the Bacteriological Research laboratory of the Faculty of Health
79 Science between November 2017 and November 2018. We interviewed consenting patients
80 who had provided their samples priorly. We collected detailed information on the study
81 subject's drinking water, eating habits, hand washing habits, intake of antibiotics prior to
82 consultation. Interviewers asked participants to identify the initial symptoms associated with
83 their illness and the date when this symptom occurred. Interviewers next asked if the cases
84 had taken any antimicrobials in the 2 weeks prior to the onset of their first symptom. Because
85 reliable estimates of household income are difficult to obtain, we constructed two indices to
86 evaluate the relative wealth of the participants. We calculated the person-per bedroom ratio,
87 that is, the number of persons living in the household divided by the number of bedrooms.

88

89 **Laboratory Procedures**

90 To culture *S. enterica* species from stool, approximately 5g of sample was inoculated
91 immediately in Selenite F broth (Oxoid Oxoid, Basingstoke, United Kingdom) , and
92 incubated at 37 °C for 2-3 hours, the time it took to arrive at the research laboratory. They
93 samples were later subcultured on MacConkey's Agar (Oxoid, Basingstoke, United
94 Kingdom) and Salmonella enterica -shigella Agar.

95 To culture *S. enterica* from blood, 5 ml of venous blood from adults, and 1±2 ml from
96 children, was inoculated into 45 ml each of brain±heart infusion and thioglycolate broth, and
97 incubated at 37 °C for 7 days. Each bottle was examined daily for visual evidence of growth
98 and routinely subcultured to blood agar and MacConkey's agar plates (Oxoid, Basingstoke,
99 United Kingdom).

100 Non-lactose fermenting colonies on the MacConkay agar and black-decolorising
101 colonies on SS were biochemically identified as *Salmonella enterica* by using API 20E

(BioMerieux, Marcy l'Etoile, France). Serological identification was performed by slide agglutination using *Salmonella enterica* species specific antisera.

Statistical analysis

Our study sought to identify sources of contamination. Because specific foods and other exposures would be expected to be closely associated with each other, we controlled for confounding through a multivariate analysis. We placed all of the exposures with a P-value % of ± 0.05 on univariate analysis using a logistical regression to model to bring out the probability of being exposed with *Salmonella enterica* species. The dependent variable was whether or not *Salmonella enterica* species were present in the isolates of patients and the independent variables were age, area, location, water supply, auto medication, expiration date of drugs. The multivariable logistic model using χ^2 of Pearson test estimated the p-value of the whole model is 0.227, meaning that all predictors in the model might be approved with 5% level of confidence.

Results

The study included 510 persons, most of whom were outpatients 503 (98.6%), with 217(42.6%) males and 293 (57.5%) females. The median age was 25 years old. Data were collected in rural (4.3%), suburban (60%) and urban (35.7%) areas in the localities of Buea, Mutengene, Tiko1 and Tiko2. Most of patients were single (59%), other were married (35.1%) and the rest were either widowed (1.2%) or divorced (4.7%).

The prevalence of Salmonellosis was **9.8%**, that is, about 1 person in 10 is likely to be infected with *Salmonella* species. The prevalence is higher for patients aged between 0-20 and 46-82 than those aged between 21-45 years.

According to this survey, population of South-West region of Cameroon, living in the above-mentioned localities obtain drinking water from streams (7.7%), fountains (13.1%), CDC boreholes (29.6%), CDE taps (53.3%) and mineral water (14.3%) found on the market. *Folere* (57.7%) and *Kossam* or yoghurt (38.2%) are locally prepared drinks frequently consumed (**Table 1**).

134 **Table 1: Prevalence estimates of Salmonellosis among patients in Fako division of**
135 **Cameroon (N=510), 2018**

Characteristics	Frequency (%)	Prevalence of salmonella infection	
		Number of positive isolates	(% of positive)
Gender			
Male	217(42.6)	20	9.2
Female	293(57.4)	30	10.2
Age groups			
0-5	47(9.2)	7	14.9
6-10	47(9.2)	6	12.8
11-15	51(10.0)	7	13.7
16-20	52(10.2)	6	11.5
21-25	66(12.9)	3	4.6
26-30	55(10.8)	4	7.3
31-35	37(7.3)	5	13.5
36-40	40(7.8)	1	2.5
41-45	24(4.7)	0	0.0
46-50	25(4.9)	4	16.0
51-55	16(3.1)	3	18.8
56-60	11(2.2)	2	18.2
More than 60	39(7.5)	2	5.1
Marital status			
Single	301(59.0)	25	8.3
Divorced	6(1.2)	0	0.0
Married	179(35.1)	24	13.4
Widow/Widower	24(4.7)	1	4.2
Area of residence			
Urban	182(35.7)	11	6.0
Suburban	306(60.0)	36	11.8
Rural	22(4.3)	3	13.6
Site of Colleection			
Mutengene	33(6.4)	2	6.1
Buea	167(32.8)	15	8.9
Tiko1+Tiko2	310(60.8)	33	10.7
Roommates			
One per room	218(42.8)	17	7.8
Two per room	185(36.3)	24	12.9
More than two	107(20.9)	9	8.4
Source of Drinking Water			
Tap			
Yes	272(53.3)	24	8.8
No	238(46.7)	26	10.9
CDC			
Yes	151(29.6)	15	9.9
No	359(70.4)	35	9.6
Stream			
Yes	39(7.7)	2	5.1
No	471(93.7)	48	10.2
Fountain			
Yes	67(13.1)	5	7.5
No	443(86.9)	45	10.2

Mineral			
Yes	73(14.3)	8	10.9
No	437(85.7)	42	9.6
Other			
Yes	44(8.6)	3	6.8
No	466(91.4)	47	10.1
Locally prepared drinks			
Folere			
Yes	294(57.7)	29	9.9
No	216(42.3)	21	9.7
Kossam			
Yes	195(38.2)	19	9.7
No	315(61.8)	31	9.8
Hygienic conditions			
Wash hands			
Yes	337(66.1)	34	10.1
No	173(33.9)	16	9.3
Eat food outside			
Never= 1	21(4.1)	4	19.1
Occasionally	156(30.6)	19	12.2
always	333(65.3)	27	8.1
Drug consumption			
Auto medication			
Not=1	217(42.6)	23	10.6
Use leftover drugs	72(14.1)	7	9.7
Roadside vendors	40(7.8)	3	7.5
Drugstore	66(12.9)	11	16.7
Pharmacy	115(22.6)	6	5.2
Expiration date			
Never=1	184(36.1)	22	11.9
Sometimes	145(28.4)	10	6.9
Always	181(35.5)	18	9.9
Total	510	50	9.80

136

137 We brought out the following as risk factors for Salmonellosis: area of residence;
138 suburban OR=5.7 95% CI (1.1-30.03) and rural OR=2.3 95% CI (0.91-5.76), overcrowding
139 (> a person in a room) OR=2.3 95% CI (1.01-5.41) and OR=1.2 95% CI(0.43-3.28);
140 consuming locally prepared yoghurt or *Kossam* OR=1.52 95% CI (0.68-3.37); occasionally
141 eating out OR=2.15 95% CI(0.37-12.34) and daily eating out OR=1.13 95% CI (0.2-6.34);
142 auto-medication by taking leftover drugs OR=1.07 95% CI (0.32-3.55) and buying drugs
143 from the drugstore OR=2.39 95% CI(0.76-7.56 as being relatively significant risk
144 factors(**Table 2**).

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146

147 **Table 2: Prevalence and Odds ratios of risk factors for Salmonellosis in multivariable**
148 **logistic regression model (N=510), 2018.**

Characteristics	Prevalence (%)	Coef. (Std Err.)	p-value	Odds ratio (95% CI)
Age		-0.03 (0.01)	0.049	0.97(0.94-0.99)
Area				
Urban=1	6.04	1		1
Suburban	11.8	1.8(0.8)	0.037	5.7 (1.1-30.03)
Rural	13.6	0.8(0.5)	0.077	2.3 (0.91-5.759)
Area of Residence				
Mutengene=1	6.06	1		1
Buea	8.9	-0.2(0.9)	0.825	0.81(0.13-5.03)
Tiko1+Tiko2	10.7	0.1(0.9)	0.900	1.11 (0.21-5.95)
Number of persons in a room				
One per room =1	7.8	1		1
Two per room	12.9	0.9(0.4)	0.047	2.3 (1.01-5.41)
More than two	8.4	0.2(0.5)	0.739	1.2 (0.43-3.28)
Source of drinking water				
Tap (yes=1)	8.9	-1(0.4)	0.032	0.38(.16-.092)
CDC (yes=1)	9.9	-.3(0.5)	0.594	0.76(0.29-2.01)
Stream (yes=1)	5.1	-1.2(0.9)	0.194	0.29(0.046-1.86)
Fountain (yes=1)	7.5	-0.4(0.6)	0.522	0.68(0.21-2.18)
Mineral (yes=1)	10.9	-0.3(0.6)	0.588	0.74(0.24-2.21)
Locally prepared drinks				
Folere (yes=1)	9.9	0.5(0.4)	0.292	0.68(0.67-3.81)
Kossam (yes=1)	9.7	0.4(0.4)	0.302	1.52 (0.68-3.37)
Handwashing habit (yes=1)	10.1	-0.2(0.5)	0.656	0.8(0.32-2.05)
Eat food outside home				
Never =1	19.1	1		1
Occasionally	12.2	0.8(0.9)	0.392	2.15 (0.37-12.34)
Daily	8.1	0.1(0.9)	0.887	1.13 (0.2-6.34)
Auto medication				
No=1	10.6	1		1
Yes, Use leftover drugs	9.7	0.07(0.6)	0.906	1.07 (0.32-3.55)

Yes, from Roadside vendors	7.5	-1.2(1.1)	0.245	0.28(0.03-2.37)
Yes, from Drugstore	16.7	0.9(0.6)	0.136	2.39 (0.76-7.56)
Yes, from Pharmacy	5.2	-1.1(0.6)	0.079	0.35(0.11-1.13)
Look at the expiration date				
Never =1	11.9	1		1
Sometimes	6.9	-0.5(0.5)	0.270	0.58(0.22-1.53)
Always	9.9	-0.6(0.5)	0.203	0.54(0.21-1.39)
Intercept		-2.1(1.5)	0.166	0.13(0-2.34)
Number of observations =510				
LR Chi2(23) =37.65				
Prob > Chi2 =0.0277				

Discussion

The prevalence of Salmonellosis in South-West region of Cameroon, was **9.8%**, about 1 person in 10 is likely to be infected with *Salmonella* species. The prevalence is higher for patients aged between 0-20 and 46-82 than those aged between 21-45 years [20]. We also revealed that married people were the most infected, with 13.4% infection rate. Age was computed to have a negative significant ($p<0.05$) effect on the presence of *Salmonella enterica*, meaning that the older a patient is, the less likely is the possibility of contracting Salmonellosis.

The risk factors identified in this study are: age, area of residence; overcrowding; consuming locally prepared yoghurt or Kosam; eating out or auto-medication by taking leftover drugs

Area of collection is a significant factor in the prediction of the presence of Salmonellosis. Considering the area of residence, we singled out patients residing in suburban areas OR=5.7 95% CI (1.1-30.03) and rural OR=2.3 95% CI (0.91-5.76) like Tiko OR **1.11**(0.21-5.95). People of rural area(13%), suburban area (11.8%) have 2.3; 5.7 more odds respectively to be have Salmonellosis, when compared to those living in urban areas(6.0%).

Another relevant factor of *Salmonella enterica* infection is water supply. Drinking water from five of the most common sources in the locality was evaluated, and it revealed that water from the Cameroon development Corporation (CDC) catchment area has a lesser likelihood of contaminating its consumers with the Salmonellosis($p<0.05$). We computed that 8.8% of people who had their source of water to be tap water had Salmonellosis, 9.9% of those who consumed CDC water, 5.1% fountain, 7.5% streams, 10.9% mineral water, and

6.8% other sources such as wells. Even with the aforementioned positive cases for Salmonellosis, only those who drank water from the tap had a statistical significance of 0.03

We further evaluated overcrowding as a risk factor which significant statistically ($p<0.05$) computed from the number of people who actually sleep on a bed. It was measured as two or more people sharing a bedroom. We noticed that the risk is higher when at least two people share a room, OR 2.3-1.2. People who attested to eating out frequently had a slightly greater chance (OR=1.13 95% CI (0.2-6.34) of getting Salmonellosis in contrast to occasionally eating out with OR=2.15 95% CI (0.37-12.34). It is probable the hygienic conditions of the commercial food handlers [18, 19] is generally not optimal and have been reported as being vehicles for the transmission of Salmonellosis and these depends on the infective dose, in this case, the frequency of eating out

Lastly we note the consumption of drugs as being a risk factor. Auto-medication is a comparatively statistically significant factor ($p=0.07$) as it might increase the susceptibility to infections with the *Salmonella* bacilli. This can be attributed to the fact the drugs taken might not be of the correct type, potency and dosage and might further lead to resistance. However, auto medication using drugs from pharmacy reduces the probability of being infected. It means that in the case of auto-medication, drugs from the pharmacy are probably more reliable in the treatment against *Salmonella enteric*. It was noted that 42% of patients seek for consultation with a physician when they are ill and others auto-medicate, taking leftover drugs, or collect drugs and medicine from roadside vendors, drugstores and pharmacies. However, 36.1% of them do not verify the expiration date of drugs before taking them. In addition, *Salmonella* infection is very prevalent in patients who buy their drugs in a drugstore (16%). Patients who take left over drugs OR=1.07 95% CI (0.32-3.55) having a slightly high risk and those who buy from drugstores OR=2.39 95% CI (0.76-7.56 explained by the fact that consuming drugs without a consultation might mean not taking the appropriate drugs for the illness for which they suffer, or not taking the right dosage, drug not being stored under the right conditions amongst so many other reasons

Conclusion

As the prevalence *Salmonella enterica species* continues to increase, clinicians in countries caring for patients with presumed Salmonellosis are often forced to treat patients without a confirmatory diagnosis of Salmonellosis, we would encourage the usage of vigorous screening tools for the diseases' symptoms and further on, the usage of more sensitive tools for diagnosis such as blood and stool cultures.

We recommend policy makers and governments to accentuate on health education especially in schools. Health authorities should discourage the intake of drugs without appropriate medical consultation and also the purchase of drugs from uncensored sources.

Clearly, the best approach is prevention. Infrastructure and economic development is most effective and should be encouraged. Continued efforts to develop and distribute low-cost vaccines that provide earlier immunity to children as well as a better and longer duration of immunity may help alleviate the problem in the intermediate term. While awaiting these developments, immediate efforts to improve commercial food hygiene in our localities such as testing and vaccination of all food handlers

REFERENCES

1. Crump JA, Luby SP, Mintz ED (2004). The global burden of typhoid fever. *Bull World Health Org* 82: 346-53.
2. Lozano R, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012; 380:2095–2128. [PubMed: 23245604]
3. Gunn JS, Marshall JM, Baker S, Dongol S, Charles RC, Ryan ET. *Salmonella* chronic carriage: epidemiology, diagnosis, and gallbladder persistence. *Trends Microbiol* (2014). doi: 10.1016/j.tim.2014.06.007
4. Luby SP, Faizan MK, Fisher-Hoch SP *et al.* (1998) Risk factors for typhoid fever in an endemic setting, Karachi, Pakistan. *Epidemiology and Infection* **120**, 129-138.
5. Gasem MH, Dolmans WM, Keuter, M. ..and Djokomoeljanto R(2001), Poor food hygiene and housing as risk factors for typhoid fever in Semarang, Indonesia. *Tropical Medicine & International Health*, 6: 484-490. doi:10.1046/j.1365-3156.2001.00734.x
6. Mead P , Slutsker L , Dietz V, et al. Food-related illness and death in the United States, *Emerg Infect Dis*, 1999, vol. 5 (pg. 607-25)
7. Adabara NU, Ezugwu, BU, Momojimoh A, Madzu A, Hashiimu Z and Damisa D, (2012). The Prevalence and Antibiotic Susceptibility Pattern of *Salmonella enterica* typhi among Patients Attending a Military Hospital in Minna, Nigeria *Hindawi Publishing Corporation* .Volume 2012, Article ID 875419, 4 pages. doi:10.1155/2012/875419
8. Hendriksen RS, Mikoleit M, Kornschöber C, Rickert RL, Van Duyn S, Kjelso C, Hasman H, Cormican M, Mevius D, Threlfall EJ, Angulo FJ, Aarestrup FM. Emergence of Multidrug-Resistant *Salmonella enterica* Concord Infections in Europe and the United States in Children Adopted From Ethiopia, 2003–2007. *Pediatr Infect Dis J*. 2009 Sep; 28 (9): 814-818.

9. Rao RS, Amarnath SK, Sujatha S. An outbreak of typhoid due to multidrug resistant *Salmonella enterica typhi* in Pondicherry. *Trans Roy Soc Trop Med Hyg* 1992; 86: 204–5.
10. Nguyen-TA, Ha-Ba-K, Nguyen-TD. La fièvre typhoïde au sud du Viet-Nam, 1990–1993. *Bull Soc Pathol Exot* 1993; 86: 476–8.
4. Oh HML, Chew SK, Monteiro EH. Multidrug-resistant typhoid fever in Singapore. *Singapore Med J* 1994; 35: 59–601.
11. Coovadia YM, Gathiram V, et al. An outbreak of multiresistant *Salmonella enterica typhi* in South Africa. *Quart J Med* 1992; 82: 91–100.
12. Sherbini AE. An outbreak of typhoid fever resistant to chloramphenicol and other drugs in Gharbeya Governate in Egypt. *J Trop Pediatr* 1992; 38: 331–4.
13. Elshafie SS, Rafay AM. Chloramphenicol resistant typhoid fever: an emerging problem in Oman. *Scand J Infect Dis* 1992; 24: 819–20.
14. Nzouankeu A, Ngandjio A, Ejenguele G, Njine T, Wouafo MN. Multiple contaminations of chickens with *Campylobacter*, *Escherichia coli* and *Salmonella enterica* in Yaounde (Cameroon).. *Journal of Infection in Developing Countries*, Independent, 2010, 4 (9), pp.583–686.
15. Akoachere TKJ, Tanih FN, Ndip ML, Ndip NR: Phenotypic Characterization of *Salmonella enterica* Typhimurium Isolates from Food-animals and Abattoir Drains in Buea, Cameroon. *J Health Popul Nutr*. 2009, 27: 1-7
16. Njunda AL, Assob NJC, Nsaghac SD, Ndec FP, Kamga FHL, Njimbia CA, et al., Antibiotic susceptibility profile for *Salmonella enterica* in the Buea health district, *Sci J Microbiol*, 1 (2012), 97–100.
17. Ammah A, Nkuo-Akenji T, Ndip R, and Deas JE, An update on concurrent malaria and typhoid fever in Cameroon, *Trans R Soc Trop Med Hyg*, 93 (1999), 127–129.
18. Nsutebu, E. F., Martins, P., & Adiogo, D. Prevalence of typhoid fever in febrile patients with symptoms clinically compatible with typhoid fever in Cameroon. *Tropical Medicine & International Health: TM & IH*, 8(2003), 575–578.
19. <http://www.minsante.cm/site/sites/default/files/National%20Health%20Development%20Plan%202016-2020.Cameroon..pdf>
20. Feng-Ying LC, Becke JM, Groves C, et al. Restaurant associated outbreak of typhoid fever in Maryland: Identification of carrier facilitated by measurement of serum Vi antibodies. *J Clin Microbiol* 1988; 26: 1194±7.
21. Misganaw Birhaneselassie, David Williams. A Study of *Salmonella* Carriage among Asymptomatic Food-Handlers in Southern Ethiopia. *International Journal of Nutrition and Food Sciences*. Vol. 2, No. 5, 2013, pp. 243-245. doi: 10.11648/j.ijnfs.20130205.15