Original Research Article

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

Abstract – Give place and duration of study; leave the space before mentioning of unit

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 givng 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 – please correct the bacterial names – like *S.Typhi* is wrong make it *S.typhi*

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 – Make as Materials and Methods – mention the materials used

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

Sampling

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

Design

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

Laboratory Procedures – Give reference

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

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

Non-lactose fermenting colonies on the MacConkay agar and black-decolorising 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 - Khi^2 of Pearson test as Chi^2 ; please leave space before mentioning of unit

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 **Khi^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 – combine discussion to result only; please leave space before mentioning of unit

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.

133 *Folere* (57.7%) and *Kossam or* yoghurt (38.2%) are locally prepared drinks frequently
134 consumed (**Table 1**).
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138 **Table 1: Prevalence estimates of Salmonellosis among patients in Fako division of**
139 **Cameroon (N=510), 2018** - put these two as note below the table not in title

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**

We brought out the following as risk factors for Salmonellosis: area of residence; suburban OR=5.7 95% CI (1.1-30.03) and rural OR=2.3 95% CI (0.91-5.76), overcrowding (> a person in a room) OR=2.3 95% CI (1.01-5.41) and OR=1.2 95% CI(0.43-3.28); consuming locally prepared yoghurt or *Kossam* OR=1.52 95% CI (0.68-3.37); occasionally eating out OR=2.15 95% CI(0.37-12.34) and daily eating out OR=1.13 95% CI (0.2-6.34); auto-medication by taking leftover drugs OR=1.07 95% CI (0.32-3.55) and buying drugs

147 from the drugstore OR=2.39 95% CI(0.76-7.56 as being relatively significant risk
148 factors(**Table 2**).

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151 **Table 2**: **Prevalence and Odds ratios of risk factors for Salmonellosis in multivariable**
152 **logistic regression model (N=510), 2018.-** put these two as note below the table not
153 in title

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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 - combine this section with Result

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

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CONCLUSION

As the prevalence *S*almonella *enterica species* continues to increase, clinicians in countries caring for patients with presumed Salmonelosis 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

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