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Symptomatic and Asymptomatic Salmonella Infections

KEYWORDS: Salmonella; Symptomatic; Asymptomatic

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ABSTRACT *Patients infected by the Salmonella bacilli at the convalescent stage are usually asymptomatic and misdiagnoses may often occur. The distribution of Salmonella enterica organism in the samples of subjects at acute and convalescent stage was evaluated. A total of 765 subjects were enrolled (557 symptomatic and 208 asymptomatic). The total population of symptomatic and asymptomatic subjects that were positive for Salmonella enterica enterica var typhi/paratyphi was 269(48.0%) and 23(11.1%), respectively. The symptomatic and asymptomatic subjects blood samples yielded 107(19.2%) and 18(8.7%) isolates respectively, (p= 0.001), while the stool samples of the two groups yielded 90(16.2%) and 74(35.6%), P= 0.089. Definitive diagnosis is necessary for the control of typhoid fever. Absence of clinical symptoms in the endemic setting does not exclude the presence of the Salmonella enterica pathogens. Carriers and those in contact within household are usually at risk and require periodic screening and treatment*

INTRODUCTION

Typhoid and paratyphoid fevers are vastly disseminated systemic diseases commonly known as enteric fever. The disease is mostly endemic in the tropical Africa including Nigeria. High prevalence of carriers is a factor in transmission and spread of enteric fever (WHO, 2003). Typhoid and paratyphoid fevers which are diseases associated with poor personal and environmental hygiene and have largely been eliminated in many parts of the world by improved sanitation, but remains a significant health threat in developing nations (Bhan *et al.*, 2005).

Enteric fever incidence can conveniently be a barometer for gauging the environmental sanitation condition in a region or country. Incidence of typhoid decreases when the level of development of a country increases. Where these hygienic conditions are lacking, the probability of fecal contamination of water and food remains high and so is the incidence of typhoid (Santillana, 1991; Singh *et al.*, 1995). A greater population of this locality under study lack access to clean and safe water, the populace resort to untreated shallow well water from the ground, which may possibly have been contaminated by sewage. Recently, there has been a great surge and demand of commercially packaged sachet water to supplement the scarce water supply. This invariably results in high rate of consumption of such products. This too poses a threat and danger to health; as the hygienic condition and safety of such water cannot be attested for. The scarce supply of pipe-borne water which has remained the only source of drinking water is likely to be contaminated too through rusted and perforated pipes. There is a need to establish the actual index of these infections, especially in the endemic settings with high prevalence of the asymptomatic individuals.

MATERIALS AND METHODS

Subjects and Samples

The study was carried out within the urban and peri-urban cities of Enugu State in Nigeria. The analysis took place between February 2008 and September 2011. Samples were obtained from subjects living in the endemic area or directly exposed to carriers (contact cases). Criteria for selection of subjects included the provisional diagnosis of typhoid by the clinician and the symptoms expressed by the subjects. A total of 765 subjects were enrolled, made up of 557 symptomatic (symp.), 208 asymptomatic (asymp.)

Blood were drawn aseptically using 10 ml syringe and needle. About 8 ml of blood were collected from adult and 1-3 ml from each child into vacutainner (plain sterile 15 ml volume stoppered glass tubes). The blood was allowed to clot and after centrifugation the serum was separated from the clot. The serum was used for serological screening while the clot was poured into sterile universal containers and minced with scissor. Stool samples were collected in clean wide-mouthed containers with screw covers or sterile containers with wooden spatula.

Widal (Qualitative) Slide agglutination Test Method

Commercially prepared stocks of the Widal agglutination kits were stored at 2-8°C in the refrigerator. A drop of serum was placed into each row of circles on paper cards. One drop of each of the negative and positive control sera were dispensed unto two additional circles. One drop of the appropriate antigen suspension was added to each circle next to the sample to be tested. Next, the mixtures were rocked gently on the card for a period of two minutes. The reactions were observed immediately under a suitable light source for any degree of agglutination as positive reaction.

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Quantitative Widal (Tube Agglutination) Test
Six clean plain tubes were placed in racks in two rows and 0.9 ml of normal saline (0.85% sodium chloride) was delivered into the first tube in each row and 0.5ml into others. Next, 0.1 ml of the test sera were delivered into the first tubes and mixed. Quantities of 0.5 ml volume of the diluted sera from the first tubes were delivered into the second tubes to obtain two-fold dilutions. This procedure was repeated up till the last tube (tube 6) and 0.5 ml of the diluted sera was discarded from tube 6. (At this stage the serial doubling dilutions of 1 in 20 to 1 in 640 was obtained). Similar set-up were prepared for the positive and negative control sera. Then, 0.5 of appropriate antigen suspension was added to each (containing serum dilution). The two racks were shaken carefully to mix the antigens and the sera. Experiments with somatic O antigen were incubated at 50°c for 4 hours in water bath while those containing the flagella H antigen were incubated for 2 hours in the same temperature. After incubation the result of control tubes were read first by examining the pattern of the sediment and then shaking the tubes gently.

Bacterial Isolation: Processing of Blood Samples.
Blood clots were lysed by mincing with sterile scissors to free any salmonella organisms trapped in the clot. About 5 ml of the prepared bile salt broth were dropped into each of the containers. These were incubated overnight at 37°c for 18 to 24 hours. After this subcultures were made from each of the containers unto freshly prepared and dried Salmonella Shigella Agar (Biotec) and incubated at 37°c.

Processing of Stool Samples
The stool samples were first inoculated into the enrichment medium (Selenite–F broth) and after incubation for 24 hours at 37°C each was sub-cultured into Salmonella-Shigella agar (SSA). The plates were incubated overnight at 37°C and examined for growth.

Sugar fermentation tests
Bijou bottles containing the basal medium and appropriate prepared carbohydrate (mannitol, maltose, dulcitol, sucrose and glucose) were inoculated with drop of the nutrient broth suspension of the test isolate and were loosely capped and incubated at 35°C overnight. Each was observed for change in colour from amber to red and for gas production (in the medium filled inverted Durham tube).

Urease Test
The test organisms were inoculated heavily on the entire slope surface of the urea agar slants prepared in caped tubes. The tubes were placed in racks and incubated at 37°C up to 48 hours. Tubes were examined for change of colour from plain to pink.

Hydrogen Sulphide Production
Test organisms were inoculated into the triple sugar iron agar (Lab. M) slants contained in test tubes. These were incubated at 35°- 37°C for up to 48 hours. After incubation the Triple Sugar Iron agar media were checked for blackening and change in colour from amber to red at the bottom (butt) of the tube.

Serological Identification (Serotyping)
One to two loopfulls of the agar cultures were mixed with normal saline on clean microscope slides to form a paste. A drop each of the O and H polyvalent sera were added and further mixed with the organisms on the slide. Positive results were indicated by visible agglutination within 30 seconds. Slide tests were repeated for the positive cultures using single factor sera.

Statistical Analysis
This was carried out using SPSS version 15. Cross tabulation and two by two contingency tables format were used. Correlation coefficient and Pearson chi square was used to compare data at a probability value of 0.05 significant levels.

RESULTS
Colonies growths on the SSA, were morphologically positive for salmonella species when it showed 1-2 mm in diameter, appearing pale, colourless, translucent with some black centres. For sugar fermentation tests, each was observed for change in colour from amber to red and for gas production (in the medium filled inverted Durham tube). Each isolate that was positive for glucose, dulcitol and mannitol but negative for sucrose were also regarded as salmonella species. The urease negative appearing pale yellow were also suspected to be positive isolates. After incubation the TSI agar media were checked for blackening and change in colour from amber to red at the bottom (butt) of the tube. Additionally, production of gas (perforation) in the butt of the tubes gave the identity of Salmonella paratyphi serotypes. Positive result of slide tests with polyvalent and single factor sera were indicated by visible agglutination within 30 seconds. For Widal test titre of <1 in 20 were regarded as negative.

Among the 557 samples from symptomatic cases screened, distribution of sera positive for O and H antibodies at titre of ≥1:160 was as follows; 56 (10.1%) for *S. paratyphi* A, 35(6.3%) for *S. paratyphi* B, 30 (5.4%) for *S. paratyphi* C and 118 (21.2%) for *S. typhi* D. Similarly, among the 208 asymptomatic individuals screened, 7(3.4%) had O and or H antibodies against *S. paratyphi A*, 6 (2.9%) for *S. paratyphi* B, 3 (1.4%) for *S. paratyphi C* and 16(7.7%) for *S. typhi* D (Figure 1).

Table 1 shows that a total of 107 (19.7%) *S. enterica* organisms were isolated from the blood of symptomatic individuals and 18 (9.8%) from the asymptomatic; but from stool cultures, 90 (16.2%) were isolated from the symptomatic and 74 (35.6%) from the asymptomatic. There were no isolates obtained from the stool and blood samples of the control subjects. Table 2 shows the isolation rate of the salmonella organism from the stool and blood of subjects, 31(86.1%) were from symptomatic males and 5(13.9%) from asymptomatic males. Similarly, 34(87.2%) and 5(12.8%) were from the female counterparts, respectively. 32 (13.9%) and 52(61.9%) salmonella organisms were isolated singly from symp and asymp male stool samples. and from that of females 58(72.5 %) and 22(27.5 %). Stool samples of symptomatic and asymptomatic females had 72(92.3%) and 6(7.7%) isolates, respectively.

DISCUSSION
Blood and stool samples for culture were selected based on finding the clinical symptoms for enteric fever in patients. However, *Salmonella typhi D and paratyphi (A, B or C)* variants were isolated from apparently healthy individuals but exposed subjects.

Isolation of the Salmonella variants from blood samples of a significantly higher proportion of symptomatic individuals than asymptomatic ones. This is not surprising since the symptomatic cases were in the acute state of infection. Symptoms of the disease remain positive predictors for choosing which samples to culture in event of scarce

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resources or large number of samples requiring evaluation. This does not underrate the public health importance of isolation of the organism from apparently healthy individuals in an epidemiological survey setting. Most asymptomatic individuals showed no detectable anti-O and/or H antibodies or the antibodies were detected at low titres (1 in 20/1in 40) in some of the cases and could only be confirmed by isolation of the Salmonella organism from the stool or blood. This shows the unreliability of Widal test technigue in screening carriers. This fact is established in report that only about 70% adults formed antibody within the first week of infection (Colle *et al*, 1996). Furthermore, it is specifically stated that anti-O agglutinins are often detected after eight day of the disease while the anti-H agglutinins showed up after 10 days (Le Minor, 2006). It is not very clear why some individuals harboring the organism could not exhibit detectable antibodies or what factors suppressed expression of antibodies when they were provoked. Typically, antibodies to O and H antigens start appearing during the end of the first week and peaks at the end of the third week (Levine et al., 2001). As explained earlier, these organisms can be isolated at this early incubation stage but later manifest symptoms with the rising antibodies. The significance of these findings lies in the chances of missing these cases when Widal test method is employed exclusively for diagnosis of enteric fevers.

The prevalence of salmonella isolates from stools of the asymptomatic alongside those of the symptomatic subjects may suggest carrier status. Those positive with stool culture alone were likely to be chronic carriers. Such carriers may or may not have had history of typhoid fever. It was reported that up to 25% of established chronic carrier cases have no history of clinical enteric fever (Parry, 2008). Many people become carriers as a result of inadequate medication following diagnosis and self-medication, a practice which is prevalent in Nigeria. There was, however, significant difference in the rate of isolation from stool samples from the symptomatic and asymptomatic individuals. This implies that stool culture may also be of value for the diagnosis of acute infection; though not very specific. Some cases of typhoid infection were obviously transmitted through carrier contact as recorded in this study. About 1-5% of people who are infected with *S. typhi* become asymptomatic chronic carriers, also about 10% of untreated typhoid fever patients excrete the bacteria for 3 months after onset of symptoms (APHA, 2002).

CONCLUSION

Imperative and definitive diagnosis is necessary for the control of typhoid fever, especially in patients with confounding clinical feature. Absence of clinical symptoms in the endemic setting does not exclude the presence of the *Salmonella enterica* bacilli. Carriers and those in contact within household are usually at risk and require periodic screening and treatment.

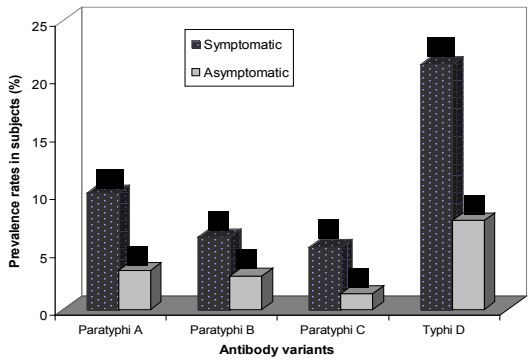

FIGURE 1
Distribution of Antibody Variants in Symptomatic and Asymptomatic Subjects (Titre ≥ 1/160)

TABLE 1
Distriution of Salmonella enterica in Blood Samples of the Sypmtomatic and Asymptomatic Subjects.

Subjects.	Nos.Screened.	Positive in stool (%)	Positive in blood (%)	Positive in stool/ blood (%)
Symptomatic	557(72.8)	90(16.2)	107(19.2)	65(11.7)
Asymptomatic	208(27.2)	74(35.6)	18(8.7)	10(4.8)
Total value	765(100.0)	164(21.4)	125(16.3)	75(9.8)
P value		0.035	0.001	0.001

TABLE 2
Distribution of Salmonella enterica in Samples of Symptomatic and Asymptomatic Subjects by Gender

Subjects	Stool pos (%)	Bld pos (%)	Stl/bld pos (%)	Total
Male (symp)	32(38.1)	35(74.5)	31(86.1)	98(58.7)
Male (asymp)	52(61.9)	12(25.5)	5(13.9)	69(35.9)
Total	84(50.3)	47(28.1)	36(21.6)	167(100.0)
P value	0.082	0.001*	0.001*	
Female (symp)	58(72.5	72(92.3)	34(87.2)	164(83.2)
Female (asymp)	22(27.5)	6(7.7)	5(12.8)	33(16.8)
Total	80(40.6)	78(39.6)	39(19.8)	197(100.0)
P value	0.436	0.005*	0.044*	

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