



Original Article

Detection of Salmonella species by Automated Blood Culture Method in Suspected Cases of Enteric Fever

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Abstract

Background: Enteric fever caused by *Salmonella* species is a systemic febrile illness that can be fatal since its characterization in 1880. Enteric fever may remain asymptomatic carrier and sometimes fatal that it can be difficult to diagnose based on symptoms alone because they are similar to those of other febrile disorders. In developing countries, enteric fever is common global health problem due to poor standard of personal hygiene, contaminated food, inadequate safe water and sanitation.

Methods: This is a cross-sectional study that was conducted in the Microbiology Department of Rajshahi Medical College Hospital for one year from January 2021 to December 2021 to identify *Salmonella* species by Automated Blood Culture (Fastidious Antibiotic Neutralization method) followed by subculture in MacConkey's agar, Xylose lysine deoxycholate agar, and *Salmonella Shigella* agar media in suspected enteric fever cases. This study included suspected cases of enteric fever as determined by the physician who fulfilled the inclusion and exclusion criteria.

Results: Among 127 suspected cases of enteric fever 64(50.39%) culture positive bacteria isolated by automated blood culture (FAN) method and out of that *Salmonella* species 38 (59.37%) and others bacteria 26(40.63%) following subculture in MacConkey's agar, Xylose lysine deoxycholate agar and *Salmonella Shigella* agar media. Regarding gender, the majorities (61.4%) of culture-positive patients were male, and the majority (65.35%) came from rural areas.

Conclusion: Especially for patients who have received antibiotics, we propose that the automated blood culture approach followed by subculture could be employed as a novel diagnostic method for typhoid fever.

Key words: *Salmonella* species, Fastidious Antibiotic Neutralization (FAN), Enteric Fever cases.

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Introduction

Typhoid fever remains a significant public health concern in developing nations.¹ *Salmonella typhi* and paratyphi are responsible for the disease. Both

belong to the family Enterobacteriaceae.² *S. typhi* is exclusive to humans and is transmitted via the fecal-oral route. Internationally, it is estimated that about 26 million cases of enteric fever and five million cases of paratyphoid infection result in

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215,000 deaths annually.³ The incidence of enteric fever is greater in low- and middle-income countries,⁴ Bangladesh, the Indian subcontinent, South and Central America, and Southern Africa are among areas where this is more prevalent than in affluent nations.^{1, 5-8} Since 1907, blood culture has been the gold standard⁹ for detecting enteric fever.^{10,11} The diagnoses of typhoid fever on clinical conditions presenting symptoms are diverse with other febrile sickness. Therefore, laboratory-based investigations are necessary for supporting the diagnosis of the condition. The gold standard for diagnosis of typhoid fever is the isolation of *Salmonella* species from suitable samples including blood, feces and urine. The most reliable method for diagnosing enteric fever is the isolation of the pathogenic bacteria in a patient's blood culture, which is typically positive within the first few days after sample collection. Culture is the most reliable way of detecting typhoid in infected patients, and usually via blood culture.¹² Widal test is most often used because to its low cost, especially in impoverished countries like Bangladesh; nonetheless, it does not have adequate sensitivity and specificity. Contrarily, the Fastidious Antibiotic Neutralization (FAN) technique demonstrates excellent sensitivity and specificity. Antibiotics and other sample inhibitors can be rendered inactive by the test.¹³ If a reliable diagnostic test were available, limiting typhoid fever diagnosis to patients with severe symptoms would significantly reduce diagnostic expenses without compromising accuracy techniques like FAN and molecular study, which might be

developed at Rajshahi Medical College Hospital, would be useful for future investigation.

Materials and Methods

This was a descriptive cross-sectional study conducted in the Department of Microbiology at the Rajshahi Medical College. The study lasted from January through December of 2021. The study population consisted of clinically suspected cases of enteric fever from both the inpatient and outpatient Medicine departments at Rajshahi Medical College Hospital, Rajshahi, during the study period. A total of 127 patients were enrolled in this study, which used a strategy of purposeful sampling. Out of 127 automated blood culture positive cases for identification *Salmonella* spp (N=64). Culture positive *Salmonella* spp. on different media (N=38). Identification of others bacteria by automated blood culture positive cases (N=26). Clinically suspected patient complaints of step ladder pattern fever lasting from 3 to 7 days with or without myalgia, fatigue, constipation, psychosis and other symptoms by the physician. Patients of both sexes 18 years and above were considered. Those who were unwilling to participate or showed symptoms of a different sickness were not included. Patients with enteric fever who were admitted to Rajshahi Medical College Hospital or visited the hospital's outpatient clinic were interviewed face-to-face using a semi-structured questionnaire to collect data. The Rajshahi Medical College Hospital's Institutional Review Board and the relevant authority approved the study protocol prior to its implementation.

Results

Total number 127 suspected cases it was identified *Salmonella* spp 38 (59.37%) and 26 (40.63%) others bacteria. Regarding other bacteria by automated blood culture positive cases, out of 26 population 16 (61.53%) *E. coli*, 5 (19.23%) *S. aureus*, 4 (15.38%) *Pseudomonas* spp. and 1 (3.86%) *Proteus* spp were found. Concerning culture positive *Salmonella* spp. on different media it was found that 38 (100.0%) cases positive on conventional culture, 38 (100.0%) found positive on XLD media and 38 (100.0%) found positive on SS media (Table I-III).

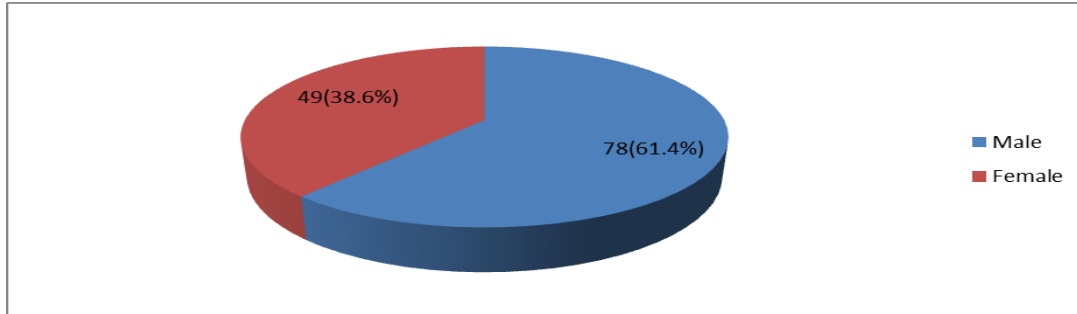


Figure -I: Distribution of the respondents on sex (N=127).

In Fig-I, distribution of study population on sex showed that 78 (61.4%) were male and 49 (38.6%) were female.

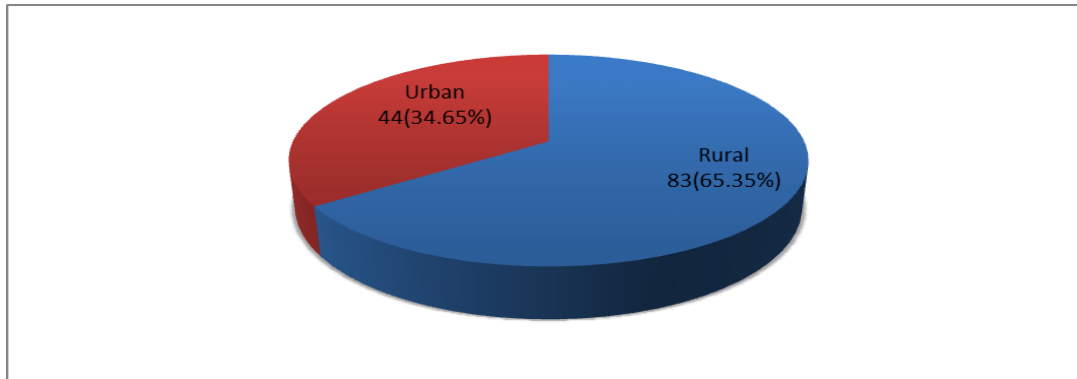


Figure -II: Distribution of the respondents on residence (N=127).

It was generated that most 83 (65.35%) of the population were from rural area and the rest 44 (34.65%) were from urban area.

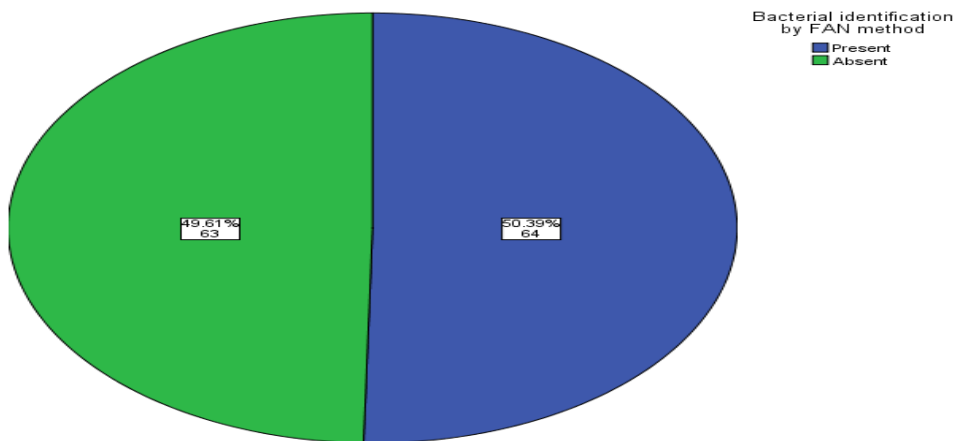


Figure- III: Bacterial identification by automated blood culture (N=127).

Automated blood culture were found positive in 64 (50.39%) population and negative were 63 (49.61%) of population.

Table 1: Distribution of automated blood culture positive cases for identification *Salmonella spp* (N=64).

Identification of <i>Salmonella spp</i> from automated blood culture positive cases (N=64)	Population	
	No.	%
<i>Salmonella spp</i>	38	59.37
Others bacteria	26	40.63
Total	64	100.0

Table 2: Distribution of other bacteria by automated blood culture positive cases (N=26).

Other bacteria by automated blood culture positive cases (N=26)	Population	
	No.	%
<i>E. coli</i>	16	61.53
<i>S. aureus</i>	5	19.23
<i>Pseudomonas spp.</i>	4	15.38
<i>Proteus spp.</i>	1	3.86
Total	26	100.0

Table 3: Distribution of culture positive *Salmonella spp.* on different media (N=38).

Number of <i>Salmonella spp.</i>	Conventional culture		XLD		SS	
	No.	%	No.	%	No.	%
	38	100.0	38	100.0	38	100.0

Discussion

Fastidious Antibiotic Neutralization (FAN) was used in this investigation to identify *Salmonella* species in instances of probable enteric fever at Rajshahi Medical College Hospital. Figure I also revealed that men made up a little larger proportion 78 (62.42%) of the population than women did. In this research, males were found to have a higher incidence of enteric fever than females. This was similar with the study of in 2014 (60.19%) in Vietnam and in 2016 (61.8%) in Laos.^{14, 15} Similar to this study was one conducted in 2012 that found that males whose jobs required them to travel around the country were more likely to contract enteric fever.¹⁶ A 2012 research found that 51.7% of Cambodians are female; this statistic differs from the South Asian average mostly

because more women in Cambodia are self-employed there.¹⁷ In this study, most 83 (65.35%) of the population were from rural area (Figure II). Similar results were reported by Divyashree et al. (2016), 70.28%, and Gaiind et al. (2006), 61.3%, both from India.^{18, 19} Our findings are consistent with those of a 2016 survey done in the USA, which also indicated that the majority of the population now resides in metropolitan areas (60.89%). The majority of people in a country like Bangladesh, which is still developing, reside in rural areas.¹⁵ The current investigation found that out of 127 samples, 64 (50.39%) were culture positive, which is quite comparable to the results observed by Saleh et al. (2008) in Bangladesh (48.19%), Gautam et al. (2002) in northern India (37.7%), and Maharjan et al. (2008) in Nepal

(48.96%).^{20,21,22} Comparatively, a 2018 study in Bangladesh found an isolation rate of 63.33 percent, which was significantly higher than the present study.²³ This high rate of isolation may be due to the prevalence of automated blood culture techniques. Blood culture positivity rates among febrile cases have been reported to be 8.5%, 5%, and 4.2% in Vietnam, 5%, and 4.2% in Delhi, and 4% in Egypt, respectively, despite diverse population-based experiences of the occurrence of typhoid fever in these countries.^{24, 25} From the total of 64 culture-positive samples, subculture and biochemical testing positively identified 38 (50.37%) as *Salmonella* spp. (Table II). In another study, 2009 in Pakistan a total of 29 (51.6%) cases were found *Salmonella* spp.²⁶ From a total of 131 culture-positive individuals, this finding was comparable. But the rate of positive cases were dissimilar with the study of Qureshi *et al.*, 2020 in Pakistan and their culture positive *Salmonella* spp. rate was 40.18%.²⁷ Possible causes include anaerobic bacterial infection, the presence of additional blood-borne organisms besides *Salmonella* spp., and sloppy blood collection and culture techniques. Out of 26 positive blood culture results for additional bacteria, it was discovered that 16 (61.53%) were *E. coli* and 1 (3.86%) were *Proteus* spp (Table III). The results of the current investigation were strikingly comparable to those of a 2013 study (60.9%) that discovered *E. coli* in Bangladesh.²⁸ Other investigations by Divyashree *et al.* (2016) and Gaid *et al.* (2006) reported finding *Proteus* spp. at 5.24% and 2.19%, respectively.^{18,19} Regarding culture-positive *Salmonella* spp. on various media, 38 (100.0%) cases were positive on conventional culture media, 38 (100.0%) cases were positive on XLD media, and 38 (100.0%) cases were positive on SS media. Gomes *et al.*, 2017 in Spain conducted a study and which was similar with the findings (91.15%) in conventional culture media. Positive results for *Salmonella* spp. were reported to be 92.9% on XLD media and 89.3% on SS media, which is consistent with a study conducted in Pondicherry, India.^{29, 30}

Conclusion

This investigation looks at how well the FAN technique works as a diagnostic test for *Salmonella* species in enteric fever, how the search for better diagnostic tests is going, and how hard it is to find new perfect tests. Nonetheless, these findings will provide the basis for further studies. So, it is essential to either find cheaper and easier ways to do this or change these technologies into something easier to use. Because it is easy to use and doesn't cost much, the FAN method is the best way to find *Salmonella* species in areas where they are familiar. Finally, albeit it is more complicated, future work should include establishing diagnostic tools for identifying healthy/asymptomatic chronic carriers of these human-specific diseases.

Conflict of interest: None declared

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