Review Article

The laboratory diagnosis of enteric fever

John Wain[1], Salih Hosoglu[2]

[1]*Wellcome Trust Sanger Institute, Hinxton, United Kingdom*
[2]*Dicle University Hospital, Diyarbakir, Turkey*

Abstract

The diagnosis of enteric fever currently depends upon the isolation of *Salmonella* from a patient, most commonly by blood culture. This facility is not available in many areas where the disease is endemic. Serodiagnosis depends upon the 100-year-old Widal test, and other serological diagnostic tools have limitations because of their low sensitivity and/or specificity. The most promising recently published results are from PCR-based amplification of DNA from the blood of enteric fever patients but again this technique is not available where it is most needed. Antigen detection has not been investigated for well over three decades and detecting an immune response specific for typhoid fever has been done only with antibody detection. There is an urgent need for the rational design and evaluation of effective and appropriate diagnostics for enteric fever which must include the emerging threat of *S.* Paratyphi A.

Key Words: Laboratory diagnosis, typhoid fever, enteric fever, *Salmonella* Typhi, serology, molecular tests, PCR diagnosis

J Infect Developing Countries 2008; 2(6):421-425.

Received 16 July 2008 - Accepted 20 August 2008

Introduction

The World Health Organization (WHO) estimate for annual global incidence of typhoid fever, about 21 million cases [1], is probably an underestimate because of poor diagnostics. Several options exist for diagnosing enteric fever: clinical signs and symptoms; serological markers; bacterial culture; antigen detection; and DNA amplification. None is entirely satisfactory. The clinical diagnosis of typhoid fever is difficult because the manifestations of the disease are diverse [2] and there are many causes of prolonged fever in typhoid endemic regions [3]. Signs such as relative bradychardia or leucopoenia may be useful [4] but give a low specificity. The culture of blood, bone marrow and stool are the most reliable diagnostic methods but these are expensive techniques and the infecting organism may be dead on arrival at the hospital if the patient has taken antibiotics before clinical samples can be taken. Serological diagnosis is predominantly by the Felix-Widal test, first standardised in the 1950s, [5]. Although ELISA [6] and immunoblotting [7] suggest possibilities, the commercially available kits for the serodiagnosis of enteric fever have not performed well in large studies [8]. There has been very little commercial interest in developing antigen detection tests.

Diagnosis by culture

Culture of the causative organism remains the most effective diagnostic procedure in suspected enteric fever and where culture is available typhoid fever may account for two thirds of cases of community-acquired septicaemia admitted to hospital [9,10]. Blood has been the mainstay of culture for *S.* Typhi since 1900. In 1907 Coleman published the first review of blood cultures in typhoid fever [11] and recommended the use of ox bile broth. In 1911 its superior qualities were attributed to the inhibition of the antibacterial activity of fresh blood caused by lysis of blood cells rather than direct enhancement of growth by the bile salts [12].

Reports of the evaluation of different blood culture media suggest that ox bile broth is superior to rich nutrient media, for the isolation of *S.* Typhi from blood, even when compared to modern blood culture media [13]. While useful for studies on typhoid fever, this media has not found widespread use in general diagnostic laboratories because only bile resistant organisms can be isolated. The addition of saponin to modern blood culture media allows blood to be lysed without inhibiting bile sensitive bacteria; this method was developed in the late 1980s [14], but is not well reported. The isolation of *S.* Typhi from the bone marrow is considered to be the gold standard method

for the diagnosis of typhoid fever and is reported as more sensitive than blood culture by most, [15,16] but not all, authors [17]. There is a larger number of bacteria found in the bone marrow, tenfold more per volume than in blood, and they may be protected from the presence of systemic antibiotics [18]; however, if enough blood is cultured it may be possible to increase the sensitivity of blood culture to that of bone marrow culture. The use of the blood clot from serum collected for serology is possible but the experience of the authors (JW) is that contamination is problematic and that buffy coat collection provides a higher level of concentration. Stool culture is also an important adjunct for diagnosis; it may be positive when blood culture is negative [19] and it is also important for the monitoring of carriage of *S.* Typhi after apparent clinical cure, a risk factor for the families of cases [20].

Enrichment media containing selenite are used to isolate *S.* Typhi from stool because of very large numbers of competing bacteria, especially *Escherichia coli* [21, 22]. Other sites have been cultured but are not used routinely as diagnostic specimens: culture of the upper gastrointestinal tract using a duodenal string can be valuable but the technique is poorly tolerated by young children [23]. *S.* Typhi can be grown from rose spots [24] but rose spots are often difficult to see and may only be present in 4% of cases [25]. *S.* Typhi can also be grown from urine but may be associated with urinary tract infection rather than typhoid fever [26].

Antibody detection tests (serology)

The Widal

The Widal agglutination test, suggested by Widal more than one hundred years ago for the diagnosis of typhoid fever [27,28], detects serum antibodies to the O=9,12 somatic, the H=d flagellar, and the "Vi" capsular antigens of *S.* Typhi. The interpretation of the Widal test remains problematic to this day, with a great number of articles reporting different cut-offs [29] and the test has lost some popularity in recent years as antigenic determinants of both typhoid and non-typhoid *Salmonella* organisms are now characterised.

In many places, instead of the standard Widal test, a quantitative slide agglutination test [30] is used but this should always be interpreted with reference to clinical data. According to the original papers, a rise in titre over time or a single high test result is diagnostically significant and this is supported by modern studies using ELISA. False negative results may occur if the blood is collected too early in the disease; therefore, negative results do not rule out typhoid fever [31] and may be best used as a baseline for subsequent comparative titrations [31]. False positive results may be associated with a past history of immunization for typhoid fever, cross-reacting antibodies [6], or a whole host of infections and conditions.

The detection of Vi antibodies can be used for detection of carriers during specific investigations [32,33] but is not routinely performed in most diagnostic laboratories and the use of a Vi Widal reagent using tube agglutination has not been well reported.

Haemagglutination (HA) Tests

Many researchers have evaluated the usefulness of HA tests in different countries. In a study from India, the anti LPS HA test showed a sensitivity of 60% and specificity of 98.2%. The positive predictive value and negative predictive value were 66.7% and 96.7% respectively. In the same study, the haemagglutination inhibition test targeted *Salmonella* antigens and was found useful for helping the early detection of S. Typhi in culture [34]. In another study, a Reverse Passive Haemagglutination Test (RPHA) was designed for the detection of *S.* Typhi antigen. The test was found to be 70% sensitive and 92% specific for acute typhoid fever diagnosis [35]. These studies indicate that the passive HA test is comparable with the Widal test and may be a useful alternative to the Widal test for the serological diagnosis of typhoid fever in busy microbiology laboratories in areas in which the disease is endemic [36].

Countercurrent Immunoelectophoresis (CIE)

This test is based on electrophoresis and the visualization of the precipitin band of antigen-antibody complexes that form. The sensitivity is similar to that of the Widal test and the procedure may be quicker if tests are batched (about one hour for a gel), but bands are often difficult to see, the cost is higher than that of the Widal, and some studies conclude that CIE has a low sensitivity with Vi antigen. A panel of antigens (somatic (O), flagellar (H) and capsular polysaccharide (Vi) antigens of Salmonella typhi) is recommended for rapid diagnosis of typhoid fever [37].

Rapid tests

The clinical application of a dot blot test to detect IgG (88% sensitivity and specificity) and IgM (12.1% sensitivity and 97% specificity) against the flagellar antigen from *Salmonella enterica* serovar Typhi has been performed in Peruvian and Colombian patients

with 100% specificity [38]. The TyphiDot is a DOT enzyme immunoassay (Typhidott and Typhidot-Mt; Malaysian Biodiagnostic Research SDN BHD, Kuala Lumpur, Malaysia) that detects either IgM or IgG antibodies against a specific antigen on the outer membrane protein of serotype Typhi. This test is designed for the rapid diagnosis of typhoid fever even in areas with limited resources. Some studies showed that the Typhidott and Typhidot-Mt gave superior results to the Widal test in their diagnostic sensitivity and specificity [39] but a large study of over 6,000 cases of fever found no advantage over the Widal test and reported problems with the quality control of different batches of kits [8]. A dipstick assay that was developed for use in developing countries has been trialed and, although superior to the Widal, again lacks either sensitivity or specificity (sensitivity 77%, specificity 95%) (58% and 98.1%) [40]. The advantages of the dipstick assay are that the result can be obtained on the same day, allowing a prompt treatment; only a small volume of serum is needed; no special laboratory equipment is needed to perform the assay; and the reagents remain stable when stored at room temperature [41].

TUBEX (IDL Biotech, Sollentuna, Sweden) is a semiquantitative test that uses polystyrene particle agglutination to detect IgM antibodies to the O9 antigen. TUBEX detects antibodies from a patient's serum by the ability of these antibodies to inhibit the binding between an indicator antibody-bound particle and a magnetic antigen-bound particle. The TUBEX test uses a colorimetric reaction which may be masked in hemolyzed samples, and false positives may occur in persons with recent *S.* Enteritidis infection [42]. Of the currently available commercial kits trialed in developing countries, TUBEX seems to perform best [43] but none may actually be better than the 100-year-old Widal test [8]. It is a very rare study that reports specificity and sensitivity above 95%. This means that at least 1 out of 20 patients is misdiagnosed; surely in the 21st century this is unacceptable for such a widespread disease as enteric fever.

Antigen detection tests

There is clearly a demand for a simple diagnostic test for enteric fever. An ideal test is reliable, simple, and affordable for the countries where the need is greatest. Many of the affected countries are poor, and some places do not have electricity. Perhaps the under-explored antigen detection, rather than antibody detection, could provide such a test.

Protein antigens and Vi

S. Typhi antigen can be detected in the urine of some typhoid patients by co-agglutination [30] and ELISA [44,45] but specificity varies from 25-90%. Testing of urine during the first week of fever onset for Vi antigen using the ELISA with a monoclonal Vi capture antibody detects most patients with typhoid fever [46].

DNA detection tests

Given the problems associated with the diagnosis of enteric fever by both culture and serological techniques, many authors have explored the use of PCR for detecting specific DNA sequence in clinical specimens from patients. The food industry has used PCR technology for several decades and guidelines are published for quantitative detection of *Salmonella* in food by PCR [47]. While there are several very good studies looking at the detection of *S.* Typhi DNA from clinical material, medical science is not at the same level of standardisation as the food industry, and there is very little published data on the detection of *S.* Paratyphi A. Below is a survey of recent literature on DNA amplification techniques but direct comparison is impossible at this stage. What is needed is a laboratory comparison of the different target DNA sequences used for diagnosis so that the most appropriate can be recommended and hopefully used.

Studies using single [48] or nested [49] PCR primers for *fliC* of *S.* Typhi have reported good results from PCR. Using samples from 40 clinically suspected cases of typhoid fever, 20/20 culture positive and 12/ 20 culture negative cases were positive by PCR in Delhi, India [50]. Using single primer PCR in South Sulawesi, Indonesia, 46/73 blood samples collected from patients with clinically suspected typhoid fever were positive compared to 13.7% positive by blood culture [51]. In Varnassi, India, nested PCR (53/57 positive) was again better than blood culture (17/53 positive) on specimens from 63 clinical typhoid fever cases and 25 healthy controls [52].

A large, well-designed study in Indonesia investigated 131 patients with a clinical diagnosis of typhoid fever and found diagnosis by PCR of DNA from blood (84.5%) and urine (69.3%) to be slightly more sensitive than blood culture (61.8%) [53]. A study from Nepal on specimens from 71 children with suspected typhoid fever reports 82.7% positivity for PCR from blood and urine, both much higher than blood culture (26.9%). In Pakistan, 55 cases of

suspected typhoid fever and a control group of 20 healthy persons (PCR versus blood culture) gave 58.2% versus 14.5% positivity, respectively [54]. The authors of the study conclude that the sensitivity of PCR as compared with that of blood culture was significantly better [54]. Again in Pakistan, a multiplex PCR targeting five different genes for differential diagnosis of typhoidal pathogens has been developed for use directly on clinical blood samples. Of 42 multiplex PCR-positive blood samples, 35 were positive for S. Typhi and two for *S.* Paratyphi A. Interestingly, five patients were found to have mixed infection [55].

Despite this wealth of data, we have reviewed only some of the more recent articles; PCR has not become an established method for diagnosis typhoid fever.

Conclusions

The diagnosis of enteric fever depends upon techniques, blood culture and PCR, which are not available in the very areas were the disease is most common. The Widal test and other serological diagnostic tools have limitations because of their low sensitivity and/or specificity.

There is an urgent need for the rational design and evaluation of effective and appropriate diagnostics for enteric fever. These must include the emerging threat of *S.* Paratyphi A.

Acknowledgements
JW is supported by the Wellcome Trust of Great Britain.

References

1. Crump JA, Luby SP, Mintz ED (2004) The global burden of typhoid fever. Bull World Health Organ 82:346-53.
2. Stuart BM, Pullen RL (1946) Typhoid fever; clinical analysis of three hundred and sixty cases. Arch Intern Med 78:629-61.
3. Petit PL, Wamola IA (1994) Typhoid fever: a review of its impact and diagnostic problems. East Afr Med J 71:183-188.
4. Khan M, Coovadia YM, Connoly C, Sturm AW 1998. The early diagnosis of typhoid fever prior to the Widal test and bacteriological culture results. Acta Tropica 69:165-73.
5. Felix A, Standardization of diagnostic agglutination tests: typhoid and paratyphoid A and B fevers (1950) Bull WHO 2:643-9.
6. House D, Wain J, Ho VA, Diep TS, Chinh NT, Bay PV, Vinh H, Duc M, Parry CM, Dougan G, White NJ, Hien TT, Farrar JJ (2001) Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. J Clin Microbiol 39:1002-7.
7. Chart H, Cheasty T, de Pinna E, Siorvanes L, Wain J, Alam D, Nizami Q, Bhutta Z, Threlfall EJ (2007) Serodiagnosis of Salmonella enterica serovar Typhi and S. enterica serovars Paratyphi A, B and C human infections. J Med Microbiol 56:1161-6.
8. Dutta S, Sur D, Manna B, Sen B, Deb AK, Deen JL, Wain J, Von Seidlein L, Ochiai L, Clemens JD, Kumar Bhattacharya S (2006) Evaluation of new-generation serologic tests for the diagnosis of typhoid fever: data from a community-based surveillance in Calcutta, India. Diagn Microbiol Infect Dis 56:359-65.
9. Hoa NT, Diep TS, Wain J, Parry CM, Hien TT, Smith MD, Walsh AL, White NJ (1998) Community-acquired septicaemia in southern Viet Nam: the importance of multidrug-resistant Salmonella typhi. Trans R Soc Trop Med Hyg,. 92:503-8.
10. Anderson KE, Joseph SW, Nasution R, Sunoto, Butler T, Van Peenen PF, Irving GS, Saroso JS, Watten RH (1976) Febrile illnesses resulting in hospital admission: a bacteriological and serological study in Jakarta, Indonesia. Am J Trop Med Hyg 25:116-121.
11. Coleman W, Buxton BH (1907) The bacteriology of the blood in typhoid fever. Amer J Med Sci 133:896-903.
12. Cummins SL 1911 The antibacteriocidal action of the bile salts. J Hyg Camb XI:25-380.
13. Foster WD (1975) Laboratory diagnosis of typhoid fever. Lancet 2:80.
14. Murray P, Spizzo A, Niles A (1991) Clinical comparison of the recoveries of bloodstream pathogens in septi-check, brain heart infusion broth with saponin, septi-check tryptic soy broth, and the isolator lysis-centrifugation system. J Clin Microbiol. 29:901-905.
15. Farooqui BJ, Khurshid M, Ashfaq MK, Khan MA (1991) Comparative yield of Salmonella typhi from blood and bone marrow cultures in patients with fever of unknown origin. J Clin Pathol 44:258-259.
16. Gasem MH, Dolmans WM, Isbandrio BB, Wahyono H, Keuter M, Djokomoeljanto R (1995) Culture of Salmonella typhi and Salmonella paratyphi from blood and bone marrow in suspected typhoid fever. Trop Geogr Med. 47:164-167.
17. Soewandojo E, Suharto U, Hadi P, Prihartini F (1997) Comparative result between bone marrow culture and blood culture in the diagnosis of typhoid fever. Third Asia-Pacific symposium on typhoid fever and other Salmonellosis, Bali. Abstract No. D2-3: p. 83.
18. Wain J, Pham VB, Ha V, Nguyen NM, To SD, Walsh AL, Parry CM, Hasserjian RP, HoHo VA, Tran TH, Farrar J, White NJ, Day NP (2001) Quantitation of Bacteria in Bone Marrow from Patients with Typhoid Fever: Relationship between Counts and Clinical Features. J Clin Microbiol 39:1571-6.
19. Buck RL, Escamilla J, Sangalang RP, Cabanban AB, Santiago LT, Ranoa CP, Cross JH (1984) Comparative study of three blood culture systems for isolation of enteric fever Salmonella. Southeast Asian J Trop Med Public Health 15:161-166.
20. Luxemburger C, Chau MC, Mai NL, Wain J, Tran TH, Simpson JA, Le HK, Nguyen TT, White NJ, Farrar JJ (2001) Risk factors for typhoid fever in the Mekong delta, southern Viet Nam: a case-control study. Trans R Soc Trop Med Hyg 95:19-23.
21. Hobbs B, Allison V (1945) Studies on the isolation of Bact. typhosum and Bact. paratyphosum. B. Bull Minist Health 4:12-19.
22. Moriñigo MA, Muñoz MA, Martinez-Manzanares E, Sánchez JM, Borrego JJ (1993) Laboratory study of several enrichment broths for the detection of Salmonella spp. particularly in relation to water samples. J Appl Bacteriol 74:330-335.
23. Vallenas C, Hernandez H, Kay B, Black R, Gotuzzo E (1985) Efficacy of bone marrow, blood, stool and duodenal contents cultures for bacteriologic confirmation of typhoid fever in children. Pediatr Infect Dis 4:496-498.
24. Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB (1975) Relative efficacy of blood, urine, rectal

swab, bone-marrow, and rose- spot cultures for recovery of Salmonella typhi in typhoid fever. Lancet 1(7918)::1211-1213.
25. Wain J, Diep TS, Ho VA, Walsh AM, Nguyen TT, Parry CM, White NJ (1998) Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility, and antibiotic resistance. J Clin Microbiol 36:1683-7.
26. Mathai E, John TJ, Rani M, Mathai D, Chacko N, Nath V, Cherian AM (1995) Significance of Salmonella typhi bacteriuria. J Clin Microbiol 33:1791-2.
27. Widal F 1896 Serodiagnostic del la fievretyphoide. Bull Soc Med Hop 13:561-566.
28. Widal G, Sicard A (1896) Researches de la reaction agglutinate dans le sang et le serum desseches des typhiques et dans la serosite des vesications. Bull Soc Med Paris (3rd series) 13:681-682.
29. Olopoenia LA, King AL 2000 Widal agglutination test - 100 years later: still plagued by controversy. Postgrad Med J 76:80-4.
30. West B, Richens JE, Howard PF (1989) Evaluation in Papua New Guinea of a urine coagglutination test and a Widal slide agglutination test for rapid diagnosis of typhoid fever. Trans R Soc Trop Med Hyg 83:715-717.
31. House D, Chinh NT, Diep TS, Parry CM, Wain J, Dougan G, White NJ, Hien TT, Farrar JJ (2005) Use of paired serum samples for serodiagnosis of typhoid fever. J Clin Microbiol 43:4889-90.
32. Losonsky GA, Ferreccio C, Kotloff KL, Kaintuck S, Robbins JB, Levine MM (1987) Development and evaluation of an enzyme-linked immunosorbent assay for serum Vi antibodies for detection of chronic Salmonella typhi carriers. J Clin Microbiol 25:2266-2269.
33. Lin FY, Becke JM, Groves C, Lim BP, Israel E, Becker EF, Helfrich RM, Swetter DS, Cramton T, Robbins JB (1988) Restaurant-associated outbreak of typhoid fever in Maryland: identification of carrier facilitated by measurement of serum Vi antibodies. J Clin Microbiol 26:1194-7.
34. Shukla S, Chitnis DS (1997) Haemagglutination system for the simultaneous detection of LPS and anti LPS antibodies of S. typhi. India J Med Scien 51:265-9.
35. Kalhan R, Kaur I, Singh RP, Gupta HC (1998) Rapid diagnosis of typhoid fever. India J Pediatr 65:561-4.
36. Coovadia YM, Singh V, Bhana RH, Moodley N (1986) Comparison of passive haemagglutination test with Widal agglutination test for serological diagnosis of typhoid fever in an endemic area. J Clin Pathol 39:680-683.
37. Sharma M, Datta U, Roy P, Verma S, Sehgal S (1997) Low sensitivity of counter-current immuno-electrophoresis for serodiagnosis of typhoid fever. J Med Microbiol 46:1039-42.
38. Jesudason MV, Sridharan G, Arulselvan R, Babu PG, John TJ (1998) Diagnosis of typhoid fever by the detection of anti-LPS & anti-flagellin antibodies by ELISA. India J Med Res 107: 204-7. http://www.biomednet.com/db/medline/98335273:
39. Bhutta ZA, Mansurali N (1999) Rapid serologic diagnosis of pediatric typhoid fever in an endemic area: a prospective comparative evaluation of two dot-enzyme immunoassays and the Widal test. Am J Trop Med Hyg 61:654-7.
40. Hatta M, Goris MG, Heerkens E, Gooskens J, Smits HL (2002) Simple dipstick assay for the detection of Salmonella typhi-specific IgM antibodies and the evolution of the immune response in patients with typhoid fever. Am J Trop Med Hyg 66:416-21.
41. Hatta M, Mubin H, Abdoel T, Smits HL (2002) Antibody response in typhoid fever in endemic Indonesia and the relevance of serology and culture to diagnosis. Southeast Asian J Trop Med Public Health 33:742-51.
42. Oracz G, Feleszko W, Golicka D, Maksymiuk J, Klonowska A, Szajewska H (2003) Rapid diagnosis of acute Salmonella gastrointestinal infection. Clin Infect Dis 36:112-5.
43. Kawano RL, Leano SA, Agdamag DM (2007) Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. J Clin Microbiol 45:246-7.
44. Banchuin N, Appassakij H, Sarasombath S, Manatsathit S, Rungpitarangsi B, Komolpit P, Sukosol T (1987) Detection of Salmonella typhi protein antigen in serum and urine: a value for diagnosis of typhoid fever in an endemic area. Asian Pac J Allergy Immunol 5:155-159.
45. Chaicumpa W, Ruangkunaporn Y, Burr D, Chongsa Nguan M, Echeverria P (1992) Diagnosis of typhoid fever by detection of Salmonella typhi antigen in urine. J Clin Microbiol 30:2513-2515.
46. Fadeel MA, Crump JA, Mahoney FJ, Nakhla IA, Mansour AM, Reyad B, El Melegi D, Sultan Y, Mintz ED, Bibb WF (2004) Rapid diagnosis of typhoid fever by enzyme-linked immunosorbent assay detection of Salmonella serotype typhi antigens in urine. Am J Trop Med Hyg 70:323-8.
47. Malorny B, Lofstrom C, Wagner M, Kramer N, Hoorfar J (2008) Enumeration of salmonella bacteria in food and feed samples by real-time PCR for quantitative microbial risk assessment. Appl Environ Microbiol 74:1299-304.
48. Song JH, Cho H, Park MY, Na DS, Moon HB, Pai CH (1993) Detection of Salmonella typhi in the blood of patients with typhoid fever by polymerase chain reaction. J Clin Microbiol 31:1439-1443.
49. Frankel G, (1994) Detection of Salmonella typhi by PCR. J.Clin.Microbiol 32:1415.
50. Kumar A, Arora V, Bashamboo A, Ali S (2002) Detection of Salmonella typhi by polymerase chain reaction: implications in diagnosis of typhoid fever. Infect Genet Evol 2:107-10.
51. Massi MN, Shirakawa T, Gotoh A, Bishnu A, Hatta M, Kawabata M (2003) Rapid diagnosis of typhoid fever by PCR assay using one pair of primers from flagellin gene of Salmonella typhi. J Infect Chemother 9:233-7.
52. Prakash P, Mishra OP, Singh AK, Gulati AK, Nath G (2005) Evaluation of nested PCR in diagnosis of typhoid fever. J Clin Microbiol 43:431-2.
53. Hatta M, Smits HL (2007) Detection of Salmonella typhi by nested polymerase chain reaction in blood, urine, and stool samples. Am J Trop Med Hyg 76:139-43.
54. Haque A, Ahmed N, Peerzada A, Raza A, Bashir S, Abbas G (2001) Utility of PCR in diagnosis of problematic cases of typhoid. Jpn J Infect Dis 54:237-9.
55. Ali A, Haque A, Haque A, Sarwar Y, Mohsin M, Bashir S, Tariq A (2008) Multiplex PCR for differential diagnosis of emerging typhoidal pathogens directly from blood samples. Epidemiol Infect 2009;137:102-7.

Corresponding Author: Salih Hosoglu, Dicle University Hospital, Diyarbakir, Turkey
E-mail: hosoglu@hotmail.com

Conflict of interest: No conflict of interest is declared.