

## Diagnosis of septicaemia with special reference to enteric fever

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**Abstract:** Sepsis is the most common cause of death in the non-coronary intensive care unit. The mortality ranges from 16% in patients with sepsis to 40% in patients with septic shock. In some cases, the focus is known, e.g. pneumococcal pneumonia, while in some cases, it is unknown. The organisms most often come from the patient's own flora. Sepsis is becoming more common in immunocompromised and critically ill patients than ever before. Drug resistance is rampant in the isolates making therapy difficult and a good number of cases show no growth in conventional culture systems, obviating the need for special techniques to look for fastidious organisms that may be involved in such cases.

### Introduction

While dealing with cases of blood stream infection, the terms bacteraemia, septicaemia, sepsis, etc. are often used synonymously and indiscriminately. This causes much confusion in understanding the disease process and can hamper proper management. The recommended definitions are:<sup>1</sup>

**Bacteraemia:** The presence of viable bacteria in the blood.

**Sepsis:** The systemic response to infection that is manifested by two or more of the following conditions as a result of infection: temperature  $>38^{\circ}\text{C}$  or  $<36^{\circ}\text{C}$ ; heart rate  $>90/\text{min}$ ; respiratory rate  $>20/\text{min}$  or  $\text{PaCO}_2 <32$  torr;  $\text{WBC} >12\ 000$  cells/cmm,  $<4000$  cells/cmm or 10% immature (band) forms.

**Septic shock:** Sepsis with hypotension, despite adequate fluid resuscitation, along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration of mental status.

In a study it was found that of the total cases with septicaemia, 50% were due to community-acquired infections; the commonest isolate was *Salmonella* species out of which one-third were *S. typhi*.<sup>2</sup> Multidrug-resistant *S. typhi* has emerged in various parts of the world since 1989. Such strains have been isolated from Asian countries such as India and Bangladesh as well as from the western world. In India, several epidemics of typhoid have broken out in the past two decades. These isolates are often resistant to chloramphenicol, ampicillin and co-trimoxazole. In 1983, an outbreak of *S. typhi* infection in Chandigarh was caused by chloramphenicol-resistant strains.<sup>3</sup> An increase in the number of cases caused by *S. paratyphi* A has been noticed in Delhi since 1996 and an outbreak caused by this bacteria was detected in 1999. About 32% of the 16 strains isolated were resistant to more than two antibiotics.<sup>4</sup> Plasmid-mediated chloramphenicol resistance was detected in the isolates of *S. typhi* since the early 1970s, both in the

northern and southern parts of India.<sup>3,5</sup> Armed with drug-resistant genes, typhoid fever continues to be a rogue disease affecting over 20 million people each year resulting in 700 000 deaths worldwide. In a SENTRY antimicrobial surveillance programme in Europe during 1997–98, *Escherichia coli*, *Staphylococcus aureus* and coagulase-negative *Staphylococcus* topped the list of pathogens isolated from the blood.<sup>6</sup> In children and those admitted in the neonatal intensive care unit (ICU), Gram-negative bacteria are the most frequent invaders of the blood stream with *Klebsiella pneumoniae* being the most frequent isolate. There have been several reports of outbreaks of Gram-negative septicaemia among neonates caused by *K. pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* species and *Enterobacter*. Invasive procedures such as exchange transfusions via the umbilical vein potentially expose the neonate to nosocomial infection. Blood stream infections are the most common mode of nosocomial infection.

From January 2001 to July 2002, a clear predominance of Gram-negative bacteria (67.8% of isolates) over Gram-positive cocci (32.2%) isolated from the blood was seen in the Sanjay Gandhi Post Graduate Institute of Medical Sciences, a tertiary-care hospital. *E. coli* was the most common Gram-negative bacteria in the samples from the ward and ICU, and *S. aureus* was the commonest Gram-positive organism from these places. Kapoor *et al.* reported a predominance of Gram-negative isolates (67.9%) in the ICU and nurseries.<sup>7</sup> In the burns unit, there was a fall of *Pseudomonas* and rise of *K. pneumoniae* isolates. In other reports, bacteraemia caused by *P. aeruginosa* was the major cause of death in burn patients.<sup>8</sup>

Another Gram-negative bacteria that is emerging as an important nosocomial pathogen is the *Enterobacter* species. As far as hospital-acquired blood stream infections are concerned, coagulase-negative staphylococci have made a comeback and their presence in the blood can no longer be regarded as being mere contaminants, especially among patients in critical care units. Arterial lines, central lines and other artificial devices are a major source of this infection.

## Pathophysiology and clinical features

The complications of septicaemia can range from hypotension, bleeding, leucopenia, thrombocytopenia, haemolytic uraemic syndrome to organ failure. The whole range of manifestations is the result of an interaction between bacterial products and host factors. Bacterial endotoxin is one of the best studied products, though others such as formyl peptides, exotoxins and proteases from Gram-negative bacteria, and exotoxins, enterotoxins, haemolysins, peptidoglycans and lipoteichoic acid from Gram-positive bacteria may also be involved. These toxins stimulate the release of cytokines from macrophages that trigger off a systemic inflammatory cascade. Tumour necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 play a major role in this process.

The most important mediator of septic shock seems to be nitric oxide, which is a potent vasodilator. The expression of adhesion molecules may be upregulated on endothelial cells and neutrophils during septic shock. These include CD11/CD18 on neutrophils and intercellular adhesion molecules (ICAM)-1 and ICAM-2 on endothelial cells. Adhesion and transmigration of neutrophils to the extravascular space lead to microvascular and tissue injury. There may be activation of the extrinsic pathways leading to disseminated intravascular coagulation. The host factors which can increase the risk of septicaemia include invasive procedures, extremes of age and immune suppression. Acquired immunodeficiency syndrome (AIDS) and/or severe immunosuppression can be associated with an increased risk of Gram-negative septicaemia, especially among young children.

Bacteria causing bacteraemia can come from a variety of sources. They can be from an infective focus in the body such as pericarditis, peritonitis, pneumonia, osteomyelitis, skin and soft tissue infections, or they may come from medical devices and the hospital environment. Microbes such as *Staph. epidermidis* are frequent colonizers of catheters and can easily invade the blood stream. In neonates, low birth weight, presence of a central venous pressure (CVP) line, paediatric risk of mortality (PRISM) score and length of stay in the hospital have been identified as notable risk factors.

Septicaemia in enteric fever deserves a detailed discussion. Enteric fever caused by *S. typhi* and *S. paratyphi* (A, B and C) has been defined as a generalized infection of the reticuloendothelial system and intestinal lymphoid tissue accompanied by sustained fever and bacteraemia. Typhoid fever is more common than paratyphoid fever and rarely other serovars of *S. enterica* may cause a similar clinical condition. Bacteraemia occurs twice during the course of infection—once, when the infectious dose reaches the intestine, the bacteria pass through the epithelium without damaging it to enter the lymphatic channels and then into the blood stream producing a transient and usually symptomless primary bacteraemia. The second episode of bacteraemia results when the bacteria

multiply at the end of the incubation period in the reticuloendothelial system and spill over into the blood stream producing obvious signs and symptoms. It does not produce a fulminant clinical picture resembling septic shock; rather, it begins as a subacute illness and progresses gradually to the acute stage. The patients are bacteraemic during the first and second weeks of the illness but after that, when the disease worsens, the patients are mostly free of bacteraemia. Also, in typhoid fever, compared with other Gram-negative bacteraemic illnesses, the bacterial load is low ( $<10^3$  bacteria/ml), whereas a count of  $\geq 10^5$ /ml is usual for other Gram-negative bacteraemia. In spite of the low level of circulating lipopolysaccharides (LPS) in typhoid fever, the toxin may still act locally in the liver, spleen and elsewhere to release endogenous pyrogens into the blood.

A key factor in the pathogenesis of enteric fever is the survival of the organisms within macrophages. After reaching the small bowel, the bacteria are rapidly internalized by the host cells and transported to the submucosal lymphatic tissue.<sup>9</sup> Internalization occurs by what is called bacteria-mediated endocytosis. In this process, the bacteria are internalized into the membrane-bound vacuoles through which they are transcytosed from the apical to the basolateral surface. In the lymphoid tissue, they interact with the macrophages. They gain entry inside the macrophages by the induction of macropinocytosis rather than receptor-mediated endocytosis. The macropinosomes may fuse to give rise to spacious phagosomes containing *Salmonella*. *Salmonella* can induce death of the macrophages after phagocytosis and can also induce phagocytosis by macrophages, thus, surviving engulfment by neutrophils, which are very effective killers of *Salmonella*. Survival within macrophages is mediated by regulatory proteins PhoP/PhoQ that regulate the gene complex, which is also responsible for resistance to cationic antimicrobial proteins, acid pH and invasion of epithelial cells. Regulatory genes implicated in the pathogenesis include *crp/cya*, *ompR*, *envZ* and *katF*.<sup>9</sup>

## Diagnosis of septicaemia

**Blood culture:** The role of blood culture in typhoid fever cannot be overemphasized. In 1907, a review of the literature reported that 89% of blood cultures were positive in the first week of typhoid fever; 73% were positive in the second; 60% were positive in the third; and only 26% were positive in the fourth week and thereafter.<sup>10</sup>

**Collection:** Blood samples should be collected prior to the administration of antibiotics. Blood meant for culture must be collected under aseptic conditions. Though collection by venepuncture is preferred over collection from indwelling intravenous or intra-arterial catheters, a comparison of these two practices showed 96% correlation in positive cultures and 98% correlation with negative cultures. It has been recommended that less than 15 colony forming units (CFU) of bacteria obtained by catheter culture is less likely to reflect true septicaemia.

**Culture methods:** It has been observed that in about 10%–30% cases, the aetiological agents of septicaemia cannot be grown in culture medium. There can be several reasons for this: (i) prior antimicrobial therapy; (ii) presence of fastidious bacteria in the blood that need a special growth environment; (iii) slow-growing bacteria, e.g. *Brucella*, which needs longer incubation; and, (iv) inappropriate anticoagulant that may be inhibitory to some bacteria, e.g. sodium amylosulfate (SAS) is inhibitory to *K. pneumoniae*, and sodium citrate (0.5%–1.0%) may be inhibitory to some Gram-positive cocci. The lysis centrifugation system is particularly good for fungaemia.

Of the automated blood culture systems, both BacT/Alert and the BACTEC 9440/9120 detect changes in the CO<sub>2</sub> concentration in the blood–broth mixture. While BacT/Alert uses spectral light to detect the change, BACTEC uses fluorescent light. The extra sensing power (ESP) blood culture system monitors multiple factors such as CO<sub>2</sub> pressure, changes in the concentrations of H<sub>2</sub> and O<sub>2</sub> in addition to CO<sub>2</sub>. The Vital blood culture system differs from BACTEC in the incorporation of a soluble fluorescent molecule directly in the broth. The OASIS blood culture system measures headspace gas pressure by a scanning laser sensor.

Apart from these automated systems, there are also some manual systems designed for the rapid detection of growth of bacteria in blood. These include the Oxoid signal system and the Septi-Check system. Some comparative studies indicate that BacT/Alert is superior or equivalent to BACTEC.<sup>11</sup> The FAN bottles of BacT/Alert have a higher rate of positive results while the patient is on antibiotics. The BACTEC Aerobic Plus/F culture system is more rapid in detection than Septi-Check, though the latter shows a greater rate of recovery<sup>12</sup> and the ESP system detects growth much earlier than Septi-Check.<sup>13</sup>

The non-culture techniques include latex agglutination tests for group B streptococci, *Haemophilus influenzae* type B, *S. pneumoniae*, *Neisseria meningitidis* and staphylococcal teichoic acids. The limulus amoebocyte lysate assay is a highly sensitive test for endotoxin detection. Gas liquid chromatography and lysis filtration techniques have also been tried.

The problem with blood culture in typhoid fever is that the bacterial load in the extracellular compartment of the blood is low, as two-thirds of the bacterial population remain confined within the phagocytic cells. Also, the proportion of patients with a positive culture decreases with increasing duration of illness and volume of blood. Isolation techniques are also important factors that determine the yield from blood cultures.

**Clot culture:** Several methods have been tried as alternatives to whole blood culture. One of these is clot culture. In this method, blood is allowed to clot, loosened with streptokinase and incubated in broth for subsequent subcultures. This gets rid of the bactericidal substances present in the serum. Also, the serum can be used for biochemical or serological testing.

**Buffy coat culture:** Wain *et al.*<sup>10</sup> used direct plating of the buffy coat as an alternative to whole blood culture. They found that the method was as sensitive as whole blood culture and allowed earlier identification of the organism and antimicrobial sensitivity testing. Other advantages included less

contamination, higher yield from a small volume of blood, especially in children having higher bacterial load and availability of plasma from the same sample, which can be used for biochemical and serological tests.

**Lysis direct plating lysis centrifugation (LDP–LC):** This method was tried by Saha *et al.* in 1992 on the blood samples of Bangladeshi children and evaluated for its effectiveness in the rapid identification of *S. typhi*.<sup>14</sup> They found the time for generating a report was less by this method than the conventional techniques.

**Bone marrow culture:** Enteric fever is the only bacterial infection in humans for which bone marrow examination is routinely recommended while investigating pyrexia of unknown origin. Bone marrow culture is superior to blood culture because it increases the diagnostic yield by about one-third compared with those from blood.<sup>15</sup> Quantitative studies have revealed that in typhoid fever blood bacterial counts do not correlate either with the outcome or the clinical and laboratory measures of severity.<sup>10</sup>

The marrow samples remain positive for up to 5 or more days after starting fluoroquinolone therapy. Cell-culture experiments have shown that serovar typhi can replicate in human macrophages to reach an average of 14 organisms per cell. In contrast, peripheral blood monocytes from patients infected with serovar typhi contain an average of only 1.3 CFU/cell. In one study, it was found that there were over 10 times more bacteria in the bone marrow than in the blood, which means that compared with 10 ml of blood, only 1 ml of bone marrow will be needed to yield a positive result.

**Molecular techniques for detection of *S. typhi* in blood:** Song *et al.*<sup>16</sup> developed a polymerase chain reaction (PCR)-based test for this purpose using a 343 bp fragment of *flagellin* gene of *S. typhi* as the target. They concluded that PCR was a rapid, simple and specific method for the early diagnosis of typhoid fever, particularly useful in culture negative-antibiotic treated cases. A nested PCR has also been developed based on the *Via B* sequence that codes for the Vi antigen, which has been found to be highly sensitive.<sup>17</sup>

## Tests for the diagnosis of enteric fever

Stool cultures are positive in less than half the patients and urine culture in even less. Stool cultures may be useful when the patient is on antibiotics and the bacteria have been cleared from the blood. Culture of biopsy specimens from rose spots can be positive in about two-thirds of the cases. The Widal test has been in use for more than 100 years but its use in the diagnosis of typhoid fever is limited.<sup>18</sup> The minimal titres defined as positive for O and H antigens should be established for individual geographical areas to obtain credible results. The test is more reliable in areas from which data on the titres in control groups without enteric fever are available. Other limitations are cross-reactions and false-positive results in the acute settings.

Though several assays to detect antibodies against

*Salmonella* have been developed, the value of enzyme immunoassay (EIA) for routine diagnostic purposes is limited because there are numerous *Salmonella* serotypes and antigens. Counter-immunoelectrophoresis to detect *Salmonella* antigens in the blood or urine lack sensitivity and specificity. Dot enzyme immunosorbent assay for rapid serodiagnosis has been described by various workers and is available commercially as easy-to-do kits.<sup>19</sup>

## Management

The choice of antimicrobials in sepsis depends on a multitude of factors such as whether the infection is community- or hospital-acquired, the organism involved, immune status of the patient, tolerance of a drug in a particular individual and the existing pattern of antimicrobial resistance in a particular ward. Multiple antibiotics are often administered and the value of such a regimen cannot be denied. Combination therapy covers a large range of organisms while the culture reports are awaited; it prevents the emergence of resistance and may act additively or synergistically. For community-acquired infections, first- and second-generation cephalosporins are reasonable choices. For nosocomial infection, aminoglycosides with  $\beta$ -lactam drugs is a better regimen. For the neutropenic patient, the  $\beta$ -lactam should be active against *Pseudomonas*. Indiscriminate use of third-generation cephalosporins may give rise to extended spectrum  $\beta$ -lactamase producing strains. Use of these drugs as well as imipenems in places such as ICUs should be made judiciously to prevent the emergence of resistance. The final choice obviously depends on the results of the culture and antibiogram. It should be remembered that it may take 4–5 days before the fever peaks abate, therefore, this should not be viewed as resistance or a need for combination therapy.

The use of antiserum to treat bacterial infections is not a new concept. Several such agents have been tried for sepsis, e.g. E5 monoclonal antibody to endotoxin, human monoclonal antibody HA-1A, monoclonal antibody to human TNF- $\alpha$ , etc. Other agents that have been tried include recombinant human IL-1 receptor antagonist, platelet activating factor (PAF) receptor antagonist<sup>20</sup> and activated protein C.

For enteric fever, chloramphenicol was the drug of choice until resistant strains became widespread. The most frequently used group of drugs now is the quinolones. Ciprofloxacin has good activity inside phagocytes. A short course of ofloxacin has been evaluated for multidrug-resistant *S. typhi*. Quinolones are cheap, well tolerated and have good efficacy. There has been some concern regarding their use in children but other reports say that no arthropathy or cartilage damage has been observed with their use in humans and they can be used safely even in neonatal septicaemia with good results.<sup>21</sup> The other drug with high efficacy against *Salmonella* is ceftriaxone. The shortening of the duration of fever has been observed with the use of this drug.<sup>22</sup> However, a resurgence of chloramphenicol-sensitive *S. typhi* has been noted recently, which is probably due to the restricted use of the drug for the treatment of typhoid fever.<sup>23</sup>

## References

1. American College of Chest Physicians/Society of Critical Care Medicine Consequence Conference Committee. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992;**20**:864–74.
2. Cheng AF, Fok TF, Duthie R, French GL. A five-year prospective study of septicaemia in hospitalized children in Hong Kong. *J Trop Med Hyg* 1991;**94**:295–303.
3. Kapil A, Ayyagari A, Garg RK, Agarwal KC. *S. typhi* with transferable chloramphenicol resistance isolated in Chandigarh during 1983–87. *Indian J Pathol Microbiol* 1994;**38**:179–83.
4. Chandel DS, Chaudhary R, Dhawan B, Pandey A, Dey AB. Drug resistant *Salmonella enterica* serotype *paratyphi* A in India. *Emerg Infect Dis* 2000;**6**:420–1.
5. Agarwal KC, Garg RK, Panhotra BR, Verma AD, Ayyagari A, Mehanta J. [Drug resistance in *Salmonella* isolated at Chandigarh (India) during 1972–78.] *Antonie von Leeuwenhoek* 1980;**46**:383–6.
6. Fluit A.C, Jones ME, Sckmitz FJ, Acar J, Gupta R, Verhoef J, and the SENTRY participants group. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. *Clin Infect Dis* 2000;**30**:454–60.
7. Kapoor H, Sumathi M, Aggarwal P, Jain SD, Kaur J. Spectrum of bacterial isolation in high risk areas of a tertiary care hospital: A 3-year study. *Indian J Med Microbiol* 2000;**184**:166–9.
8. McManus AT, Mason AD, McManus WE, Pruitt BA. Twenty-five year review of *Pseudomonas aeruginosa* bacteraemia in a burns centre. *Eur J Clin Microbiol* 1985;**4**:219–33.
9. Miller SI, Pegues DA. *Salmonella* species, including *Salmonella typhi*. In: Mandell GL, Bennett JE, Dolin R (eds). *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 5th ed. Washington DC: ASM Press; 2000:2344–62.
10. Wain J, Diep TS, Ho VA, Walsh AM, Hoa NTT, Parry CM, et al. Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility, and antibiotic resistance. *J Clin Microbiol* 1998;**36**:1683–7.
11. Weinstein MP, Mirrett S, Reimer LG. Controlled evaluation of BacT/Alert standard aerobic and FAN aerobic blood culture bottles for detection of bacteremia and fungemia. *J Clin Microbiol* 1995;**33**:978–81.
12. Rhoner P, Pepey B, Auckenthaler R. Comparison of BacT/Alert with Signal blood culture system. *J Clin Microbiol* 1995;**33**:313–17.
13. Welby PL, Keller DS, Storch GA. Comparison of automated Difco ESP blood culture system with biphasic BBL Septi-Check system for detection of blood stream infections in pediatric patients. *J Clin Microbiol* 1995;**33**:1084–8.
14. Saha SK, Darmstadt GL, Baqui AH, et al. Rapid identification and antibiotic susceptibility testing of *Salmonella enterica* serovar *typhi* isolated from blood: Implications for therapy. *J Clin Microbiol* 2001;**39**:3583–5.
15. Wain J, BeBay PV, Vinh HA, et al. Quantitation of bacteria in bone marrow from patients with typhoid fever: Relationship between counts and clinical features. *J Clin Microbiol* 2001;**39**:1571–6.
16. Song JH, Cho H, Park MY, et al. Detection of *Salmonella typhi* in the blood of patients with typhoid fever by polymerase chain reaction. *J Clin Microbiol* 1993;**31**:1439–43.
17. Hashimoto Y, Itho Y, Fujinaga Y, Khan AQ, Sultana F, Miyake M, et al. Development of nested PCR based on the *Via B* sequence to detect *Salmonella typhi*. *J Clin Microbiol* 1995;**33**:775–7.
18. Shukla BS, Patel B, Chitnis DS. 100 years of Widal test and its reappraisal in an endemic area. *Indian J Med Res* 1997;**105**:53–7.
19. Ismail A, Abdul Kader Z, Ong KH. Dot enzyme immunosorbent assay for the serodiagnosis of typhoid fever. *Southeast Asian J Med Pub Health* 1992;**22**:563–6.
20. Bone RC. Why sepsis trials fail. *JAMA* 1996;**276**:565–6.
21. Khaneja M, Naprawa J, Kumar A, Pieciech S. Successful treatment of late onset infection due to resistant *Klebsiella pneumoniae* in an extremely low birth weight infant using ciprofloxacin. *J Perinatol* 1999;**19**:311–14.
22. Gulati S, Marawaha RK, Prakash D, et al. Multidrug resistant *Salmonella typhi*—a need for therapeutic reappraisal. *Ann of Trop Paed* 1992;**12**:137–41.
23. Sood S, Kapil A, Das B, et al. Reemergence of chloramphenicol-sensitive *Salmonella typhi*. *Lancet* 1999;**353**:1241–2.