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Transport time for blood culture bottles: underlying factors and its consequences

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ABSTRACT

In the present study we investigated transport times for blood cultures from three tertiary-care hospitals to Karolinska University Laboratory and identified predictors of long transport times. Concomitantly, consequences of delayed incubation on total detection time (TDT) were analyzed by in vitro sepsis models. A total of 909 blood cultures were studied. The median (interquartile range) transport time was 9 (3–15) h. The hospital accommodating the microbiology laboratory had the shortest transport time compared to the other two hospitals ($P < 0.0001$). Samples taken between 16:00–24:00 had longer transport times compared to samples taken between 8:00–16:00 and 24:00–08:00 ($P < 0.0001$). In vitro experiments showed that TDT was longer for samples pre-incubated at room temperature (RT) for 19 h compared to the ones pre-incubated for 2 h or 9.5 h ($P < 0.0001$). In conclusion, off-site location, time of sampling and number of transports per day were related to, and predictors of transport time.

1. Introduction

In patients with sepsis, early detection and identification of the disease-causing microorganism is paramount for choosing the appropriate antibiotic therapy, and a direct indicator of survival rate and clinical outcome (Barenfanger et al., 1999; Valles et al., 2003). It has been shown that availability of microbiological identification and susceptibility testing results increase the proportion of appropriate antibiotic use markedly (Barenfanger et al., 1999; Berild et al., 2006; Kerremans et al., 2008). Appropriate antibiotic use and choosing narrow-spectrum drugs after identification of the organism decreases the cost of antibiotic therapy (Barenfanger et al., 1999; Berild et al., 2006). Furthermore, by avoiding over-use of broad-spectrum antibiotics we can contribute to minimizing the potential of selecting for resistant bacteria which are becoming an increasing threat to successful health care world-wide (Livermore, 2005). Accordingly, there is an urgent need to shorten the time for identification and susceptibility testing of microorganisms isolated from positive blood culture bottles (turnaround time for laboratory diagnosis of sepsis). Hitherto, heavy focus has been put on developing novel methods for rapid microorganism detection and identification in the laboratory. Development of automated blood culture systems and a number of rapid identification methods have shortened the time for identification of microorganisms from positive blood cultures (Peters et al., 2004). More recently, establishment of novel proteomic methods such as MALDI-TOF allow bacterial and yeast identification in a fraction of the time required by traditional biochemical methods (Bizzini and Greub, 2010).

In contrast to the remarkable development shortening turnaround time in the analytical part of blood culture diagnostics, surprisingly little attention has been paid to the time spent during the pre-analytical part i.e. transport time of blood cultures from the clinical ward to the laboratory (Murray and Masur, 2012; Willems et al., 2012). Transport times for blood cultures have shown to play a major role in the time it takes to put an accurate sepsis diagnosis (Kerremans et al., 2009a), but to date, few studies on the subject have been published. (Kerremans et al., 2009b; Saito et al., 2009). Consequently, the need to study transport times of blood culture samples, and its implications for total detection time is imminent. To our best knowledge, there are no published studies combining the analysis of clinical data on transport times with the evaluation of an in vitro set-up on delayed incubation of blood culture samples.

The aim of this study was to describe transport times for blood cultures from three tertiary-care hospitals to a university microbiology laboratory and identify predictors of long transport times. We analyzed the relationship between transport time and a number of parameters including type of clinic, time of sampling, day of the week, distance to laboratory and number of transports. Furthermore, the consequences of transport time on detection time of positive blood cultures were studied both in the clinical setting and by utilizing simulated blood culture models.

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2. Materials and methods

2.1. Setting

The Karolinska University Laboratory in Huddinge receives blood culture specimens from the southern part of the greater Stockholm area and surrounding cities and suburbs. The total number of blood culture bottles processed each year is ca 75,000. The laboratory opening hours, including an M.D. microbiologist consultant function are as follows: Monday–Friday: 08:00–18:00, Saturday: 08:00–15:30, Sunday: 08:00–14:00. Blood cultures taken outside of these working hours are not incubated in the blood culture systems until the next day as there are no incubators outside the laboratory. There is no on-call function for neither technicians nor MDs. Blood culture bottles are inoculated at the bedside using a closed Vacutainer needle system and stored in room temperature (RT) until transport to the laboratory. Blood culture specimens received during working hours are processed immediately and incubated in the BacT/ALERT 3D (bioMérieux, Durham, NC, USA) system aerobic (FA) and anaerobic (FN) bottles for detection of bacteria, and in the BACTEC 9240 (BD Diagnostic Systems, Sparks, MD, USA) system Mycosis (Myc) IC/F bottles for detection of yeasts.

2.2. Study design

Included in the study were the following three tertiary-care hospitals: Karolinska University Hospital in Huddinge, Stockholm South General Hospital and Södertälje Hospital in Södertälje. The clinical wards in Karolinska University Hospital in Huddinge are located within the same building as the microbiology laboratory and approximately 3–10 min walking distance away. The Stockholm South General Hospital is situated 17 km away from the Microbiology Laboratory with a travel time of approx. 21 min, and Södertälje Hospital is situated 22 km away with a travel time of approx. 18 min.

The number of transports on weekdays (Saturday, Sunday) from Karolinska University Hospital, Huddinge, Stockholm South General Hospital and Södertälje Hospital are 6 (4, 4), 4 (3, 2) and 2 (1, 1) respectively.

This study was conducted in two parts: (1) Analysis of transport times for blood culture bottles, (2) Laboratory investigation of the possible consequences of delayed entry on total detection time (TDT). The following definitions are included for clarity:

Transport time is the time difference between the time point of sampling in the hospital and the time point at which the blood culture bottles are placed in the BacT/ALERT 3D (bioMérieux) or BACTEC 9240 (Becton, Dickinson and Company) blood culture systems. Transport times are calculated for all samples.

Time to detection (TTD) is the time difference between the time point at which the samples are placed in the blood culture system and the time point at which the blood culture bottle signals for positivity. TTD was thus only calculated for positive samples.

Total Detection Time (TDT) is the time difference between the time point of sampling and the time point at which the blood culture bottle signals for positivity, thus the sum of transport time and TTD.

1) Analysis of transport times for blood culture bottles was done retrospectively for a total of 909 blood culture bottle pairs from 10 different departments from three different hospitals. The departments included were as follows: from Karolinska University Hospital: infection emergency room (ER), infection, intensive care unit (ICU), and surgery ER. From Stockholm South General Hospital: infection, internal medicine, surgery ER and ICU. From Södertälje Hospital: internal medicine and ICU.

In total, 734 negative samples that were randomly selected with approximately 10 from each day of the week were analyzed from each of the 10 departments. A further 175 samples positive for *Escherichia coli* (n = 99) and *Staphylococcus aureus* (n = 76) were included making a total of ca 90 samples from each department. Only one sample per patient was included in the study. The time of sampling and the time of incubation were recorded for each sample.

2) Laboratory investigation of TDT was performed as an in vitro analysis of the consequences of delayed entry on TTD using frozen clinical isolates of 10 different species of bacteria and 2 species of yeasts. The following bacteria were included: *S. aureus*, *E. coli*, *S. epidermidis*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Enterococcus faecium* and *Acinetobacter baumannii*. The following yeasts were included: *Candida albicans* and *C. glabrata*.

For *S. aureus*, *E. coli* and *C. albicans* 3 different strains were used for each species making a total of 18 isolates included in the study. After thawing, the isolates were cultured on a blood (B) agar plate and a Cystein-Lactose-Electrolyte-Deficient (CLED) agar plate in the case of bacterial strains, and on a B agar plate, Sabouraud dextrose agar (SAB) plate and a Chrome (Chrom) agar plate in the case of yeast strains. The resulting colonies from each of the 18 strains were individually dissolved in 1 ml phosphate-buffered saline to a final concentration of 1.0×10^3 CFU/ml for bacterial strains, and 1.0×10^4 CFU/ml for yeasts. To simulate bacteremia, 100 µl of each bacterial suspension was inoculated into one BacT/Alert FA and one BacT/Alert FN blood bottle containing 5 ml blood in order to reach a concentration of approximately 100 CFU/bottle; 100 µl of each yeast suspension was inoculated into one BacT/Alert FA and one Bactec Mycosis IC/F bottle containing 5 ml blood in order to reach a concentration of approximately 1000 CFU/bottle. For this in vitro monomicrobial sepsis model only one organism was inoculated into each blood culture bottle pair. To investigate the effect on TDT of delayed entry of the blood culture bottles into the incubator, one set of each bottle pair was left in room temperature for 2 h, 9.5 h and 19 h before being placed in the incubator. An in vitro polymicrobial sepsis model was also set up by combining 2 different microorganisms accordingly; *S. aureus* + *E. coli*, *E. faecium* + *A. baumannii*, *C. albicans* + *E. coli*, *C. albicans* + *S. aureus*, *C. glabrata* + *E. faecium*, *C. glabrata* + *S. aureus*, *C. albicans* + *C. glabrata*. One set of bottles for each combination was then placed in the incubator with a 2 h, 9.5 h and 19 h delay. The 3 different time points chosen for delayed entry of the blood culture bottles into the incubator represent the 10%tile, average and 90%tile transport time calculated from the statistical analysis in the first part of the study.

All blood culture bottles signaling for positivity were taken out of the incubator and a Gram stain was made in order to rule out any contamination. A selection of positive samples were then cultured on B + CLED plates for bacterial isolates, B + SAB/Chrom plates for yeast isolates and B + CLED + SAB/Chrom plates for mixed bacterial and yeast isolates.

2.3. Statistical methods

The transport time of blood culture bottles from the three hospitals was analyzed by non-parametric Kruskal-Wallis test. The non-parametric Mann–Whitney *U* test was used in comparison of transport times from two different hospitals. One way analysis of variance (Friedman) was used to analyze the differences in TTD and TDT in the paired groups in the in vitro experiments. A P value of < 0.05 was considered statistically significant.

3. Results

3.1. Analysis of transport times

In total, 909 blood culture bottles were analyzed. The median with interquartile range (IQR) transport time for all samples was 9 h (3–