Eur J Clin Microbiol Infect Dis (2001) 20:594–595
DOI 10.1007/s100960100559

BRIEF REPORT

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Rapid Identification of *Streptococcus pneumoniae* in Blood Cultures Using a Latex Agglutination Assay

Published online: 9 August 2001

Streptococcus pneumoniae is a major etiologic agent for bacteremia worldwide. Pneumococcemia requires early recognition in order for its prognosis to be improved [1]. Currently, the preliminary identification of pneumococci from blood cultures relies on direct Gram stain smears, but, as Merlino et al. [2] recently reported, the interpretation of these smears can be problematic, resulting in delayed initiation of appropriate therapy. In this report, we review our experience using a commercial latex agglutination (LA) assay for the preliminary identification of pneumococci directly from blood culture bottles.

Blood cultures performed on samples collected from patients at our institution were analyzed from January 1996 to June 2000. Three sets of blood cultures were performed for each adult patient in whom bacteremia was suspected. Each culture set consisted of 20 ml of blood, which was distributed equally between BacT/Alert (Organon Teknika, Spain) aerobic and anaerobic FAN bottles. On arrival in the laboratory and after venting of the aerobic bottles, all of the inoculated bottles were placed in the BacT/Alert instrument for a 5-day incubation period at 35°C. During the incubation period, the bottles were monitored following the manufacturer's recommendations. Bottles flagged as positive by the instrument were Gram stained, and aliquots of the bottles were subcultured onto adequate media. All microorganisms were identified to the species level using standard microbiologic procedures.

Blood cultures for which a direct Gram stain smear showed the presence of gram-positive cocci arranged in pairs or short chains and those with suspicious signs of pneumococcal autolysis, like gram-negative diplococci, were tested for the presence of pneumococcal capsular antigen. A commercial LA assay, Slidex Pneumo-kit (bioMérieux, Spain) was used according to the manufacturer's specifications. Briefly, 1 ml from the positive blood culture bottle was centrifuged at 1,000×*g* for 10 min. One drop of the specimen supernatant and one drop of the latex reagent were dispensed onto a clean slide. After stirring the two drops, the slide was rotated for a maximum of 2 min and was read macroscopically under normal lighting conditions. The appearance of a clearly visible, homogeneous agglutination was considered a positive result. Any other results, like slight or nonhomogeneous aggregates, were considered negative.

In order to detect possible cross-reactions, we experimentally seeded 67 strains from our collection (30 pneumococci, 9 viridans streptococci, 3 *Enterococcus faecalis*, 6 coagulase-negative staphylococci, 2 *Staphylococcus aureus*, 1 *Corynebacterium* spp., 2 *Haemophilus influenzae*, 6 *Escherichia coli*, 3 *Klebsiella pneumoniae*, 2 *Proteus mirabilis*, 2 *Pseudomonas aeruginosa*, 1 *Candida albicans*) into uninoculated bottles. Suspension equivalent to a 0.5 McFarland standard was prepared from each strain. A volume of 0.1 ml of each suspension plus 4 ml of blood was inoculated into BacT/Alert FAN aerobic bottles and introduced into the instrument. After being signaled as positive by the BacT/Alert system, they were processed as described above.

A total of 191 first positive blood culture bottles were tested (mean of 42 specimens per year, each obtained from a separate patient). The distribution of isolates obtained was as follows: 130 pneumococci, 26 viridans streptococci, 18 *Enterococcus faecalis*, 2 *Enterococcus faecium*, 6 *Streptococcus agalactiae*, 2 *Streptococcus pyogenes*, 1 group G streptococcus, 3 *Staphylococcus aureus*, 1 *Listeria monocytogenes*, 1 *Aerococcus viridans*, 1 *Lactococcus* spp. The LA test resulted positive for the 130 pneumococcal specimens and negative for the other 61 specimens. Regarding the simulated blood cultures, the assay was positive for the 30 samples known to contain pneumococci and negative for the other 37 samples.

Streptococcus pneumoniae bacteremia is associated with high morbidity and mortality. Therefore, rapid recognition of pneumococci is necessary in order to optimize therapy and improve prognosis [1]. When Gram stained, pneumococci can appear with a wide variety of

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morphological characteristics, due to the production of autolysins [2]. Interestingly, in our series no specimens with suspicious signs of autolysis were found. This might be related to the use of BacT/Alert FAN bottles, which have been reported to prevent pneumococcal autolysis [3]. The problems inherent in interpreting direct blood culture Gram stain smears can delay the diagnosis by 24–48 h [2]. Thus, complementary methods for the rapid diagnosis of pneumococcemia are required.

Several reports have focused on the use of LA tests to diagnose pneumococcal bacteremia directly from blood cultures [4, 5, 6, 7, 8, 9]. The overall sensitivity and specificity values reported were 93.3% (range, 88.3–100%) and 94.2% (range, 83.3–100%), respectively, while the positive and negative predictive values were 90% and 96.2%, respectively. These results are comparable to ours, which revealed 100% sensitivity, specificity, and positive and negative predictive values. Various publications have reported that cross-reactions with other bacteria occurred when using LA tests, mainly with group C streptococci and certain strains of viridans streptococci [4, 5, 8]. We did not encounter this problem; however, this may be due to the selective protocol we used, whereby only specimens with a Gram stain compatible with pneumococci underwent the LA test. Thus, the combined results show that LA assays are inexpensive, rapid (less than 30 min), simple to perform, easy to interpret, and can remain positive even when no viable bacteria are available in the blood culture [4, 10]. However, in order to establish a definitive diagnosis, results require confirmation by culture.

Other methods for the rapid diagnosis of pneumococcal bacteremia are also available. A well-known conventional method, the direct modified bile solubility test, is accurate but time-consuming, and expertise is required for interpretation [6, 7]. Pneumococcal genomic detection using polymerase chain reaction assays has shown promising results, as communicated in several reports [11, 12, 13]. These techniques are highly sensitive and specific, and they can provide positive results even when culture-negative samples are used [13]; however, they are still expensive, time-consuming (hours), highly complex, and lacking minimum standardization. Given these drawbacks, these methods should, perhaps, be reserved for research purposes. In an initial report, specific DNA probes showed good results when used directly in positive blood cultures [14], but these findings need to be confirmed by further studies.

In conclusion, pneumococcal latex agglutination assays performed using positive blood cultures are accurate, easy to perform and interpret, and require no sophisticated instrumentation. Thus, they can be considered a valuable tool in the rapid, preliminary diagnosis of *Streptococcus pneumoniae* bacteremia.

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