Rapid Evaluation of Pathogenic Strains by Gram Staining Using Positive Blood Cultures

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Objective

Blood culture tests are critical for cases of suspected severe infection. To allow treatment to be started, results of these tests must be reported immediately. However, at least 24 hours is needed to identify pathogenic bacteria after a blood culture becomes positive. Thus, we examined whether pathogenic bacteria could be evaluated more quickly through Gram staining of positive blood cultures, without waiting for the results of culture-based identification.

Materials

Materials were 1,004 culture bottles, proven to be positive for bacteria by blood culture, from March 2009 through April 2010.

Methods

Media, in which bacterial growth was detected with fully automatic blood culture equipment, were transferred to blood-collecting tubes and centrifuged. The tubes were subsequently decanted. Then, the residual liquids were used as bacterial solutions, which were subjected to bacterial identification based on Gram staining and culture. The concordance rates between these 2 identification methods were determined.

Gram-positive cocci identified with Gram staining were classified as follows: *Staphylococcus* spp. for clustered cultured bacteria, *Enterococcus* spp. for a mixture of diplococci and streptococci, and *Streptococcus* spp. for only long streptococci (**Fig. 1**). Gram-negative bacilli were classified as follows: *Enterobacteriaceae* for strongly gram-negative thick bacilli and glucose-nonfermenting bacteria for weakly stained elongated bacilli. The identifiable strains were classified as *Enterococcus faecalis*, *Pseudomonas aeruginosa*, or *Acinetobacter baumannii*.

Results

The concordance rates between identification based on Gram staining and that based on culture were as follows: 99% (444 of 448) for *Staphylococcus* spp., 79% (94 of 119) for *Enterococcus* spp., 76% (38 of 50) for *Streptococcus* spp., 76% (233 of 307) for *Enterobacteriaceae*, and 42% (49 of 117) for glucose-nonfermentative bacteria (**Fig. 2**). The specificity of strain identification was as follows: 44% (11 of 25) for *E. faecalis*, 71% (30 of 42) for *P. aeruginosa*, and 56% (10 of 18) for *A. baumannii*.

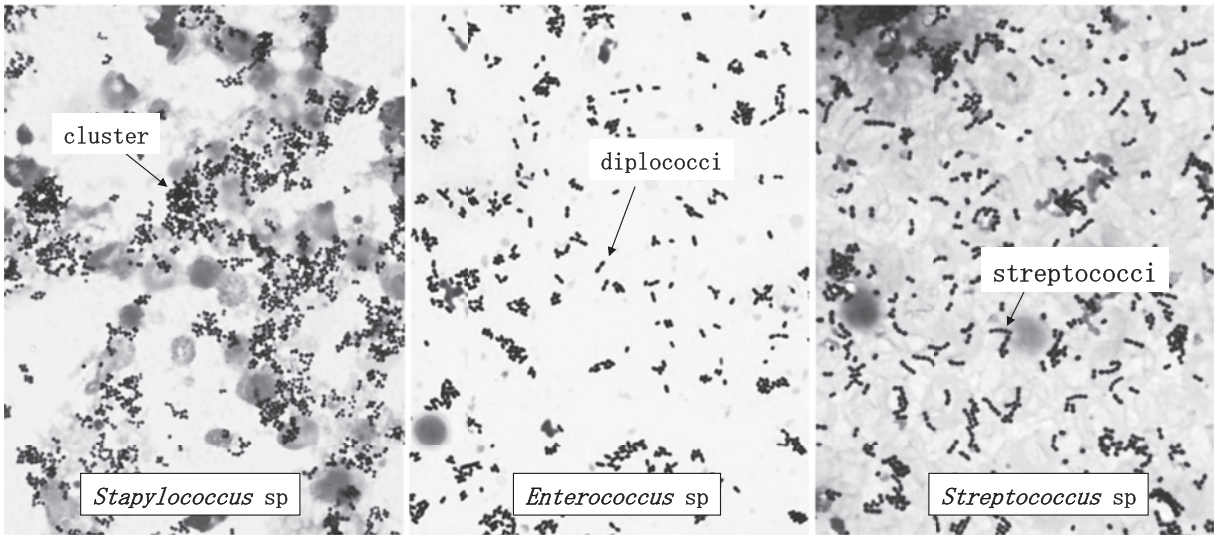

Fig. 1 Discrimination Gram-positive-coccus by Gram staining

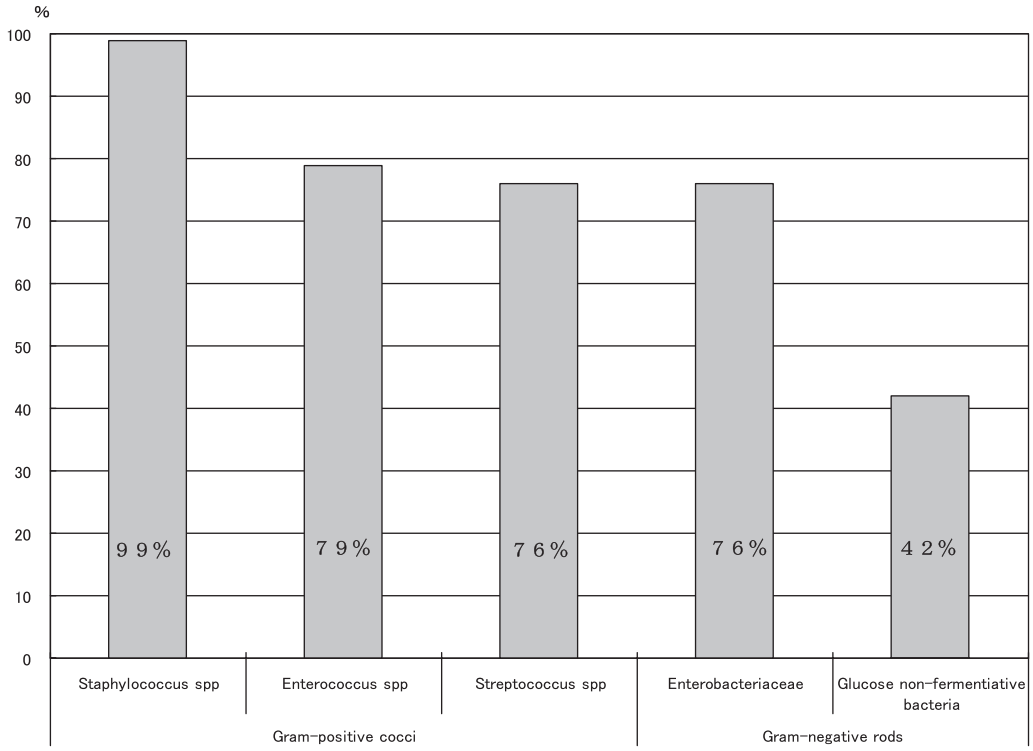

Fig. 2 Concordance rates between Gram-staining and culturebased identification.

Conclusion

The results demonstrate high concordance rates (76% or higher) for all bacteria except glucose-nonfermentative bacteria. However, *P. aeruginosa* is presumably identifiable by means of Gram staining because *P. aeruginosa* included in glucose-nonfermentive bacteria showed a specificity of 71%.

These results suggest that the Gram staining of positive blood cultures allows rapid evaluation of the pathogenic bacteria and could facilitate the selection of antibiotics for treating infections by bacteria with high concordance rates and high-level specificity, such as *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Enterobacteriaceae*, and *P. aeruginosa*.