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RESEARCH NOTE

Rapid culture and identification: a practical method for early preliminary laboratory diagnosis of sepsis

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ABSTRACT

This study describes the development of a method for rapid preliminary species identification of bacteria from positive blood culture vials. The method yielded preliminary identification results for 496 (92%) of 541 positive blood cultures within 5 h. The method was capable of identifying the most frequently isolated bacteria (i.e., *Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli*, *Streptococcus pneumoniae* and *Enterococcus* spp.) to the species level. The method can be established easily, with a materials cost of 2–5 Euros per sample.

Keywords Blood cultures, diagnosis, identification, rapid, sepsis, spot tests

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Early initiation of adequate antibiotic treatment in cases of sepsis significantly reduces mortality, morbidity and hospital costs [1]. Rapid species identification could help to correct inadequate empirical antimicrobial treatment. Rapid molecular-based species identification methods have been developed, but these are expensive, are labour-intensive and require special equipment [2,3]. The present study describes the development of a method for rapid preliminary species identification from positive blood culture vials. Significant growth of bacteria from blood culture vials was obtained within a few hours on agar plates.

Data were collected prospectively at Karolinska University Hospital, Huddinge, Sweden, during 6 months in 2006. In total, 541 positive blood cultures were included in the study, with only one culture for each patient. The method was also tested using nine samples from sterile body sites other than blood. The Bactec 9240 automated blood culture system with both aerobic and anaerobic vials (Becton Dickinson, Sparks, MD, USA) was used in the study. The results were compared with those obtained by conventional microbiological methods.

In preliminary experiments to determine the optimal incubation time and amount of inoculum for rapid culture, reference strains of *Escherichia coli* and *Staphylococcus aureus*, as well as eight clinical samples, were inoculated in blood culture vials together with 5 mL of horse blood. When the automated blood culture system signalled growth, samples of the blood culture were analysed. The results revealed that five drops yielded optimal growth and provided the best discrimination of single colonies after a 4-h incubation period.

Accordingly, five drops of blood culture (n = 541) were then subcultured in duplicate on blood agar, chocolate blood agar with haematin, and cysteine lactose electrolyte-deficient agar, followed by incubation at 37°C for 4 h in both an aerobic and a CO_2 5% v/v atmosphere. When anaerobic bacteria were suspected, similar amounts of culture were subcultured on brucella

agar plates and incubated anaerobically at 37°C for 4 h.

Preliminary identification of the infecting pathogen was performed using a panel of validated desktop spot tests, including catalase, oxidase, indole spot and L-pyrrolidonyl-β-naphthylamide (Remel Inc., Lenexa, KN, USA) tests, agglutination tests for *Staph. aureus* (Staphaurex latex test; Remel Europe Ltd, Dartford, UK), group A, B, C, D and G streptococci, *Streptococcus pneumoniae* (Oxoid, Basingstoke, UK) and *Salmonella* spp. (Reagensia, Stockholm, Sweden), and antibiotic disk tests, including nitrocefin (Biodisk, Solna, Sweden), optochin (ethyl hydrocupreine), vancomycin, aztreonam, linezolid, ampicillin and metronidazole (Oxoid). In the last phase of the study, 4-nitrophenyl-β-D-glucopyranosiduronic acid plates were used in combination with a positive indole spot test for the preliminary diagnosis of *E. coli* [4]. Ampicillin disks were used to detect ampicillin-resistant enterococci, and nitrocefin disks were used to detect β-lactamase-producing *Haemophilus* spp.

Fig. 1 shows the algorithm that was developed to guide the preliminary identification of bacteria following rapid culture. Depending on the result

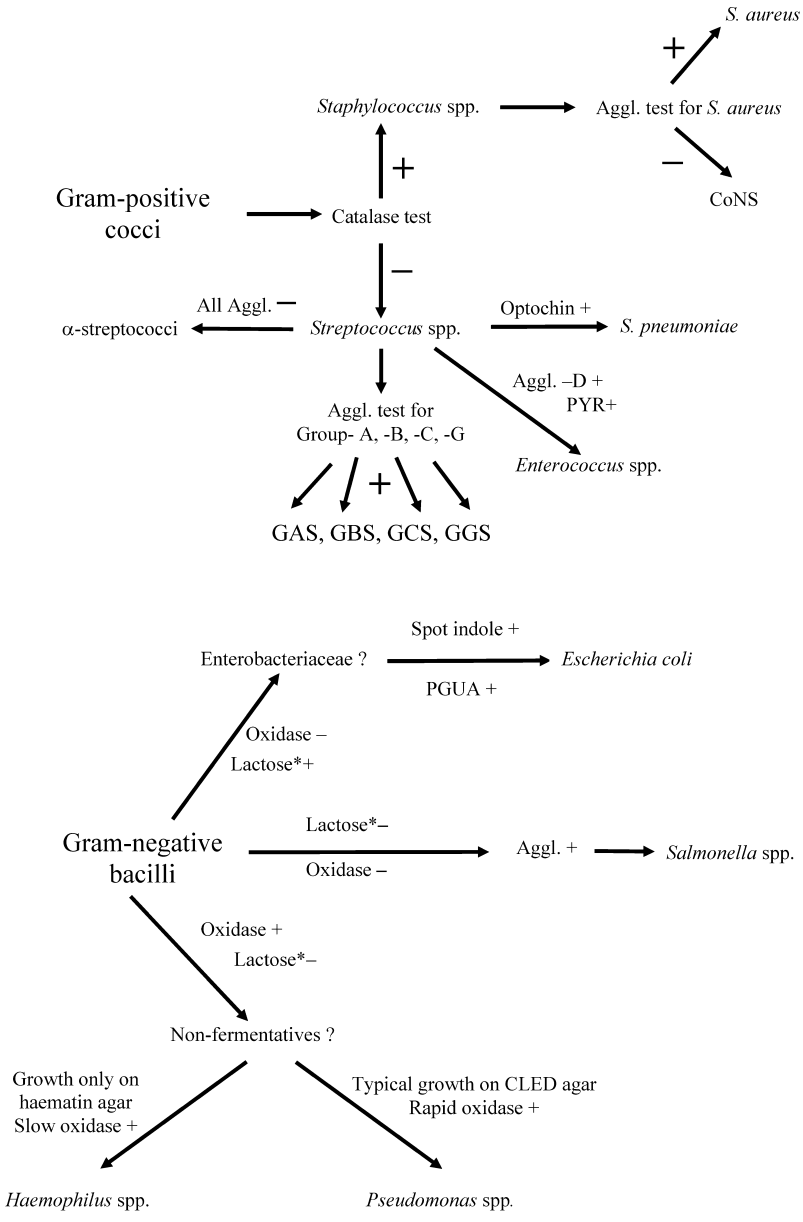

Fig. 1. Algorithm for the preliminary identification of bacteria following rapid culture. *Lactose-fermenting organisms changed the colour of the cysteine lactose electrolyte-deficient (CLED) agar from green to yellow. GAS, group A streptococci; GBS, group B streptococci; GCS, group C streptococci; GGS, group G streptococci; Aggl., agglutination; CoNS, coagulase-negative staphylococci; PGUA, 4-nitrophenyl-β-D-glucopyranosiduronic acid.

Table 1. Discrepancies in identification between the rapid culture and conventional identification methods

Rapid culture and identification	Conventional method
CoNS	*Rothia mucilaginosa*
CoNS	*Micrococcus* spp.
Group D streptococcus	*Streptococcus salivarius*
Group D streptococcus	*Enterococcus faecium*
Group D streptococcus	*Enterococcus faecium*
Staphylococcus aureus	*Micrococcus* spp.
Enterococcus faecalis	*Enterococcus faecium*
Streptococcus pneumoniae	*Streptococcus salivarius*
Streptococcus pneumoniae	α-Haemolytic streptococcus
α-Haemolytic streptococcus	*Streptococcus pneumoniae*

CoNS, coagulase-negative staphylococci.

of the initial Gram's stain, a limited number of tests were chosen and used to identify each individual pathogen following rapid culture (Fig. 1). Certain bacteria, e.g., *Streptococcus pyogenes*, could be identified to the species level, while others could only be identified to the genus level (e.g., *Enterococcus* spp.) or to the family level (e.g., certain members of the Enterobacteriaceae).

Antibiotic and diagnostic disks were used to identify bacteria. In order to confirm the results of the initial Gram's stain, aztreonam and linezolid disks were used. When *Strep. pneumoniae* was suspected, an optochin disk was placed on blood agar plates in later phases of the study, and this procedure identified correctly all 35 pneumococci [5]. Catalase and oxidase tests were used for preliminary identification of *Corynebacterium* spp. and *Neisseria meningitidis*.

Samples from positive anaerobic blood culture vials were also inoculated on brucella agar plates with metronidazole and vancomycin disks and incubated anaerobically for 4 h. An inhibition zone around the metronidazole disk, in combination with an absence of aerobic growth, indicated the presence of anaerobic bacteria. During the study period, five rapidly growing strains of *Clostridium* and *Bacteroides* spp. were identified. However, five other, slow-growing anaerobic isolates did not grow after incubation for 4 h.

The rapid culture and identification method yielded preliminary identification results for 496 (92%) of the 541 blood cultures within 5 h. Twenty (4%) isolates did not yield sufficient growth after 4 h, and a further 25 isolates grew, but 15 (3%) could not be identified using the present algorithm (Fig. 1). Ten (2%) isolates were misdiagnosed using the current algorithm (Table 1). The subsequent use of additional complementary tests (e.g., optochin disk and L-pyrrolidonyl-β-naphthylamide tests) prevented misdiagnoses in the later phases of the study. Identification to the species level was possible for 192 (39%) samples, with a further 139 (28%) and 165 (33%) isolates being identified to the group and family levels, respectively. The method was also successful with nine samples from sterile body sites other than blood, comprising four samples from bone marrow, three cerebrospinal fluids, one peritoneal dialysis effluent and one synovial fluid.

In conclusion, the present study describes a rapid 4-h method for preliminary identification of bacteria in blood culture vials. The method had a sensitivity of 92%, which made it possible to alert physicians within 5 h of the automated blood culture system detecting growth. It was possible to make a preliminary identification of 67% of the isolates to the species or group level. The method was capable of identifying the most frequently isolated bacteria, i.e., *Staph. aureus*, coagulase-negative staphylococci, *E. coli*, *Strep. pneumoniae* and *Enterococcus* spp., to the species level [6]. Preliminary identification to the group level was clinically relevant for Enterobactericeae, non-fermentative Gram-negative bacilli and anaerobic bacteria.

Unfortunately, it was not possible to identify pathogens such as methicillin-resistant *Staph. aureus*, vancomycin-resistant enterococci or enteric bacteria producing extended-spectrum β-lactamases. However, these organisms can be identified using a range of molecular methods. Another problem concerns possible misidentification of certain uncommon pathogens, e.g., *Acinetobacter*, *Stenotrophomonas* or *Listeria*. However, the method described in this report can be established easily, with <5 min required to perform the spot tests and a materials cost of only 2–5 Euros per sample. The method may be used as an alternative to, or in combination with, molecular techniques for preliminary rapid species identification of infecting pathogens in blood cultures.

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RESEARCH NOTE

Risk-factors for emerging bloodstream infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*

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ABSTRACT

Risk-factors for bloodstream infections caused by extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* were investigated using an exploratory case-double control study in which 43 cases (70% producing CTX-M enzymes) were compared with: (i) 86 patients with bacteraemia caused by non-ESBL-producing *E. coli*; and (ii) 86 hospitalised patients. Previous follow-up as an outpatient, urinary catheterisation and use of oxyimino-β-lactams or fluoroquinolones were independent risk-factors for ESBL-producing *E. coli* among patients with *E. coli* bacteraemia, and previous use of oxyimino-β-lactams or fluoroquinolones were also independent risk-factors among hospitalised patients. These findings may help in identifying patients at greater risk for bloodstream infection caused by ESBL-producing *E. coli* in endemic areas.

Keywords Bacteraemia, *Escherichia coli*, extended-spectrum β-lactamases, hospitalised patients, risk-factors

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Escherichia coli producing extended-spectrum β-lactamases (ESBLs) are an emerging cause of community and nosocomial infection, and are associated particularly with the worldwide spread of CTX-M types of ESBL [1,2]. The treatment options for patients with infections caused by these organisms are limited. As is the case with ESBL-producing *Klebsiella pneumoniae* [3], treatment with cephalosporins or fluoroquinolones of bloodstream infections caused by ESBL-producing *E. coli* is associated with a poorer prognosis than is carbapenem therapy [4]. As bacteraemia caused by *E. coli* is frequent, this could result in a significant increase in the use of carbapenems, which might contribute to further spread of carbapenem resistance. Knowledge of risk-factors for bacteraemia caused by ESBL-producing *E. coli* might help to identify patients at higher risk, who should therefore receive empirical coverage against these organisms, and could also permit the identification of potential intervention strategies. The objective of this study was therefore to investigate the risk-factors for bacteraemia caused by ESBL-producing *E. coli* in a non-epidemic situation.

A case-double control study with prospective recruitment was carried out in the Hospital Universitario Virgen Macarena, a 950-bed hospital in Sevilla, Spain that provides care for a population of 550 000. The case-patient group included all 43

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