Introduction of Gram negative microrganisms rapid identification and direct susceptibility testing to reduce turnaround time in blood cultures

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Summary

Background. Bloodstream infections and sepsis are a major cause of morbidity and mortality. The successful outcome of patients suffering from bacteremia depends on a rapid identification of the infectious agent to guide optimal antibiotic treatment. Beginning antimicrobial therapy early is vital for the treatment of bloodstream infections and sepsis. Reducing the time for microbial identification and antimicrobial susceptibility testing could decrease the average time needed for an appropriate antimicrobial therapy which leads to a decrease in mortality, a shortened hospital stay, and lower hospitalization costs.

Methods. The direct identification and the antibiotic susceptibility testing evaluated with disk diffusion directly from blood cultures provided excellent results in Gram-negative.

Results and Conclusions. Our study reveals the importance of direct methods that significantly reduce the turn around time and therefore has an impact on the successful outcome of patients suffering from bloodstream infections.

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Introduction

Sepsis is a serious medical condition caused by microbial pathogens in the blood and it is associated with high rates of morbidity and mortality (20-70%) (3, 5), it is a health emergency and clinically suspected of bloodstream infections require the prompt analysis. The identification of microorganisms in the blood is crucial for early and appropriate antimicrobial therapy (10) indeed the outcome of sepsis is dependent on appropriate and timely treatment (8, 9): the rapid identification of pathogens and availability of antimicrobial susceptibility testing (AST) is imperative. Every hour of delay in initiation of appropriate antimicrobial therapy increases the mortality by 7.6% in patients with septic shock (9). The gold standard for diagnosing sepsis is still blood culture. A serious disadvantage of this test is that it can take up to 48-72 h before the final result is available and during this period, the condition of a septic patient can rapidly deteriorate. Therefore, it is common practice to start therapy with broad-spectrum antibiotics before the results of blood culture are known. Significantly reducing turnaround time (TAT) for microbial identification and antimicrobial therapy leading to decrease in mortality, shortened hospital stay and lower hospitalization costs (2, 4). The TAT of blood culture is influenced by: i) the time that a positive signal is emitted by the automatic instrument; ii) the time necessary for bacterial identification and AST. Standardized protocol for AST from positive blood cultures require the overnight subcultures on agar to prepare a standard inoculum according to the manufacturer's guidelines. It usually takes 48-72 hours to obtain ID and AST. To overcome these disadvantages, molecular methods and mass spectrometry were evaluated for rapid identification from positive BCs. The comparison between these tecniques with standard methods showed good agreement regarding the identification (1, 11, 13), but these reflect the very important limit regarding the sensitivity of AST correlated (6). To reduce TAT, in our laboratory, we introduced rapid, reliable and inexpensive methods (7) for early identification of potential pathogens in BCs for early organism identification and detection of correlated AST (Figure 1).

This procedure expected direct identification from positive BCs by VITEK2 and AST by direct E-test which is easy to set up with low risk for contamination. The purpose of our study was to evaluate the correlation by direct method and standard procedure (VITEK and E-test by subculture). According to Jorgensen (8), we proposed that the level of acceptable accuracy for isolates falsely susceptible, Very Major Error (VME), should be ≤3% and that the combination of false resistance, major error (ME), and errors not included in the first two categories (different value MIC) minor Error (mE), should be ≤7%. Our goal is to give information to the clinicians as soon as possible, to improve the proportion of appro-

priate antimicrobial therapy once the results of the microbiological sample cultures were available.

Materials and Methods

Inclusion criteria

In this study, positive BCs of hospitalyzed patients were included (age 18-70). We analyzed a total of 291 monomicrobial BCs positive for Gram negative bacteria at microscopical examination. All BCs draw before starting antimicrobial therapy and were tested by standard procedure and *direct method*.

Standard procedure

At the onset of fever, 1 set of blood cultures (aerobic/anaerobic and fungal) was taken by sterile venipuncture and processed using Bactec 9240 (Becton Dickinson, Heidelberg, Germany) and followed up 15 min with a second set of BCs and followed up 15 min with a third set of BCs. When the BCs gave a positive signal, Gram staining was carried out. An aliquot of positive BC was plated onto solid media and incubated for 24/48 h, and identification was carried out with a Vitek 2 system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's protocol.

Direct method

Briefly, the direct method consists in drawing 8 mL of BC broth which were centrifugated in a tube with a separator gel at 3000 rpm for 15 minutes. The bacterial suspensions was dissolved in saline solution at a concentration of 0.5 Mc Farland. This inoculum was used on Vitek 2 for microorganism identification and the different antibiotics were tested with diffusion methodology on Muller Hinton agar (E-test) using a shorter incubation time (6 h for Gram negative isolates). The drugs tested in the study, according to the clinicians, were: Piperacillin/tazobactam, Cefotaxime, Gentamicin, Amikacin, Ertapenem, Imipenem, Meropenem and Colistin.

Interpretation of the data

The results obtained by direct method were compared to the standard procedure considered gold standard. The following error classes have been established: Very Major Error (VME) variation of interpretation from Sensitive (S) to Resistant (R), Major Error (ME) variation of interpretation from R to S, mE (minor Error) and errors not included in the first two categories (different value MIC).

Results

In this study, we analyzed 291 blood cultures from which we isolated the following microorganisms: *Escherichia coli* (n=135), *Klebsiella pneumoniae* (n=41), *Proteus mirabilis* (n=17), *Stenotrophomonas maltophilia* (n=6), *Enterobacter cloacae* (n=7), *Enterobacter aerogenes* (n=5), *Acinetobacter baumannii* (n=23), *Ralstonia picketii* (n=3), *Aeromonas hydrophila* (n=5), *Pseudomonas aeruginosa* (n=39), *Serratia marcescens* (n=7), *Salmonella group B* (n=1), *Campylobacter jejuni* (n=1).

Identification by Vitek2

Standard procedure vs. *direct method*

N. 274 of 291 (94.15%) Gram-negative rods were correctly identified at the species level by VITEK2 performed directly from positive blood culture (Table 1). The direct method failed to identify the following microrganisms: n.1 *E.cloacae*, n. 7 *E.coli*, n. 1 *R.pickettii*, n. 3 *A. baumannii*, n.1 *A. hydrophila*, n. 2 *K. pneumonia*, n. 1 *P. Mirabilis,* n.1 *P.aeruginosa.*

E-test standard procedure vs. *direct method*

For the AST in Enterobacteriaceae strains by direct method (reading at 6 hours), in respect to the AST evaluated with E test by

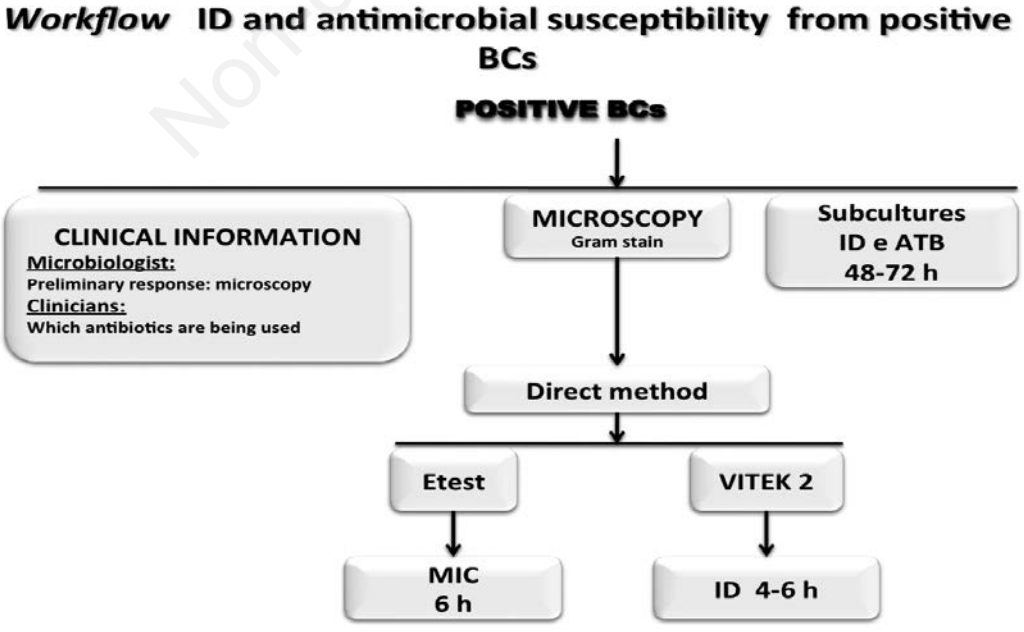

Figure 1. Direct method for identification and antimicrobial susceptibility testing.

subculture we obtained a concordance equal to 97.2% (n. 207/213) for Piperacillin/tazobactam, 99.5% (n. 212/213) for Gentamicin, Cefotaxime, 99% for Imipenem (211/213), and 100% (213/213) for Amikacin and Colistin (Figure 2).

For Piperacillin/Tazobactam, we detected n. 2 ME (0.9%) which concern *E.coli*, resistant by E-test from direct method but susceptibile by E-test from subculture; n. 4 mE (1.8%) which concern n.2 *E. coli* and n.2 *K. pneumoniae* with the value MIC higher than a dilution by E-test from rapid method in respect to subcultures but the same interpretation of sensitivity. For Imipenem we detected n.2 (1%) mE which concern *P. mirabilis* (the value MIC was higher than two dilution by E-test from rapid method in respect to subcultures, the interpretation of sensitivity was however confirmed).

For Gentamicin, we detected n.1 ME (1%) which concerns *K. pneumoniae*, (the drug resulted resistant by E-test from direct method but susceptibile by E-test from subculture). For Cefotaxime, we detected n.1 VME (1%) which concerns *K. pneumoniae*, (the drug resulted susceptibile by E-test from direct method but resistant by E-test from subculture), and n.2 mE which concern n.*2 E. coli* with the value MIC higher than a dilution by E-test from rapid method in respect to subcultures but the same interpretation of sensitivity. For the AST in other Gram negative (non Enterobacteriaceae) by direct method (reading at 6 hours), in respect to the AST evaluated with E-test by subculture we obtained a correlation equal to: 97.4% (n. 76/78) for Piperacillin/tazobactam, 98.8% (77/78) for Amikacin, Cefotaxime and Carbapenemes, 100% (n.78/78) for Gentamicin, and Colistin (Figure 2). For Pip/tazo we detected n. 2 mE (2.5%) which concerns *P.aeruginosa* (the value MIC was higher than a dilution by E-test from rapid method in respect to subcultures, the interpretation of sensitivity was however confirmed). For Cefotaxime we detected n. 1 mE which concerns *P.aeruginosa* (the value MIC was higher than a dilution by E-test from rapid method in respect to subcultures, the interpretation of sensitivity was however confirmed). For Amikacin, we detected n.1 VME (1.3%) which concerns *P.aeruginosa,* (the drug resulted susceptibile by E-test from direct method but resistant by E-test from subculture). For carbapenemes we detected n.1 ME which concern *A. baumannii*.

Table 1. Identification direct method *versus* standard procedure.

Identification microrganism	Direct method	Subculture	Total	Concordance (%)
E. coli	128	135	135	94.8
K. pneumoniae	39	41	41	95,1
P. mirabilis	16	17	17	94.1
S. marcescens	7	7	7	100
E. cloacae	6	7	7	85,7
E. aerogenes	5	5	5	100
Salmonella group B	1	1	1	100
S. maltophilia	6	6	6	100
A. baumannii	20	23	23	86,9
R. pickettii	2	3	3	66.6
A. hydrophila	4	5	5	80
P. aeruginosa	38	39	39	97.4
C. jeujuni jejunii	1	1	1	100
C. braakii	1	1	1	100

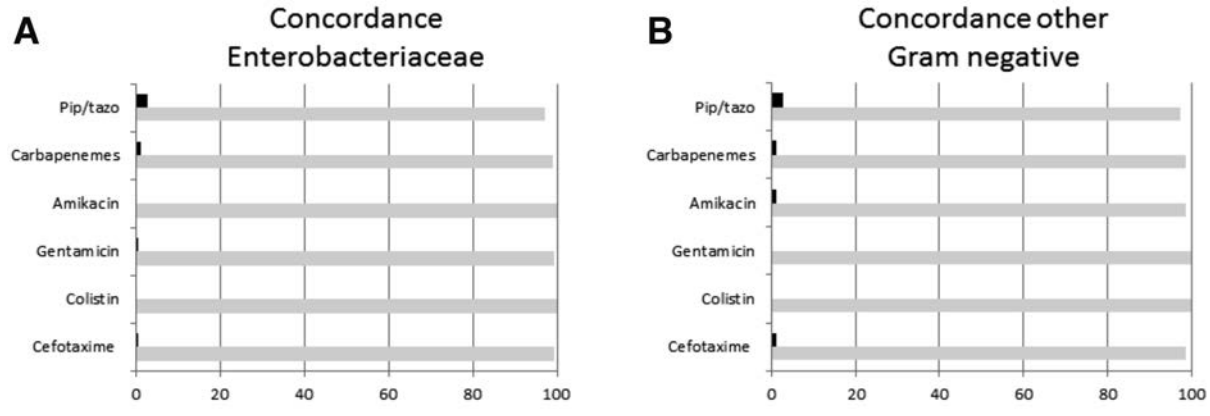

Figure 2. Concordance E-test in Enterobacteriaceae (A) and other Gram negative (B).

Discussion and Conclusions

The outcome of patients with bloosdtream infection is dependent upon rapid administration of appropriate therapy. In an era of increasing antimicrobial resistance, rapid susceptibility testing is imperative. The long TAT which characterizes traditional blood culture methods encourages the microbiologist to introduce rapid diagnostic techniques which will bring to a correct therapeutic intervention. It is essential that an antimicrobial susceptibility test system provide reproducible results and that the results generated by the system can be comparable to the results determined by an acknowledged *gold standard* reference method. In this study, we examined the validity of rapid identification and antimicrobial susceptibility testing of Gram negative microorganism directly from positive blood cultures (direct method) to reduce TAT in blood cultures. We compared the direct method to conventional procedure for etiological diagnosis of suspected bloodstream infections. Our study shows that performing identification directly from blood cultures with VITEK2 provided excellent, even though it is important specify that *Salmonella, Campylobacter* and *Citrobacter* the agreement is just based on one single results. In this study, the antibiotic susceptibility testing evaluated with E-test directly from blood cultures provided excellent results in Gram-negative. Indeed, the E-test with direct method showed a strong agreement with all tested drugs and, according to Jorgensen, our results were considered acceptable since VME was ≤3% and the combination of ME and mE was ≤7%. In our study, the level of VME (false susceptibility) with direct E test were equal to 0.47% for Cefotaxime in Enterobacteriaceae and equal to 1.3% for Amikacin *in P. aeuriginosa*.

The level of ME (false resistance) + mE (different value MIC but interpretation of resistance/sensitivity confirmed) with direct E test were equal to 2.7% for Piperacillin/Tazobactam, 0.9% for Carbapenemes and 0.47% for Gentamicin in Enterobacteriaceae and 2,5% for Piperacillin/Tazobactam, 1.3% for Cefotaxime and carbapenemes in other Gram negative microrganism. Currently, considering the results obtained, our workflow is based upon quickly reporting to the clinician the results of the microscopic examination which provides guidance on the drugs to be tested by E-test. This allows, within 12 hours, to define the microorganism identification and AST correlated, guiding the clinician towards a timely and targeted therapy. Our systematic approach of rapid diagnostic methods reduces the TAT that, in serious pathologies like sepsis, is very important to ensure the positive outcome to the patient.

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