

## ORIGINAL ARTICLE

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# Direct identification and susceptibility testing of enteric bacilli from positive blood cultures using VITEK (GNI+/GNS-GA)

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**Objective** To study the possibility of reporting results of identification and susceptibility testing of Gram-negative bacilli the same day as bacteremia is detected by using direct inoculation from positive blood cultures (Bactec 9240) into VITEK GNI+ and GNS-GA cards.

**Methods** All blood cultures with Gram-negative enteric bacillus-like morphology on microscopy found to be positive on workdays between 15 June 1999 and 29 February 2000 were included. Identification and susceptibility testing were done by three methods: the direct method using a suspension made by differential centrifugation of positive blood culture broth for inoculation of the VITEK cards; the standard method using an inoculum made from an overnight culture on a solid media; and the routine method (reference method) using conventional testing.

**Results** Of 169 isolates, the direct method resulted in 75% correct identifications, 9% misidentifications and 17% non-identifications. All misidentified isolates were *Escherichia coli*, of which 80% were reported as *Salmonella arizonae*. Five biochemical tests yielded most of the aberrant results; correcting the citrate and malonate reactions in most cases led to correct identification by the VITEK database. Despite a negative H<sub>2</sub>S reaction, 11 *E. coli* isolates were reported as *S. arizonae*. Two-thirds (69%) of identifications were reported within 6 h, and 95% of these were correct. The direct susceptibility testing method was assessable for 140 isolates. Correct results were found in 99% of isolate-antimicrobial combinations, and 85% were reported within 6 h.

**Conclusion** The direct VITEK method could correctly report identifications and susceptibility patterns within 6 h, making same-day reporting possible for almost two-thirds (63%) of bacteremic episodes with Gram-negative bacilli. These results could probably be improved by modification of the identification algorithms of the VITEK software.

**Keywords** Gram-negative blood cultures, direct identification, direct susceptibility testing, VITEK, GNI+ card, GNS-GA card

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## INTRODUCTION

Detection of bloodstream infections is one of the most relevant tasks performed by the clinical microbiology laboratory. Rapid bacterial identifi-

cation and susceptibility testing in bacteremic patients improves patient therapy and outcome, and reduces patient cost [1–3].

Both automated blood culture systems and automated systems for identification and susceptibility testing of bacteria have been on the market for a number of years. Several studies have explored the possibility of combining these systems to achieve rapid identification and susceptibility testing by direct inoculation from positive blood cultures [4–8]. However, the clinical performance

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is dependent on which systems are used and a thorough evaluation of each combination is recommended [9]. The VITEK Gram-negative identification plus (GNI+) card (bioMérieux, La-Balme-Les-Grottes, France) has only recently been introduced to the market (in 1997). Evaluation and comparison studies have been performed [10–12], but the accuracy of identification with the VITEK GNI+ card when inoculated directly from positive blood cultures has not been studied so far.

In order to study the possibility of reporting results of identification and susceptibility testing of Gram-negative bacilli on the same day as the bacteremia is detected, we evaluated the accuracy and speed of direct identification (GNI+ card) and susceptibility testing (GNS-GA card) using the VITEK.

## MATERIAL AND METHODS

Blood cultures were screened for growth of microorganisms using the Bactec 9240 system (Becton Dickinson, Cockeysville, MD, USA). The study period was daytime on workdays between 15 June 1999 and 29 February 2000. One blood culture set consisted of two Bactec PLUS aerobic/F\* bottles and one Bactec PLUS anaerobic/F\* bottle. One bottle per positive set was investigated by the direct VITEK method (aerobic bottle preferable). Only blood culture sets with Gram-negative enteric bacillus-like morphology on microscopy were included. The VITEK instrument was used with software release R06.01.

### Identification

Identification was performed using three different methods. By the direct method a 5-mL sample from the positive blood culture bottle was centrifuged at 160 g for 5 min to pellet blood cells and particulate culture material. The supernatant was then centrifuged at 650 g for 10 min to pellet bacteria. This pellet was used for an oxidase test and to make a McFarland no. 1 standard suspension in 1.8 mL of 0.45% saline using the VITEK Colorimeter. The suspension was then inoculated into the VITEK GNI+ card and processed according to the manufacturer's recommendations. The procedure for the standard method of identification was as above, except that a suspension (McFarland no. 1 standard) was made from an overnight agar culture. The routine method for identification of

Gram-negative bacteria was based on extensive conventional characterization and was considered to be the standard [13,14].

Six *Salmonella arizonae/diarizonae* strains were used in simulated blood cultures to study the H<sub>2</sub>S reaction of these strains in the GNI+ card by the direct method.

### Susceptibility testing

Susceptibility testing was also performed using three different methods. For both the direct method and the standard method, 50- $\mu$ L samples of the respective McFarland no. 1 standard suspension used for the GNI+ card were diluted in 1.8 mL 0.45% saline and inoculated into the VITEK GNS-GA card. The routine method for susceptibility testing employed Neo-Sensitabs (User's Guide to Neo-Sensitabs, 11 edn, Rosco, Taastrup, Denmark) on Danish Blood agar (SSI Diagnostika, Hillerød, Denmark) using a semi-confluent inoculum and reading after overnight incubation at 37°C in ambient air. The following antibiotics were tested: ampicillin, cefuroxime, ceftriaxone, ceftazidime, gentamicin, tobramycin and ciprofloxacin. In case of disagreement between the routine tablet diffusion method and the VITEK methods (including when this was due to differences in interpretative breakpoints), the susceptibility of the isolate was retested using the Etest on Mueller Hinton agar (Becton Dickinson) according to the manufacturer's guidelines (AB Biodisk, Etest manual, edn. 2000). The result of the Etest, which uses the same interpretative breakpoints as the VITEK, was taken as the standard.

### Definitions and analysis

In each bacteremic episode, only one positive blood culture set of any given species was included (no repeat cultures). Two positive blood culture sets (same species and susceptibility pattern) from the same patient were by definition considered to belong to the same bacteremic episode if the interval between blood culture samplings was 14 days or less. Isolates with unacceptable identification results (probability numbers of <90%) were regarded as not identified [10], and these isolates described as having a 'Questionable biopattern', or 'Good confidence, marginal separation' were grouped together with the unidentified isolates ('No identification').

**Table 1** Identification of 169 isolates from positive blood culture by direct inoculation of the GNI+ card using VITEK as compared to routine (conventional) identification

Species	Correct identification	No identification	Misidentification	Total number
<i>Escherichia coli</i>	71	17	15	103
<i>Klebsiella pneumoniae</i>	18	1	0	19
<i>Salmonella</i> spp.	6	5	0	11
<i>Proteus mirabilis</i>	6	2	0	8
<i>Pseudomonas aeruginosa</i>	9	2	0	11
<i>Enterobacter cloacae</i>	4	0	0	4
<i>Klebsiella oxytoca</i>	8	0	0	8
<i>Citrobacter koseri</i>	2	0	0	2
<i>Proteus vulgaris</i>	1	1	0	2
<i>Serratia marcescens</i>	1	0	0	1
Total number	126	28	15	169

Only isolates identified with a probability number  $\geq 90\%$  were evaluated in the susceptibility testing part of the present study, since the VITEK algorithm for susceptibility testing is dependent on species-specific growth curves. Susceptibility discrepancies were reported as very major errors (false susceptibility), major errors (false resistance) or minor errors (susceptible/resistant vs. intermediate susceptibility).

## RESULTS

Of 186 blood culture sets included by microscopy, 17 were excluded due to growth of Gram-negative anaerobes ( $n = 8$ ), Gram-negative fastidious bacilli ( $n = 4$ ), Gram-positive bacilli ( $n = 1$ ), or polymicrobial growth ( $n = 4$ ). The 12 anaerobic or fastidious bacilli did not grow well enough to generate identification and susceptibility data. One *Bacillus* sp. (Gram-negative on initial Gram stain) and one *Enterobacter cloacae*-*Enterococcus faecalis* mixture among four polymicrobial cultures were reported as unidentified. The other three polymicrobial cultures were reported with identification of the bacterium most active biochemically, and with the susceptibility pattern of the most resistant bacterium. Thus, a total of 169 blood culture sets from 165 patients were able to be evaluated for assessment of the accuracy and speed of direct identification and susceptibility testing.

### Identification

The oxidase test is a prerequisite for the VITEK identification algorithm. Identical results were

obtained with pelleted bacteria (blood cultures) and with colony material (agar plates).

In Table 1, the direct VITEK method is compared with the routine method. A total of 126 (75%) isolates were correctly identified, 28 (17%) were unidentified, and 15 (9%) were misidentified (all *Escherichia coli*). The unidentified group consisted of 22 isolates reported as unidentified and six isolates identified to species or genus level with probability numbers  $< 90\%$ .

Table 2 shows the results of the five biochemical tests that yielded for most of the aberrant results: urease,  $H_2S$  and utilization of citrate and malonate (false-positive results) and lysine decarboxylase (false-negative results) for the six isolates with probability numbers  $< 90\%$  and the 15 misidentified isolates. A total of 20 of the 21 isolates were *E. coli*. The 21 isolates were mainly misidentified or achieved probability numbers  $< 90\%$  due to false-positive reactions in the malonate ( $n = 17$ ) and the citrate ( $n = 16$ ) tests. By manually changing positive results to negative for citrate and malonate in the VITEK bionumber, 12 of these 21 isolates were correctly identified by the VITEK database.

Only one of 12 *E. coli* isolates reported as *S. arizonae* was  $H_2S$  positive, while all of the six *S. arizonae/diarizonae* strains from simulated blood cultures tested by the direct method were  $H_2S$  positive. In contrast, the 21 isolates listed in Table 2 showed no false-positive reactions for the urease,  $H_2S$  and citrate tests and only three false-positive malonate tests when tested by the standard VITEK method.

The percentages of correctly identified, unidentified, or misidentified isolates in relation to

**Table 2** Results of five biochemical tests using the direct VITEK method for 15 misidentified isolates and six isolates with low probability number (<90%)

Strain number	Conventional identification	VITEK identification <sup>a</sup>	VITEK results				
			Urease	H <sub>2</sub> S	Citrate	Malonate	Lysine
41	<i>Escherichia coli</i>	<i>Kluyvera</i> sp. <sup>b</sup>	-	-	+	+	+
50	<i>Escherichia coli</i>	<i>Citrobacter amalonaticus</i>	-	-	-	-	-
52	<i>Escherichia coli</i>	<i>Citrobacter farmeri</i>	-	-	-	-	-
69	<i>Escherichia coli</i>	<i>Salmonella</i> sp.	-	-	+	+	+
116	<i>Escherichia coli</i>	<i>Salmonella arizonae</i>	-	+	+	-	+
121	<i>Escherichia coli</i>	<i>Enterobacter amnigenus</i> <sup>c</sup>	-	-	+	+	-
130	<i>Salmonella</i> sp.	<i>Citrobacter freundii</i> <sup>d</sup>	-	+	+	-	-
131	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>	+	-	+	+	-
133	<i>Escherichia coli</i>	<i>S. arizonae</i>	-	-	+	+	-
138	<i>Escherichia coli</i>	<i>S. arizonae</i>	-	-	+	+	+
150	<i>Escherichia coli</i>	<i>E. amnigenus</i> <sup>e</sup>	-	-	+	+	-
157	<i>Escherichia coli</i>	<i>S. arizonae</i>	-	-	-	+	+
159	<i>Escherichia coli</i>	<i>E. amnigenus</i> <sup>f</sup>	+	-	+	+	-
173	<i>Escherichia coli</i>	<i>S. arizonae</i> <sup>g</sup>	-	-	+	+	-
175	<i>Escherichia coli</i>	<i>S. arizonae</i>	-	-	-	+	+
176	<i>Escherichia coli</i>	<i>S. arizonae</i>	-	-	-	+	+
184	<i>Escherichia coli</i>	<i>S. arizonae</i>	-	-	+	+	+
199	<i>Escherichia coli</i>	<i>S. arizonae</i>	+	-	+	+	+
200	<i>Escherichia coli</i>	<i>S. arizonae</i>	+	-	+	+	+
205	<i>Escherichia coli</i>	<i>S. arizonae</i>	+	-	+	+	+
206	<i>Escherichia coli</i>	<i>S. arizonae</i>	-	-	+	+	+

<sup>a</sup>Probability number >90% if otherwise not stated.

<sup>b</sup>45% *Kluyvera* species. 'Good confidence, marginal separation.'

<sup>c</sup>40% *Enterobacter amnigenus* biogroup 2. 'Questionable biopattern.'

<sup>d</sup>60% *Citrobacter freundii/youngae*. 'Good confidence, marginal separation.'

<sup>e</sup>40% *Enterobacter amnigenus* biogroup 2. 'Questionable biopattern.'

<sup>f</sup>57% *Enterobacter amnigenus* biogroup 2. 'Good confidence, marginal separation.'

<sup>g</sup>72% *Salmonella arizonae*. 'Questionable biopattern.'

detection time are shown in Table 3. Sixty-nine percent (117/169) of the VITEK identifications were made within 6 h, and 95% (111/117) of these were correct, whereas only 29% (15/52) of isolates identified after 7 or 8 h of incubation were correctly identified.

The standard VITEK method correctly identified 163 of 169 (96%) isolates. One *E. coli* isolate

was misidentified as *S. arizonae*, and one isolate of *Salmonella* sp. (serovar Schwarzengrund) was misidentified as *S. arizonae*. Three *E. coli* isolates were reported with probability numbers <90% as *S. arizonae* (86%), *Citrobacter farmeri* (73%) and *E. coli* (76%), respectively. Finally, one *S. paratyphi* B isolate was identified as a *Citrobacter freundii/youngae* with a probability number of 61%.

**Table 3** Percentages of strains correctly identified, not identified or misidentified as a function of detection time

Identification time (h)	Correct identification (%)	No identification (%)	Misidentification (%)	Total number
2	100	0	0	14
3	95	0	5	79
4	92	0	8	12
5	83	0	17	6
6	100	0	0	6
7	0	0	100	1
8	29	55	16	51
Total number				169

### Susceptibility testing

Of the 169 isolates, 140 (125 correctly identified and 15 misidentified) were able to be evaluated for the direct susceptibility testing method. A total of 29 isolates were excluded, of which 28 were due to probability numbers of <90% or lack of identification, and one was a *Serratia marcescens*, where the VITEK expert system cautioned about the combination of species and susceptibility pattern found. For the standard susceptibility testing method, 165 (98%) isolates were able to be evaluated, as four isolates with probability numbers <90% were excluded.

The overall resistance as determined by the routine method ( $n=169$ ) was low: ampicillin 51%, cefuroxime 10%, ceftriaxone 3%, ceftazidime 1%, ciprofloxacin 1%, gentamicin <1%, and tobramycin <1%.

The 140 isolates were able to be evaluated for direct susceptibility testing were assessed for seven antimicrobial agents, resulting in a total of 980 isolate-antimicrobial combinations. VITEK gave correct results for 969 (99%) of these combinations. The 11 discrepancies were as follows: one major error for ciprofloxacin with one *E. coli* isolate; one major error with another *E. coli* isolate for ceftazidime, cefuroxime and gentamicin (contamination was ruled out by retesting from the control well of the GNS-GA card); major errors for ampicillin with three misidentified isolates (*E. coli*); and finally, minor errors with four isolates (one *Klebsiella pneumoniae* and one *E. coli* for cefuroxime and two *Pseudomonas aeruginosa* for ceftriaxone and ceftazidime, respectively). No difference in accuracy was found between correctly identified isolates (99%, 867/875) and misidentified isolates (97%, 102/105). Direct susceptibility testing took from 3 to 14 h. In fact, 85% were completed within 6 h, and of these, 99% were correct.

For the 165 isolates able to be evaluated for the standard susceptibility testing method a correct result was obtained for 1146 (99%) of the 1155 isolate-antimicrobial combinations. Discrepancies were found in nine combinations, five of which involved cefuroxime.

### Isolates with both identification and susceptibility data

After 6 and 8 h, direct testing resulted in identifications and susceptibility patterns for 106 and 109

of the 169 isolates, respectively. After both 6 and 8 h, five isolates were misidentified and three isolates yielded false susceptibility testing results, one very major, one major, and one minor error.

### DISCUSSION

In Denmark, we have few problems initiating antibiotic treatment based on microscopy of blood cultures with Gram-positive bacteria due to the low incidences of methicillin-resistant *Staphylococcus aureus* (<1%) and *Streptococcus pneumoniae* with reduced susceptibility to penicillin (<4%) [15]. This is in contrast to bacteremia caused by Gram-negative enteric bacilli, where susceptibility to antimicrobial agents varies to a greater extent. Consequently, we assessed the accuracy and speed of identification and susceptibility testing using a direct inoculation method, the GNI+/GNS-GA cards, as a prelude to exploring the possibility of same-day reporting of identification and antibiotic susceptibility testing of blood cultures positive with Gram-negative bacilli.

In the present study using the direct method, 75% of isolates were correctly identified, which is considerably lower than when testing the same isolates using the standard method, where 96% were correctly identified. It is also lower than the findings of other studies of direct inoculation of positive blood cultures, in which 92–96% were correctly identified [4–6], although Waites et al. only found 72% for the rapid MicroScan panel [7]. Comparison with the results of the present study is, however, difficult as different blood culturing systems or identification systems or both were used.

*E. coli* caused the largest problem of identification in the present study; most of the aberrant identifications were *S. arizonae*. Two factors gave suspicion of misidentification. First, *S. arizonae* is seldom a cause of bacteremia and has not hitherto been seen in Denmark; and secondly, 11 of 12 strains misidentified or identified with a low probability number as *S. arizonae* had negative H<sub>2</sub>S reaction and this reaction is 99% positive for *S. arizonae* tested conventionally [13], and was also positive in all six *S. arizonae/diarizonae* strains tested from simulated blood cultures. Manually changing positive reactions in the citrate and/or malonate tests resulted in correct identification for a majority of *E. coli* isolates.

By the standard VITEK method, the two misidentifications and two of the four non-identifica-

tions due to low probability numbers were also caused by positive malonate tests, pointing at an inherent tendency of the GNI+ card in this regard. The GNI+ card is an enhanced version of the GNI card with faster reporting, improved identification accuracy, and a larger corresponding database [10]. These improvements, however, might have made the GNI+ card more prone to false-positive results. One Danish clinical microbiology laboratory has stopped using the GNI+ card and is using the GNI card instead because of problems with *E. coli* identification (Jens K Møller, Århus University Hospital, personal communication). We find that these results justify a request to the manufacturer to investigate and possibly modify the identification algorithm of the VITEK software.

Two other factors might be the cause of the low percentage of correct identifications. A washing step was not included in the protocol of the present study, in contrast to previous studies [5,6]. Thus, it is possible that traces of blood culture broth or blood components have interfered with the biochemical reactions.

The results of the antibiotic susceptibility testings were very accurate for both the direct and the standard method and were as good as those found by other investigators [6,8,16]. A shortcoming of these results is that the low level of antibiotic resistance in Denmark resulted in only a small number of isolate-antimicrobial combinations that challenged the system.

The present study was initiated to explore the possibility of same-day reporting of preliminary identification and susceptibility results to clinicians. We chose to accept only identifications made in 6 h or less, as at this point 95% of the identifications were correct, and extending the time limit beyond 6 h only increased the percentage of misidentifications. Irrespective of detection time, the direct inoculation into the VITEK of organisms incompatible with the GNI+ card (one *Bacillus* sp. and 12 anaerobic or fastidious Gram-negative bacilli) did not result in misidentifications, as all were reported as unidentified or nonviable organisms. In the case of the four polymicrobial blood cultures, the VITEK reported one as unidentified, while the three cultures with different *Enterobacteriaceae* were reported within 6 h with the therapeutically 'appropriate' susceptibility pattern, i.e. the pattern of the most resistant bacterium.

At 6 h the VITEK had reported identifications plus susceptibility patterns of 106 isolates,

corresponding to 63% of 169 mono-bacteremic blood cultures with growth of Gram-negative bacilli intended for the GNI+ card. The cost of this was five misidentifications without therapeutic consequences, and three susceptibility testing errors.

A practical advantage of the direct procedure is the fast oxidase test result, which may have direct implications for the choice of pre-emptive antimicrobial chemotherapy, i.e. inclusion of an anti-pseudomonas antibiotic on suspicion of *P. aeruginosa* bacteremia.

The direct inoculation method from positive blood cultures cannot replace approved methods of identification and susceptibility testing because of the above-mentioned shortcomings of the accuracy of identification, and because the VITEK cannot identify anaerobes, fastidious Gram-negative bacilli, etc., erroneously inoculated after microscopy. But as we believe that same-day reporting of preliminary blood culture results for about two-thirds of our patients with Gram-negative bacteremia would ensure better patient management, we plan to work further with this method in order to improve the clinical utility of our microbiological testing.

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