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Bloodstream infections related to Gram-negative rods. Usefulness of the VITEK ® 2C System for direct identification and susceptibility testing from positive BacT-ALERT® blood cultures.

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Background

Despite recent advances in our understanding of the pathophysiological mechanisms of sepsis and improved antimicrobial therapy, the mortality rate from Gram-negative sepsis remains high (20-50%), particularly after the onset of shock.
Gram-negative rods such as *Enterobacteriaceae* and *Pseudomonas aeruginosa* are the leading causes of nosocomial bloodstream infections and there has been a marked increase in the incidence of resistant strains in recent years.
Appropriate antimicrobial therapy has been shown to reduce mortality among patients with Gram-negative bacteremia and, when initiated early, to have a favorable effect on outcome in critically ill patients.

Objectives

1- Compare the results of identification and antimicrobial susceptibility testing for Gram-negative rods, obtained directly by VITEK® 2 Compact (VITEK® 2C) from the positive blood culture bottle (BacT-ALERT® system), with those performed according to manufacturer's instructions from isolated colonies.
2- Determine the usefulness of these results when the initial antimicrobial treatment was inappropriate.

Methods

A prospective observational study was conducted in two hospitals of Buenos Aires city (Argentina); 191 clinically significant monomicrobial Gram-negative bloodstream infections were included, corresponding to 189 patients.
The median patient age was 72.5 years (range 1-94), 51% were males and 49% females.
The present study demonstrates the uses of the BacT-ALERT Blood culture system combined with the VITEK 2C system.
Organism identification and susceptibility results obtained directly from the blood culture bottle using a serum separator tube SST (Becton Dickinson) to prepare the inoculum were compared with those obtained from cards inoculated with a standardized bacterial suspension obtained following subculture to agar.
A blood culture bottle that was positive with the BacT-ALERT system was removed from the instrument, the contents were gently mixed, and 6 ml of fluid was aspirated into an SST. The filled SST was centrifuged at 2,000 X g for 10 min. Following centrifugation, the supernatant was discarded, the bacterial pellet was resuspended in 3 ml of 0.45% saline solution and the inoculum was adjusted to a 0.5-0.63 McFarland standard by using the DENSICHEK densitometer (bioMérieux).
The cards were then inoculated following manufacturer's instructions.
Species identification was carried out with the GNI card and antibiotic susceptibility testing was determined by the AST-082 card of the VITEK 2C System (bioMérieux, Marcy, France).
During the study period, a total of 191 non-repetitive clinically significant isolates of Gram-negative rods, including 73 *E.coli*, 37 *K.pneumoniae*, 22 *P.aeruginosa*, 15 *A.baumannii*, 14 *P.mirabilis*, 5 *E.cloacae*, 5 *S.marcescens*, 3 *M.morganii*, 3 *S.maltophilia*, 2 *Salmonella* spp, 2 *C.freundii*, 2 *E.aerogenes*, 2 *C.koseri*, 2 *R.picketti*, 1 *E.meningoseptica*, 1 *P.agglomerans*, 1 *B.cepacia* and 1 *A.lwoffi* were tested.
The initial empirical antimicrobial therapy was considered appropriate if the initial antibiotics were administered within 24 h after acquisition of a blood culture sample, including cases where at least one active antibiotic *in vitro* was given using the correct dosage and pathway according to current medical standards.

Results

The median time to blood culture positivity was 14.1 h (range: 2-71 h)
The median time to obtain the final result of identification and susceptibility testing directly from the blood culture bottle by VITEK 2C was 8.2 h (range: 3.75-18 h).
By using the identifications obtained from pure cultures with the VITEK 2C system (reference method), the agreement between the reference method and the VITEK 2C system tested directly using blood cultures from patients was 99%.
For the antimicrobial susceptibility test, the overall accuracy at category level was 99%, with 0.21% very major errors, 0.17% major errors and 0.61% of minor errors.
The overall 14-day mortality rate for all patients was 24.3%. Of the 189 bloodstream infection episodes, 108 (57%) received appropriate initial empirical antibiotics. The appropriate initial antimicrobial therapy group had a 17.5% mortality rate, whereas the inappropriate therapy group had a 33% mortality rate (p:0.07).
After the result obtained directly from the bottle was reported, antimicrobial therapy was changed in 116 (60.7%) of the bloodstream infection cases..

Species distribution and frequency of identification errors by species.

Microorganisms	N°	Correct identification N°	Incorrect identification N°
E.coli	73	73 (100)	0
K.pneumoniae	37	37 (100)	0
P.aeruginosa	22	21 (99)	1
P.mirabilis	14	14 (100)	0
A.baumannii	15	15 (100)	0
E.cloacae	5	5 (100)	0
S.maltophilia	3	3 (100)	0
S.marcescens	5	5 (100)	0
Salmonella spp	2	2 (100)	0
C.freundii	2	1 (100)	1
M.morganii	3	3 (100)	0
B.cepacia	1	1 (100)	0
E.aerogenes	2	2 (100)	0
E.meningoseptica	1	1 (100)	0
R.picketii	2	2 (100)	0
C.koseri	2	2 (100)	0
P.agglomerans	1	1 (100)	0
A.lwoffi	1	1 (100)	0
Total	**191**	**189 (99)**	**2 (1%)**

VITEK 2C. Average time for direct identification and susceptibility from the blood culture bottle

Microorganisms	Range of time VTK 2C (hours)	Average time VTK 2C(hours)
E.coli	3.75-18	7
K.pneumoniae	3.75-10	6.7
P.aeruginosa	5-17	12.3
P.mirabilis	04-12	5.2
A.baumannii	4-13.2	9.8
E.cloacae	7.5-8.8	8.15
S.maltophilia	18	18
S.marcescens	7.75-9	8.13
Salmonella spp	08-9	8.4
C.freundii	5-8.25	6.5
M.morganii	7.25-18	10.8
B.cepacia	13.75-16	14.8
E.aerogenes	7.5-7.75	7.6
E.meningoseptica	7.20	7.20
R.picketii	13	13
C.koseri	7.5-7.7	7.6
P.agglomerans	8.2	8.2
A.lwoffi	9-9.25	9.2
Total	**3.75-18**	**8.2**

VITEK 2C. Perfomance of the direct susceptibility from the blood culture bottle

ATB	N° Tested	MINOR ERROR	MAJOR ERROR	VERY MAJOR
AMP	191	1 (0.55%)	0 (0%)	0 (0%)
AMS	191	4 (2.2%)	1 (0.55%)	1 (0.55%)
PTZ	191	0 (0%)	1 (0.55%)	4 (2.2%)
CTX	191	1 (0.55 %)	0 (0%)	0 (0%)
CAZ	191	5 (2.6%)	0 (0%)	0 (0%)
FEP	191	1 (0.55%)	1 (0.55%)	0 (0%)
IMI	191	1 (0.55%)	1 (0.55%)	0 (0%)
MER	191	0 (0%)	0 (0%)	0 (0%)
COL	191	0 (0%)	0 (0%)	0 (0%)
CIP	191	0 (0%)	0 (0%)	0 (0%)
GEN	191	1 (0.55%)	0 (0%)	0 (0%)
AKN	191	0 (0%)	0 (0%)	0 (0%)
TOTAL	192	14 (0.61%)	4 (0.17%)	5 (0.21%)

AMP: ampicillin; AMS:ampicilin-sulbactam; PTZ:piperacillin-tazobactam; CTX: cefotaxime; CAz:ceftazidime; FEP:cefepime; IMI:imipenem; MER:meropenem: COL:colistin; CIP:ciprofloxacin; GEN:gentamicin; AKN:amikacin

Usefulness of the direct susceptibility from blood culture in the adequacy of antibiotic treatment (ATB).

Episodes	N (%)
Antimicrobial changes	116 (60.7%)
- to a lower spectrum ATB	21 (18%)
- to a broader spectrum ATB	30 (26%)
-Without ATB to appropriate ATB	32 (27.5%)
-Change Beta lactam ←→ quinolone/colistin	33 (28%)
Died within 24 hs of obtained blood cultures	14 (7%)
Without antimicrobial changes (initial appropriate treatment)	61 (32%)
Total	191

Relation between mortality and adequacy of the antimicrobial treatment

Patients	Initial adequate treatment N (%)	Initial incorrect treatment or without antibiotics N (%)	Total
Non-survivors	19 (17.6)	27 (33.3)	46
Survivors	89 (82.4)	54 (66.6)	143
Total	108	81	189

p= 0.013

Relation between mortality and focus of infection

Focus of infection	Non-survivors	Survivors	Total
Urinary tract infections	17	68	85
Unknown	4	19	23
Skin and soft tissues	3	10	13
Abdominal	7	18	25
Catheter	12	21	33
Pneumonia	3	6	9
Endocarditis	0	1	1
Diarrhea	0	2	2
Total	46	145	191

Conclusion

The identification and antimicrobial susceptibility testing results obtained by VITEK 2C directly from the blood culture bottles were reliable and had an important clinical impact when the choice of the initial antimicrobial treatment was inappropriate.
Our data show an increased mortality in the group of patients who received inappropriate empirical treatment for Gram-negative bacteremia.