

Revised Abstract

OBJECTIVES: Bloodstream infections (BSI) caused by gram-negative bacilli (GNB) can be life threatening, particularly in hospitalized patients. Rapid identification (ID) of the most common gram-negative pathogens can impact appropriate empiric therapy and is therefore critical to improved patient care. This study evaluated the performance of the first rapid assay to identify 3 major GNB, *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP) and *Pseudomonas aeruginosa* (PA) directly from newly positive blood cultures (BC). Empiric therapy for PA differs from that for EC or KP and rapid detection of class A carbapenemases (KPC) for enteric GNB by real-time PCR further impacts prudent therapeutic and infection control decisions.

METHODS: From October 2009 to January 2010, 198 blood culture (BC) bottles (98 patients) newly smear-positive for GNB were tested with a new rapid tri-color fluorescence *in situ* hybridization (FISH) assay, the GNR Traffic Light PNA FISH (GNRTL) (AdvanDx). The peptide nucleic acid fluorescent (PNA) probes target rRNA sequences of EC, KP and PA which appear as green, yellow or red bacilli, respectively, when examined by fluorescent microscopy. Culture ID was performed using the Vitek 2 system (bioMérieux) and KPC production was tested directly from GNB-positive BC by real-time PCR. Patient charts were reviewed retrospectively for therapeutic changes based on pathogen ID.

RESULTS: Of 98 patients with BSI due to GNB, 76% were infected with the targeted pathogens, which included 27 EC, 29 KP, 10 PA, 7 EC + KP and 1 EC + PA. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) for the Traffic Light PNA FISH compared to culture were 100% for EC and KP. No false-positive or false-negative results occurred with co-infections of EC and KP and EC and PA. One false-positive PA result occurred with a mixed culture of *Acinetobacter radioresistens* and *Enterococcus faecalis*. The assay sensitivity and NPV for PA were 100%, the specificity was 99% and the PPV was 93.7%. KPCs were detected in BCs containing EC (7.4%) and KP (13.8%) but not in those with PA. The rapid identification of EC and KP can lead to discontinuing tobramycin and vancomycin and detection of KPC in EC or KP may prompt the addition of polymyxin B, rifampin or a carbapenem. Final treatment is dependent upon antimicrobial susceptibility reports.

CONCLUSIONS: The Traffic Light PNA FISH is a highly sensitive and specific assay for identifying the most common GNB from BC in real-time. The ability to distinguish PA from enteric GNB can have clinical utility by targeting appropriate pathogen specific empiric therapy. Three major GNB blood pathogens were accurately identified from newly positive BC within 1.5 hrs compared to 1-3 days using culture methods.

Introduction

Bloodstream infections (BSI) are associated with considerable morbidity and mortality and are the primary cause of death in intensive care units. A prime objective of clinical microbiology laboratories is to provide timely identification of bloodstream pathogens; however, these efforts are limited by culture-based methods that require 1-3 days to obtain results. Delayed reports to clinicians can impact patient care, contribute to the overuse/misuse of broad spectrum antimicrobics and hinder effective empiric therapy selection. In this regard, gram-negative bacilli (GNB) present a major challenge due to their species-specific drug resistance and limited therapeutic options. Early identification of the three predominant GNB causing BSI, *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP) and *Pseudomonas aeruginosa* (PA) can aide the clinician in the choice of the most appropriate empiric therapy. This study evaluates a new tri-color Traffic Light PNA FISH assay that is unique in its ability to rapidly detect EC, KP and PA in blood cultures within 1.5 hrs after blood cultures are flagged as positive. Results of this novel assay were compared to those derived by routine culture identification techniques. In addition, detection of KPCs in EC and KP isolates was also coupled with the PNA FISH assay to determine the impact on therapy.

Methods

PNA FISH ASSAY:

The GNR Traffic Light PNA FISH (AdvanDx, Woburn, MA) is a new tri-color fluorescence *in situ* hybridization (FISH) test using fluorescence labeled peptide nucleic acid (PNA) probes targeting specific rRNA

sequences in EC, KP and PA. The test was performed directly on smears from 198 blood culture bottles positive for GNB collected from 98 patients from October 2009 to January 2010. Smears were made by mixing a drop of fixation solution with a drop of blood from the positive bottle in a well on a slide; these were heat fixed on a heat block. A hybridization solution containing a mixture of a fluorescein-labeled GNB species-specific PNA probes was added to the smear. After incubation for 30 min. (55°C), unbound probe was removed by washing with a stringent wash solution at 55°C (30 min). Slides were then examined on a fluorescent microscope at 1000X using a FITC/Texas Red filter. EC, KP and PC were identified as bright green, yellow and red fluorescent bacilli, respectively. No fluorescence was read as a negative result.

CULTURE IDENTIFICATION:

Blood culture bottles (Bactec Aerobic Plus, BD Diagnostics, Sparks, MD) positive for GNB were subcultured onto trypticase soy with 5% sheep blood, chocolate and MacConkey agar plates (BD, Sparks, MD) and incubated for 24 hrs. Pure cultures of all GNB were inoculated onto Vitek 2 (bioMérieux, Durham NC) cards for identification and antimicrobial susceptibility testing. Discrepant results were confirmed with 16S gene sequencing.

PCR ASSAY FOR CARBAPENEMASE:

DNA was extracted from blood culture aliquots positive for GNB using the BioRobot EZ1 automated system (Qiagen, Inc., Valencia, CA). Real-time PCR was performed on a SmartCycler (Cepheid, Sunnyvale, CA), using primers and fluorescently-labeled probes that target a plasmid sequence common to the currently known KPC genotypes.

Results

TABLE 1: The prevalence of GNB isolated from BC in 2009 is as follows: EC (29%), KP (17%), PA (6%), *Acinetobacter* sp. (7%), *Enterobacter* sp. (7%) and *Serratia* sp. (4%). The results of the identification of all three GNB by the Traffic Light PNA FISH correlated 100% with those from culture, even when they were found in mixed culture (EC with KP and EC with PA). There was one false-positive PNA PA FISH result that was found by culture and confirmatory 16S gene sequencing to be a mixed culture of *Acinetobacter radioresistens* and *Enterococcus faecalis*. It is believed that there is cross reactivity of this uncommon *Acinetobacter* sp with PA.

TABLE 2: The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for EC and KP were 100%. PA resulted in 100% sensitivity and NPV; however due to the one false-positive PA specimen, the specificity and PPV were 99% and 93.7%, respectively.

TABLE 3: KPC production directly from BC was detected in 7.4% of EC, 13.8% KP and 0% in PA.

TABLE 4: Empiric therapy for BSI due to GNB varies geographically and is influenced by the institutions' microbial susceptibility patterns summarized in annual cumulative antibiograms. The empiric therapeutic regimen prescribed when GNB are detected on Gram

stains from smear- positive BC bottles varies with the patient's underlying condition, the cumulative antibiogram pattern of each isolate and geographical location of the hospital. For 2009, our medical center antibiogram reflected 19% KPC production in KP and 1% in EC. At our medical center, piperacillin/tazobactam (P/T), tobramycin and vancomycin are commonly used to empirically treat GNB BSI. When an enteric pathogen, such as EC or KP is identified, vancomycin and tobramycin are usually discontinued. When a KPC- producing enteric pathogen is detected, polymyxin B, rifampin and a carbapenem might be added to the regimen; however for KPC negative enterics, a carbapenem is often the drug of choice. The identification of PA in BC would prompt discontinuing vancomycin and continuing tobramycin and P/T. The final optimal therapy is dependent upon the antimicrobial susceptibility report.

TABLE 1
RESULTS OF GNB TRAFFIC LIGHT PNA FISH ASSAY
COMPARED TO CULTURE

GRAM-NEGATIVE BACILLI	2009 Blood Culture Prevalence	# BOTTLES (# PATIENTS) 10/2009 – 1/2010	PNA FISH GREEN	PNA FISH YELLOW	PNA FISH RED	PNA FISH NEGATIVE
<i>E. coli</i>	29%	73 (27)	73			
<i>K. Pneumoniae</i>	17%	45 (29)		45		
<i>P. Aeruginosa</i>	6%	14 (10)			14	
<i>Acinetobacter spp</i>	7%	4 (2)				4
<i>Enterobacter spp</i>	7%	14 (8)				14
<i>Serratia spp</i>	4%	19 (3)				19
<i>E. coli</i> & <i>K. pneumoniae</i>		9 (7)	9	9		
<i>E. coli</i> & <i>P. aeruginosa</i>		1 (1)	1		1	
<i>Acinetobacter radioresistens</i> & <i>Enterococcus faecalis</i>		1 (1)			1 (false +)	
Other GNB		23 (13)				23

TABLE 2
PERFORMANCE OF THE PNA FISH ASSAY COMPARED WITH
CULTURE IDENTIFICATION

GNB Species	Performance			
	Sensitivity	Specificity	PPV	NPV
<i>E. coli</i>	100%	100%	100%	100%
<i>K. pneumoniae</i>	100%	100%	100%	100%
<i>P. aeruginosa</i>	100%	99%	93.7%	100%

TABLE 3
KPC DIRECT DETECTION OF GNB FROM BLOOD CULTURES

GNB Species	TOTAL # PTS TESTED	KPC+	KPC % OF TOTAL TESTED
<i>E. coli</i>	27	2	7.4%
<i>K. pneumoniae</i>	29	4	13.8%
<i>P. aeruginosa</i>	10	0	0

TABLE 4
IMPACT OF PNA FISH & KPC RESULTS ON
THERAPEUTIC REGIMEN

GNB EMPIRIC REGIMEN	PNA FISH ID	KPC Result	AFTER PNA FISH & KPC REPORTS	
			Antimicrobials Discontinued	Optimal Empiric Regimen
Piperacillin/ Tazobactam (P/T) Tobramycin Vanco	<i>E. coli</i>	KPC neg	Vanco, Tobra, P/T	Carbapenem
		KPC pos	Vanco, Tobra, P/T	Polymyxin B + Rifampin (+ carbapenem)
	<i>K. pneumoniae</i>	KPC neg	Vanco, Tobra, P/T	Carbapenem
		KPC pos	Vanco, Tobra, P/T	Polymyxin B + Rifampin (+ carbapenem)
<i>P. aeruginosa</i>	N/A	Vanco	P/T + Tobra	

Conclusion

This study demonstrated that the tri-color Traffic Light PNA FISH accurately and quickly identifies the 3 major GNB that cause BSI. It is currently the only commercial assay that can detect and identify gram-negative pathogens from newly positive blood cultures. Detection and notification of GNB species 1 to 3 days earlier than conventional methods can impact the selection of appropriate empiric therapy and streamline identification algorithms in the clinical microbiology laboratory. We highly recommend that the new Traffic Light FISH assay be incorporated into the BSI strategy for rapid laboratory identification of GNB due to its excellent accuracy and ease of use.

Bibliography

- Ng SY, Kwang, LL and Tan TY. Identification of gram-negative bacilli directly from positive blood culture vials. J. Med Micro. 2007 56:475-479.
- Ibrahim EH, Sherman G, Ward, S, Fraser VJ. and Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest 2000 118:146-155.
- Wenzel RP and Edmond MB. The impact of hospital-acquired bloodstream infections. Emerg Infect Dis 2001 7:174-177.
- Sogaard M., Stender H. and Schonheyder HC. Direct identification of major blood culture pathogens, including *Pseudomonas aeruginosa* and *Escherichia coli*, by a panel of fluorescence *in situ* hybridization assays using peptide nucleic acid probes. J. Clin Microbiol. 2005 43:1947-1949.
- McGregor JC, Rich SE, Harris AD, Perencevich EN, Osih R, Lodise TP, Miller RR and Furuno JP. A systematic review of the methods used to assess the association between appropriate antibiotic therapy and mortality in bacteremic patients. Clin Infect Dis 2007 45:329-337.
- Wolter DJ, Kurpiel PM, Woodford N, Palepou MGI, Goering RV and Hanson ND. Phenotypic and enzymatic comparative analysis of the novel KPC variant KPC-5 and its evolutionary variants, KPC-2 and KPC-4. Antimicrobial Agents Chemo 2009 53:557-562.