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The Impact of Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA FISH®) Rapid Diagnostic Testing on the Initiation of Appropriate Antifungal Therapy in Patients with Candida Species Bloodstream Infections

Jenna Lee Ksiazkiewicz
Butler University

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Read, approved, and signed by:

Thesis adviser(s) Kendra Damer / [Signature] 4/24/15
Date

Reader(s) CHAD KNOWLER / C Knowler 4/23/2015
Date

Reader(s) Kendra Damer / [Signature] 4/24/15
Date

Certified by Judith Harper Marvel 5/18/15
Date
Director, Honors Program

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**The Impact of Peptide Nucleic Acid
Fluorescence In Situ Hybridization (PNA FISH®)
Rapid Diagnostic Testing on the Initiation of Appropriate
Antifungal Therapy in Patients with *Candida* Species Bloodstream Infections**

A Thesis

Presented to the

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Jenna Lee Ksiazkiewicz

May 9, 2015

**The Impact of Peptide Nucleic Acid
Fluorescence In Situ Hybridization (PNA FISH®)
Rapid Diagnostic Testing on the Initiation of Appropriate
Antifungal Therapy in Patients with *Candida* Species Bloodstream Infections**

Jenna L. Ksiazkiewicz, Pharm.D. Candidate; Kendra M. Damer, Pharm.D.

ABSTRACT

Background: Traditional methods for detecting positive blood cultures include the use of broth bottles with sensitive indicators for growth. These techniques can take 24-48 hours to signal a positive test. Another 24 hours are often required to determine the species of the organism. The use of rapid diagnostic testing for *Candida* species bloodstream infections provides accurate species identification in less than two hours.

Objective: Evaluate the time to appropriate antifungal therapy in patients in which rapid diagnostic testing was utilized.

Methods: This study was a retrospective, observational cohort of patients who had positive blood cultures for *Candida* species and had PNA FISH® technology used as a rapid diagnostic test. Patients admitted to either Sidney & Lois Eskenazi or Wishard Memorial Hospital during the time frame of January 1, 2012 to November 30, 2014 were eligible for inclusion. Patients had to have at least one positive blood culture for *Candida* species. The following data was collected: demographics, microbiologic data, antimicrobial regimen, time to appropriate antimicrobial therapy, hospital length of stay and mortality.

Results: Of the 27 blood cultures in which PNA FISH® technology was used, the average time to appropriate antifungal therapy was 15.24 ± 17.7 hours. Therapy was appropriately initiated or adjusted after the PNA FISH® results in 33% of patients (9/27). The mean hospital length of stay was 24.4 ± 18.8 days and the mean intensive care unit (ICU) length of stay was 15 ± 20.7 days. All-cause mortality was 22.2% (6/27).

Conclusion: This study showed that the average time to appropriate antifungal therapy of 15.24 hours was comparable to current published studies. Due to the small number of patients in which therapy was appropriately adjusted after the PNA FISH®, it may be beneficial to educate healthcare providers about the usefulness of the PNA FISH® and how this technology may be utilized to enhance clinical practice and potentially improve patient outcomes.

BACKGROUND

Candidemia is the fourth most common cause of nosocomial bloodstream infections in the United States. *Candida* species are the most common cause of invasive fungal infections in humans.¹ Common risk factors for candidemia include: central venous catheters, parenteral nutrition, renal replacement therapy, immunosuppressive agents and broad-spectrum antibiotic use. Invasive candidiasis has an estimated mortality of 47%.¹

Traditional methods for detecting positive blood cultures for an organism include the use of broth bottles with sensitive indicators for growth. These techniques can take 24-48 hours to indicate a positive result. Then, an additional 24 hours is often required to determine the species of the organism. One can then expect another 24 hours for

antimicrobial susceptibility testing.² The use of rapid diagnostic testing for *Candida* species bloodstream infections may significantly reduce the time to appropriate antimicrobial therapy by providing correct species identification in less than two hours. Examples of rapid diagnostic tests currently available include: PNA FISH (AdvanDx), PCR (BD GeneOhm and Cepheid) and MALDI-TOF (Bruker Daltonics Inc.).²

The Yeast Traffic Light® PNA FISH® (AdvanDx, Woburn, MA) provides rapid identification of five different *Candida* species. This technology is a fluorescence in situ hybridization (FISH) method which uses PNA probes to hybridize to specific ribosomal RNA sequences of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* and *C. krusei*. This test provides identification of *C. albicans* and/or *C. parapsilosis*, *C. glabrata* and/or *C. krusei* and *C. tropicalis* from positive blood culture smears by signaling a different color for each organism under fluorescence microscope examination. *C. albicans* and/or *C. parapsilosis* will be signaled by a green-positive, *C. glabrata* and/or *C. krusei* will signal as a red-positive and *C. tropicalis* will signal as a yellow-positive. The time to a positive result is approximately 90 minutes.³ Figure 1 is a sample interpretation of the results of the assay using a fluorescence microscope.

The Yeast Traffic Light® PNA FISH® (AdvanDx, Woburn, MA) is distributed as a kit containing a fixation solution, a yeast traffic light PNA, a 60x wash solution and a mounting medium. The test is performed by combining a mixture of fluorescein and rhodamine-labeled PNA probes to a blood smear. Hybridization is performed for 30 minutes at 55° C which is followed by a post-hybridization wash with the wash solution. It is then prepared onto the mounting medium and able to be examined via fluorescence microscopy.³

Limitations of this test include the inability to differentiate between *C. albicans* or *C. parapsilosis* and *C. glabrata* or *C. krusei*. The test also cannot differentiate *Candida* species in cultures with mixed growth. The assay requires further isolation on solid growth media to determine correct species identification. An additional limitation includes a potential false-positive result that may occur due to the sequence similarity of *C. glabrata* to *C. nivariensis* and *C. bracarensis*, which may lead to a false-positive (red). A false-positive (green) may also occur with *C. orthopsilosis* and *C. metapsilosis* due to their similarity to *C. parapsilosis*.³ Finally, the assay does not provide susceptibility information.

The detection limit for all five *Candida* species has been determined to be approximately 10^5 cfu/mL by serial dilutions of positive cultures which is consistent with traditional staining techniques. Specificity testing on 75 laboratory and reference cultures containing *Candida* species were all proven to show the correct organism to be isolated. Reproducibility testing of 19 isolates showed 98.7% total agreement across three different testing sites.³

Hall and colleagues performed a study on 216 blood cultures positive for *Candida* species.⁴ Of these cultures, the Yeast Traffic Light® PNA FISH® identified the correct *Candida* species 96% of the time (207/216). Using a 30-minute hybridization time, the Yeast Traffic Light® PNA FISH® correctly identified 100% of *C. albicans* (39/39), 94% of *C. parapsilosis* (31/33), 100% of *C. glabrata* (34/34), 100% of *C. krusei* (24/24) and 94% of *C. tropicalis* (31/33). They found equivalent results using a 90-minute hybridization time.⁴ Although there is an abundance of data regarding the specificity and

sensitivity of the PNA FISH® assay, the clinical application has only been briefly examined in recent years.

Heil and colleagues compared time to targeted therapy, hospital length of stay and in-hospital mortality before the implementation of the PNA FISH® technology and after. They calculated a statistically significant shorter mean time to targeted therapy with the PNA FISH® of 0.6 day or 14.4 hours compared to 2.3 days with traditional techniques (p=0.0016). Heil and colleagues found no statistically significant difference in hospital length of stay or in-hospital mortality between the two groups.⁵

Aitken and colleagues found the average time to identification of yeast by traditional laboratory methods to be 2.2 ± 1.3 days. The authors then performed a 5,000 person Monte Carlo simulation, in which they found that the average time to initiation of antifungal therapy was 3.5 ± 2.1 days with traditional methods as compared to 2.6 ± 1.3 days with the PNA FISH®.⁶

NEED FOR THE STUDY

The current Infectious Disease Society of America guidelines for the management of candidiasis recommend initial selection of antifungal therapy based on species identification, making species identification key for clinical decision making.⁷ Most patients are not initiated on an antifungal until yeast identification after a Gram stain. Appropriate pathogen-specific therapy is generally not initiated until the *Candida* species is identified.⁷ The PNA FISH® may serve as a reliable tool to determine speciation within 90 minutes which is much faster than traditional methods of detection which can take three to five days.

One disadvantage of the PNA FISH® is cost. The equipment cost is approximately \$1,000 and \$30-\$80 is required per test.⁸ This raises the question of whether the cost is worth the potential outcome. A goal of the current study was to determine the effect of the PNA FISH® on time to appropriate antifungal therapy, appropriate therapy modification and the impact on overall clinical outcomes for patients.

Limited literature is currently available regarding the use of PNA FISH® for candidemia and subsequent clinical outcomes. Therefore, it is beneficial to explore the effect of PNA FISH® technology on time to appropriate antifungal therapy, hospital length of stay and mortality.

OBJECTIVE

The primary objective in this study was to determine the time to appropriate antifungal therapy in patients with *Candida* species bloodstream infections in which the PNA FISH® assay was utilized.

Secondary objectives include: the percent of patients in which antimicrobial therapy was appropriately adjusted after the PNA FISH® results, total hospital length of stay, intensive care unit length of stay and all-cause mortality.

METHODS

Study Design: This study was a retrospective, observational cohort of patients with positive blood cultures for *Candida* species and had PNA FISH® technology used as a rapid diagnostic test on the blood culture specimens. This study received exemption status from the Indiana University Institutional Review Board.

The process for determining final *Candida* speciation for positive blood cultures at Eskenazi Health is as follows: After a positive Gram stain for yeast is identified from a blood culture, the PNA FISH® is performed by the laboratory. These results are then reported to the physician. The final step in the process is to complete standardized testing to obtain final speciation using the VITEK2 system.

Inclusion Criteria: Patients admitted to either Sidney & Lois Eskenazi or Wishard Memorial Hospital during the time frame of January 1, 2012 to November 30, 2014 were eligible for inclusion. Patients had to have at least one positive blood culture for *Candida* species. If patients had multiple positive cultures for the same *Candida* species during the same admission, only the first positive culture was included.

Exclusion Criteria: Patients were excluded if they did not have the PNA FISH® rapid diagnostic test used on their blood culture. Patients who were younger than 18 years of age, pregnant or prisoners were also excluded.

Data Source: Data was extracted from electronic medical records of eligible patients admitted to Sidney & Lois Eskenazi Hospital or Wishard Memorial Hospital during the time frame of January 1, 2012 to November 30, 2014. The data collected included: age, gender, allergies, past medical history, location of the blood draw, hospital length of stay, ICU length of stay, microbiologic data, antimicrobial regimen details, time to appropriate antifungal therapy and all-cause mortality during hospital admission.

Definition of Appropriate Antifungal Therapy: In accordance with the Infectious Disease Society of America's clinical practice guidelines for the treatment of candidiasis, appropriate antifungal therapy for *C. albicans*, *C. parapsilosis*, and *C. tropicalis* was

defined as fluconazole and appropriate antifungal therapy for *C. glabrata* and *C. krusei* was defined as an echinocandin¹ (the formulary option being caspofungin at Eskenazi Health). If the patient was started on appropriate empiric therapy then de-escalated based on susceptibilities, time to appropriate therapy was then based on appropriate empiric therapy.

Statistical Analysis: Descriptive statistics were utilized for the primary and secondary objectives of this study.

RESULTS

A total of 52 blood cultures in 52 patients which were positive for *Candida* species were evaluated. Of these, 25 blood cultures were excluded because PNA FISH® technology was not used to analyze the blood culture. This left a sample of 27 blood cultures positive for *Candida* species in which PNA FISH® technology was used. Table 1 outlines patient demographic information.

Figure 2 shows the number of different *Candida* species identified by the PNA FISH® compared to the final speciation results. The species determined by the PNA FISH® were consistent with the final speciation results 100% of the time (27/27). Susceptibility testing for every *Candida* species blood cultures is not routine practice at Eskenazi Health, however, susceptibility testing was performed on seven of the positive cultures: five *C. glabrata* isolates, one *C. krusei* isolate and one *C. parapsilosis* isolate.

The average time to appropriate antifungal therapy was 15.24 ± 17.7 hours. The mean hospital length of stay was 24.4 ± 18.8 days. Twenty-three of 27 patients (85%) were admitted to the ICU during their hospital stay, while four patients stayed on the

general medical units. Of the ICU patients, the mean ICU length of stay was 15 ± 20.7 days. All-cause mortality was 22.2% (6/27). Table 2 outlines the primary and secondary objectives. Out of the 27 included blood cultures, the infectious diseases consult service was formally consulted and had documented recommendations in 24/27 of the positive cultures. The recommendations were accepted in all 24 cases in which the infectious diseases consult service was consulted.

Appropriate empiric therapy was started in 92.6% of positive blood cultures (25/27). Therapy was initiated or changed after the PNA FISH® for 33% of positive blood cultures (9/27). Table 3 outlines the timing of antifungal therapy initiation and Table 4 describes the antifungal therapy regimens by *Candida* species in all 27 positive blood cultures.

DISCUSSION

Heil and colleagues evaluated the PNA FISH® assay and its impact on the treatment of *Candida* species bloodstream infections.⁵ The study evaluated the time to species identification, time to targeted therapy, clinical outcomes (culture clearance, hospital length of stay and hospital mortality) and the cost effectiveness of the PNA FISH® before and after test implementation. The results found a statistically significant decrease in time to targeted therapy after implementation of PNA FISH®. In the pre-implementation of the PNA FISH® group, time to targeted therapy was 2.3 days compared to 0.6 day (14.4 hours) in the post-implementation of the PNA FISH® group ($p=0.0016$).⁵ These findings are comparable to the current study findings for average time to appropriate antifungal therapy of 15.24 ± 17.7 hours.

The study done by Heil and colleagues found no statistically significant difference in hospital length of stay between the pre-implementation group and the post-implementation group (25 days versus 12 days; $p=0.82$). This study also found no statistically significant difference in mortality between the pre-implementation group and the post-implementation group (19% versus 5%; $p>0.99$).⁵ Hospital length of stay was much longer in the current study compared to the study done by Heil and colleagues. In terms of mortality, the current study is comparable to the study done by Heil and colleagues (19% compared to 22.2%) with the current study's measure of all-cause mortality being slightly higher.

Aitken and colleagues performed a study to assess the role of rapid diagnostic technologies in antifungal stewardship.⁶ The study included 162 patients with culture proven candidemia. The researchers evaluated the time to initiation of therapy and found the average to be 3.5 ± 2.1 days. Using this data they compared their findings to a 5,000 person Monte Carlo simulation. They found that the average time to initiation of therapy was 2.6 ± 1.3 days with the PNA FISH®.⁶ The average time to initiation of therapy was much higher than what was found in the current study (2.6 ± 1.3 days compared to 15.24 ± 17.7 hours).

The current study had multiple limitations including retrospective design, small sample size and being a single center study. In 2012, a change was made in the reporting strategy for positive cultures by removing the wording "by PNA FISH®" as it was believed the statement may lead to misinterpretation of the results. This may have resulted in exclusion of cultures in which the PNA FISH® was used. The authors assumed the PNA FISH® was used if the medical record notes indicated *C. albicans*

and/or *C. parapsilosis* or *C. glabrata* and/or *C. krusei*. There were multiple potential inconsistencies in result reporting as provided by the medical records.

CONCLUSIONS

In conclusion, this study found an average time to appropriate therapy of 15.24 ± 17.7 hours in patients in which PNA FISH® technology was used as a rapid diagnostic test for *Candida* species bloodstream infections. This result is comparable to the results of a study done by Heil and colleagues, in which the average time to targeted antifungal therapy after PNA FISH® implementation was 14.4 hours.

It would be beneficial in the future to evaluate the time to appropriate antifungal therapy before and after PNA FISH® implementation. This would provide the ability to run data analyses and determine statistical significance, as this study was limited by only being able to evaluate descriptive statistics.

This study found that only 33% of positive blood cultures had therapy changed or initiated after the PNA FISH® results were reported to the physician. This shows a possible area for improvement in patient care. Educational efforts should be expanded to health care providers to provide information on the use of the PNA FISH® as a rapid diagnostic test. This may improve time to appropriate therapy in patients with candidemia in which species identification is key in determining appropriate antifungal therapy.

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Table 1: Patient Demographics

	n=27
Age (years) ^a	57.15 (16.9)
Male ^b	19 (70.4)
Primary team ^b	Medical: 20 (74) Surgical: 5 (19) Other: 2 (7)

a = data reported as average (\pm SD)

b = data reported as n (%)

Table 2: Primary and Secondary Objective Outcomes

	n=27
Time to appropriate therapy (hours) ^a	15.24 (17.7)
Appropriate therapy after PNA FISH® ^b	9 (33)
Not appropriate therapy after PNA FISH® ^b	1 (3.7)
Hospital length of stay (days) ^a	24.4 (18.8)
ICU admission ^b	23 (85.2)
ICU length of stay (days) ^a	15 (20.7)
All-cause mortality ^b	6 (22.2)

a = data reported as average (\pm SD)

b = data reported as n (%)

Table 3: Timing of Antifungal Therapy Initiation

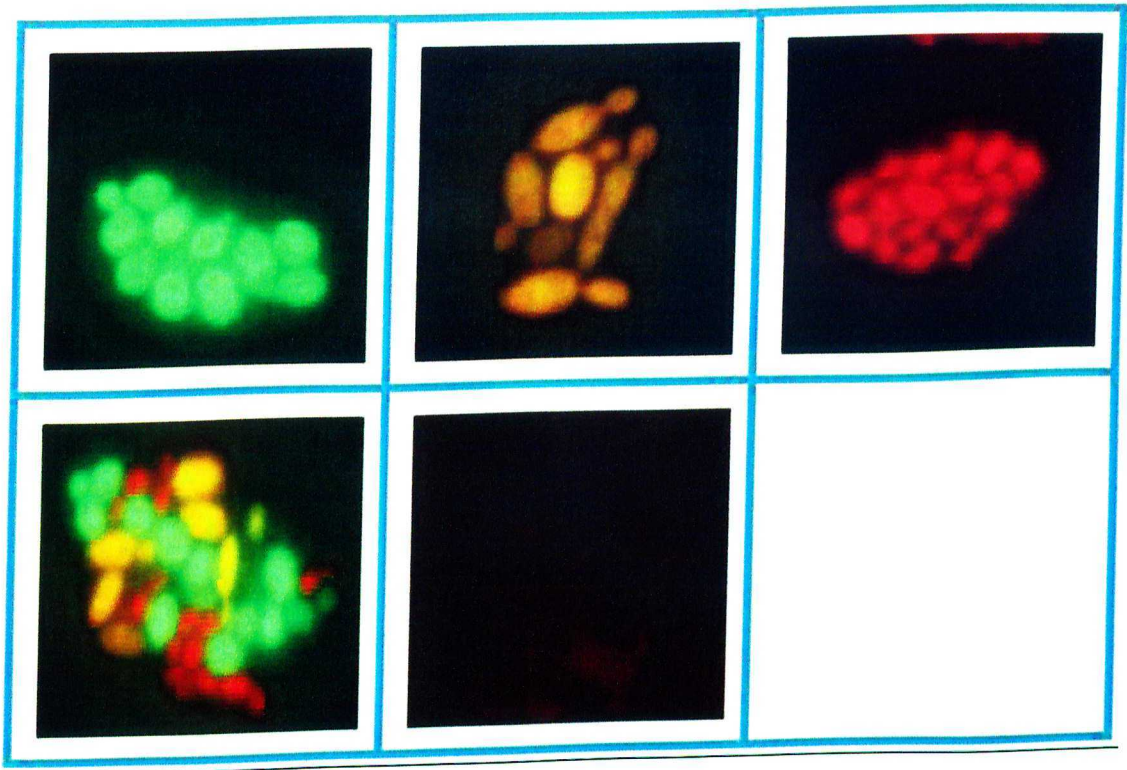
	<i>n = 27</i>
Therapy changed or initiated after PNA FISH® ^a	9 (33)
Therapy started after final speciation ^a	7 (26)
Therapy started before physician notification ^a	7 (26)
No physician notification ^a	4 (15)

a= data reported as n (%)

Table 4: Empiric and Final Antifungal Therapy

Species	Empiric Therapy	Final Therapy	n (%)
<i>C. albicans</i>	Fluconazole	Fluconazole	7 (26)
<i>C. glabrata</i>	Caspofungin	Caspofungin	6 (22)
	Caspofungin	Voriconazole	3 (11)
	Caspofungin	Fluconazole	1 (3.7)
	Fluconazole	Caspofungin	1 (3.7)
	None	None	1 (3.7)
<i>C. krusei</i>	Voriconazole	Voriconazole	1 (3.7)
<i>C. parapsilosis</i>	Fluconazole	Fluconazole	4 (15)
	Caspofungin	Fluconazole	2 (7.5)
<i>C. tropicalis</i>	Fluconazole	Fluconazole	1 (3.7)

Figure 1.



C. albicans (top left), *C. tropicalis* (top middle), *C. glabrata* (top right), mixed positive (bottom left) and negative (bottom middle) adapted from: Yeast Traffic Light® PNA FISH® [package insert]. Woburn, MA: AdvanDx; 2011.

Figure 2: Species Identification

