

Impact of Peptide Nucleic Acid Fluorescence in situ Hybridization (PNA FISH) for Enterococcal bloodstream infections

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Background: Enterococci

- *E. faecalis* (85-90%), *E. faecium* (<10%)
- One of the 5 most common organisms in nosocomial infections
- Bloodstream and Urinary tract (common sites of infection)
- VRE bacteremia associated with higher mortality and costs¹
- Delay in appropriate antimicrobial therapy a risk factor for death in VRE bacteremia²

¹DiazGranados CID 2005;41;327-333

²De Perio ICHE 2006;27;28-33.

Vancomycin Resistant Enterococci (VRE)

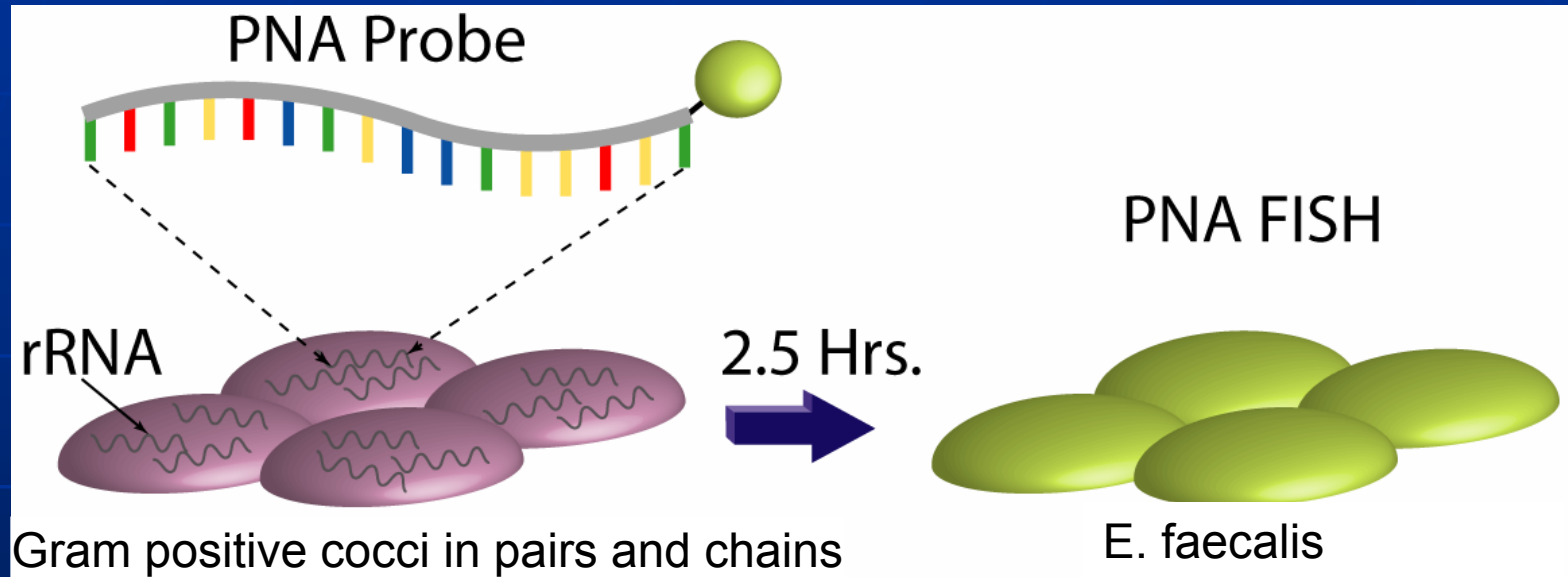
- Data NNIS¹ showed ↑ rates in vancomycin resistance in ICU's, teaching hospitals, and >500 bed hospitals (1989-1993)
- SCOPE² found 60% of *E. faecium* compared to 2% of *E. faecalis* isolates to be vancomycin resistant (1995-2002)
- Capabilities of VRE to transfer resistant genes to other organisms.

¹National Nosocomial Infection Surveillance System (NNIS). Centers for Disease Control. MMWR Morb Mortal Wkly Rep 1993; 42:S97

²Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE). Wisplinghoff H, Bischoff T, Tallent, S, et al.. Clin Infect Dis 2004; 39:309

PNA FISH

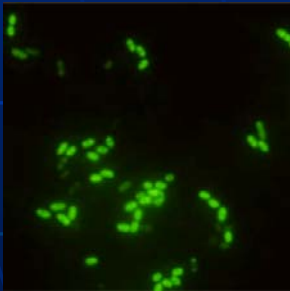
Peptide Nucleic Acid-Fluorescence In Situ Hybridization



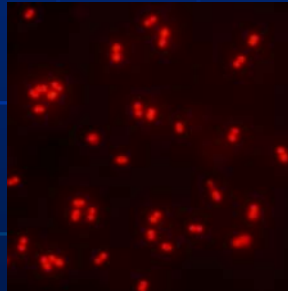
- ❑ 146 minute turnaround
- ❑ 100% specific, 98.6% sensitive
- ❑ Approved by FDA (May 2003)

PNA FISH

- Fluorescent PNA probe hybridizes to ribosomal RNA (rRNA)
- *E. faecalis* and other enterococci identification directly from GPCPC+ blood culture bottles in **2.5 hours**



E. faecalis

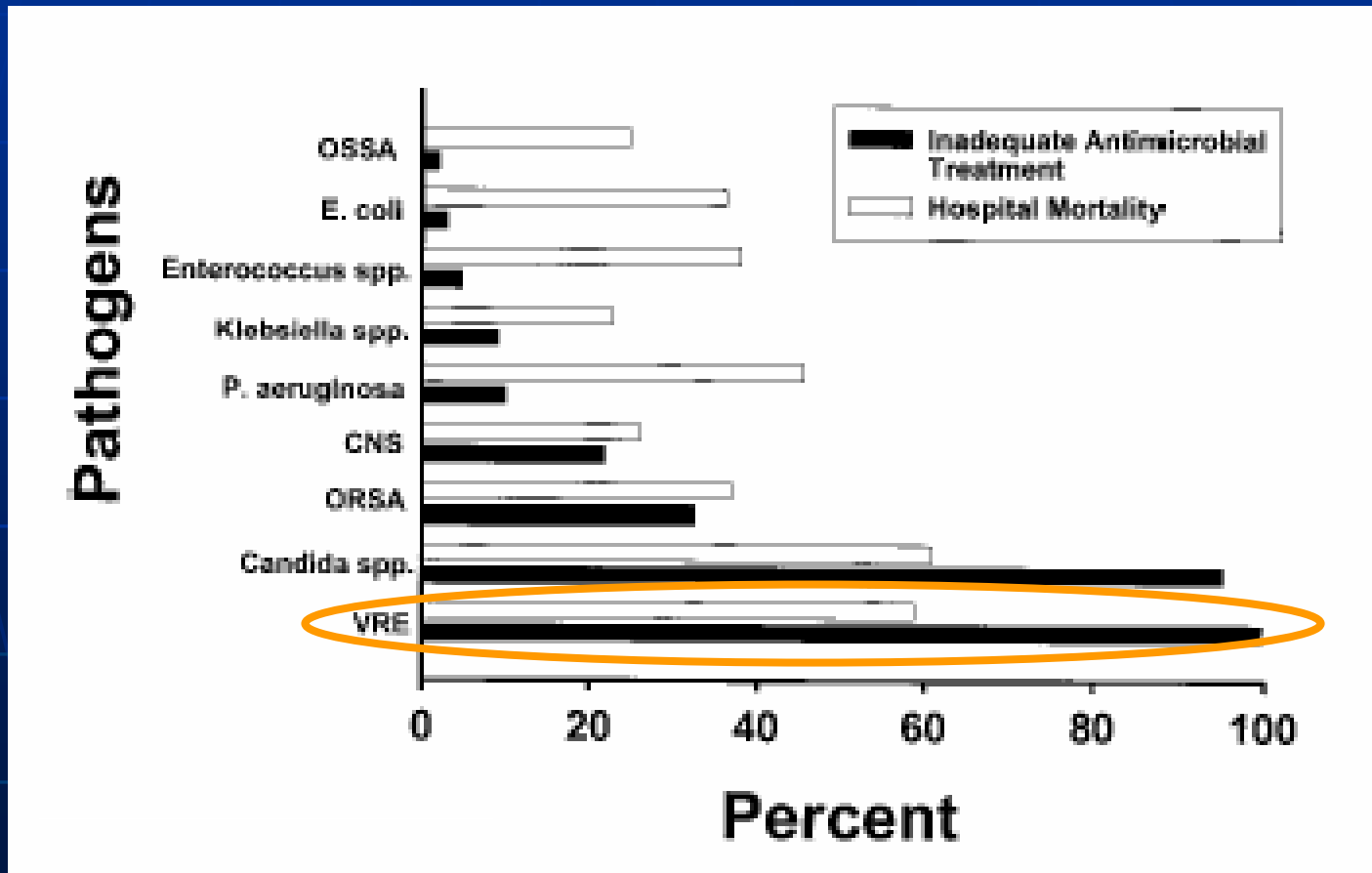


non-faecalis enterococci



non-enterococci GPCPC
(Presumptive Strep.)

Enterococcus spp. Bloodstream Infections(BSI)



The American Journal of Medicine. 2003 Nov;115(7):529-35.
Chest. 2000 Jul;118(1):146-55.

Objective

- *Primary:* Assess the impact of PNA FISH for *Enterococcus* on patient outcomes
- *Secondary:* Assess the accuracy of the PNA FISH

Methods

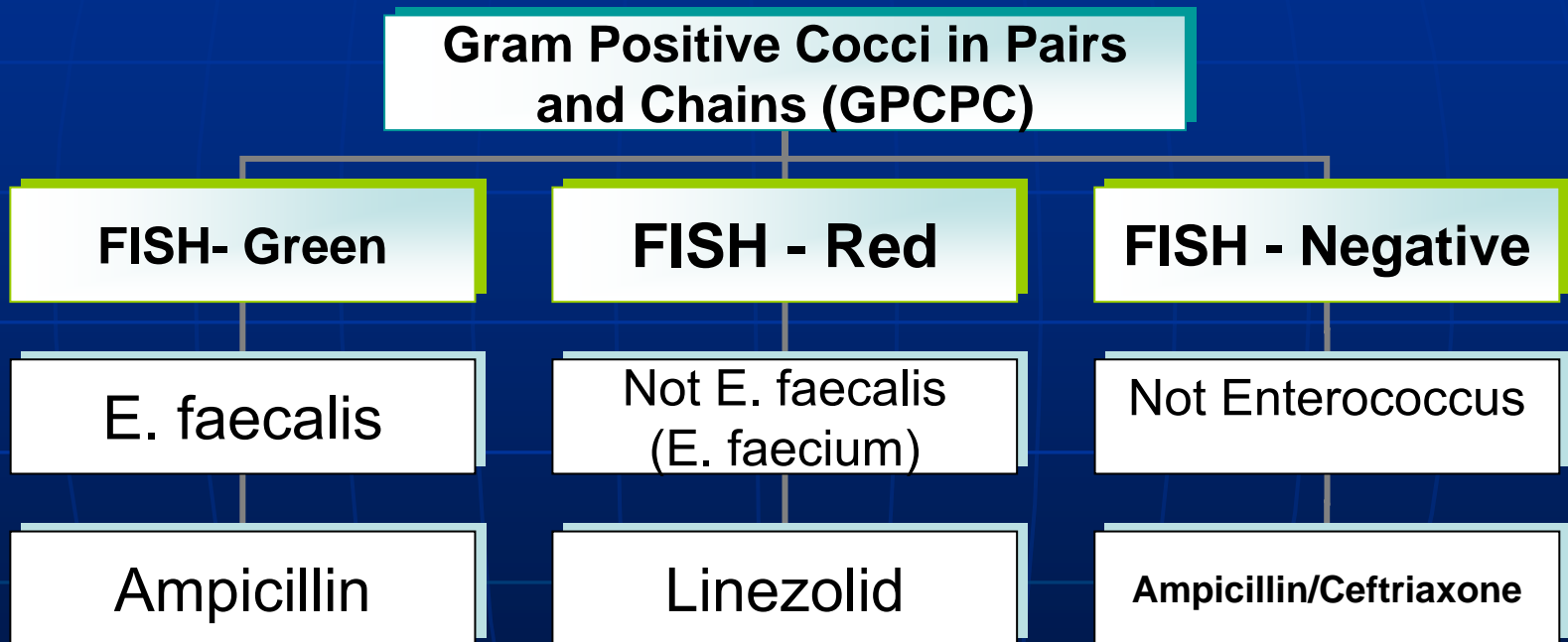
- **Retrospective:** Reviewed patient charts for Jan- Dec of 2005. Data used as historical controls.
- **Prospective:** Jan-June of 2006 prospectively identified enterococcal bloodstream cultures and active interventions were made based on PNA FISH (batched twice a day) results by the Antimicrobial Management Team

Methods

Data Collected

- Demographics: Age, Sex, Location
- Date of Gram stain, Final Culture
- Time from positive gram stain to final culture
- Time from positive gram stain to PNA Fish result
- ID of the organism
- Enterococcus resistance profile (ampicillin/vancomycin)
- White Cell Count (WCC)
- Apache II Score
- Initial Empiric Therapy (ampicillin, linezolid, vancomycin, other)
- Days to change to appropriate antimicrobial therapy
- Outcomes
- Data was analyzed using Chi-squared, Mann-Whitney and Cox regression analysis

Treatment Algorithm



Results: Accuracy of PNA FISH

- PNA FISH Test was 100% accurate in all documented test results

101 total documented test results

- 27 *E. faecalis*

- 36 non-*faecalis* (*E. faecium*)

- 38 non-enterococci (Streptococci)

12 isolates from outside hospitals

- 6 *E. faecium*

- 6 *E. faecalis*

(interventions are not within the scope of practice of UMMC antimicrobial management team)

Results

Enterococcus Faecalis

(FISH GREEN)

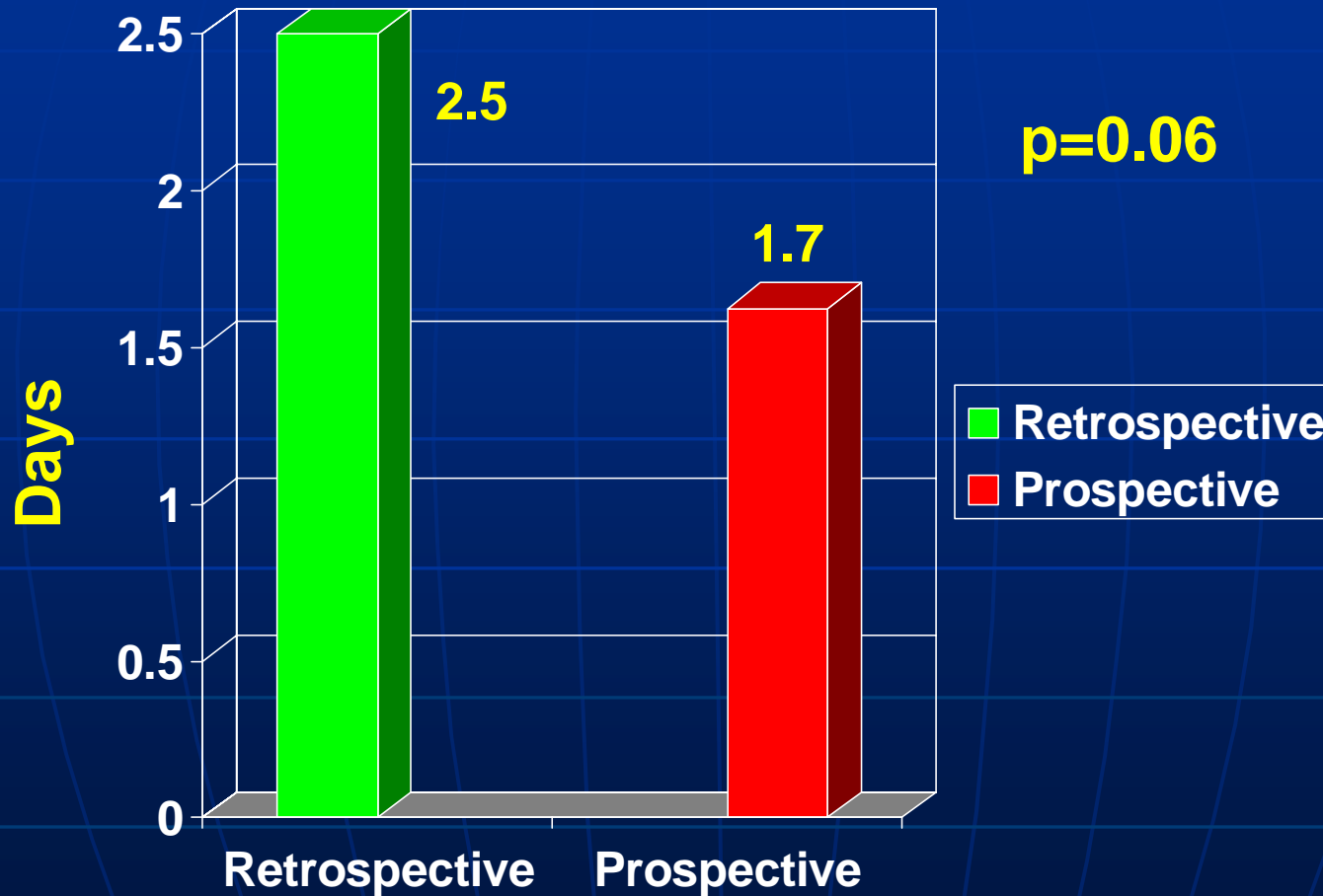
Baseline Characteristics

(*E. faecalis*)

	<i>Retrospective</i> N(%)	<i>Prospective</i> N(%)	<i>p-value</i>
	<i>n=75</i>	<i>n=21</i>	
<i>Age (years)</i>	54	42	p=0.20
<i>Male</i>	34(45%)	15(71%)	p=0.05
<i>Ampicillin-Sensitive</i>	74(99%)	21(100%)	p=1.0
<i>Initial Therapy*</i>			
<i>Ampicillin</i>	9(10%)	6(21%)	p=0.2
<i>Linezolid</i>	29(39%)	7(33%)	p=0.24
<i>Vancomycin</i>	37(43%)	14(48%)	p=0.66
<i>Broad Spectrum PCN</i>	31(36%)	6(21%)	p=0.17
<i>Inappropriate</i>	3(3%)	1(3%)	p=1.0

* Initial therapeutic regimens may contain more than 1 antibiotic

Days To Change Therapy (*E. faecalis*)



Percent Change to Ampicillin (*E. faecalis*)

	Retrospective	Prospective
<i>linezolid/vancomycin</i>	25	13*
<i>Change to ampicillin</i>	3(12%)	6(46%)
<i>P-value</i>	P=0.01	

*Note- 1 isolate ampicillin sensitive but linezolid resistant

Results

Enterococcus Faecium

(FISH RED)

Baseline Characteristics

(*E. faecium*)

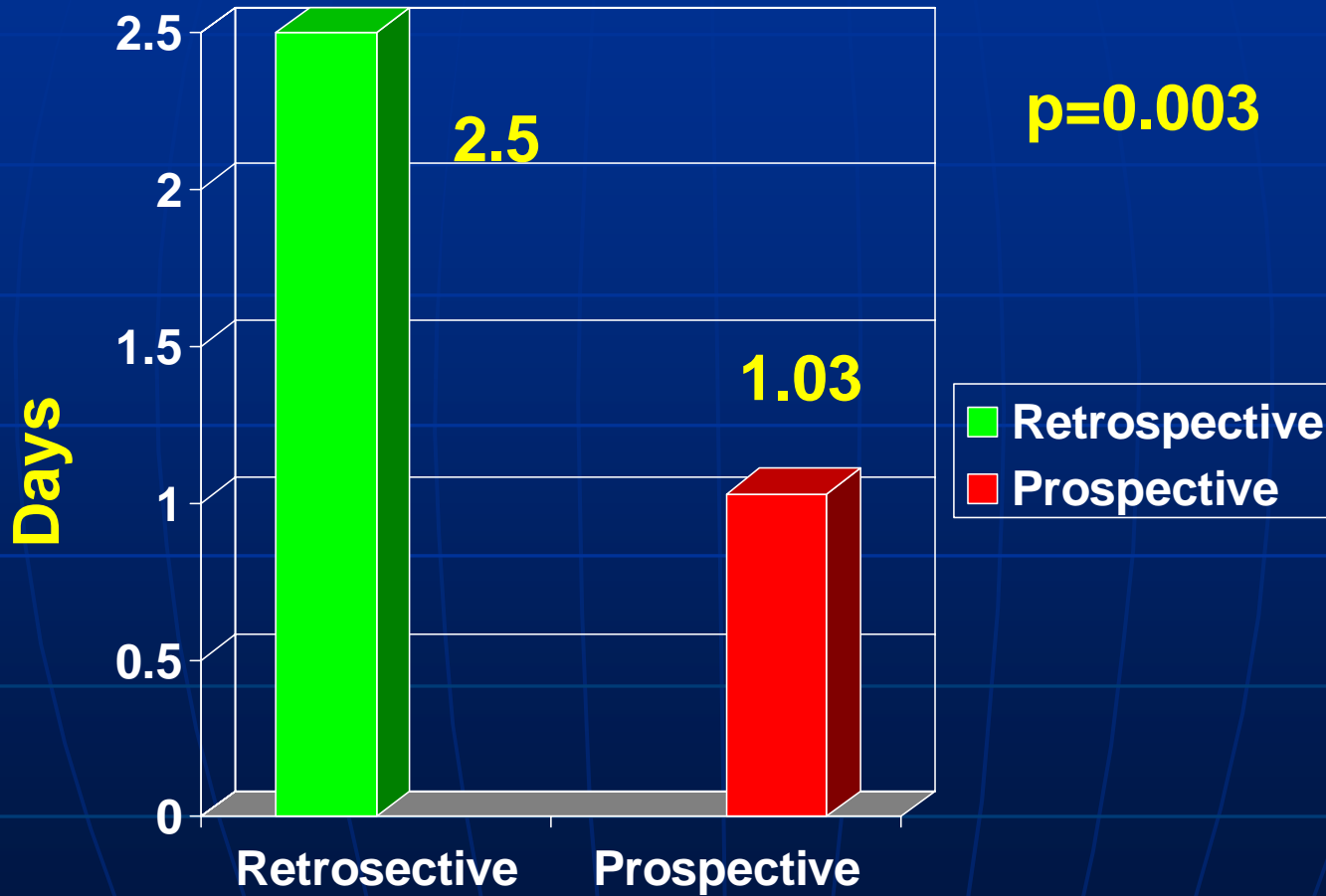
	Retrospective <i>n</i>=75	Prospective <i>n</i>=30	<i>p</i>-value
	N(%)	N(%)	
Age (med)	54	52.5	p=0.66
Sex (%male)	45(61%)	16 (55%)	p=0.27
Length of stay (med)	32 +/- 29	27 +/- 47	p=0.86
WCC	9.8+/- 10	5 +/-8	p=0.02
Creatinine	1.2 +/-2.4	1 +/-0.9	p=0.12
Apache 2	15 +/-6	17 +/-5	p=0.5
Mortality (overall)	28(38%)	8(28%)	p=0.94

Time to results and antibiotic therapy

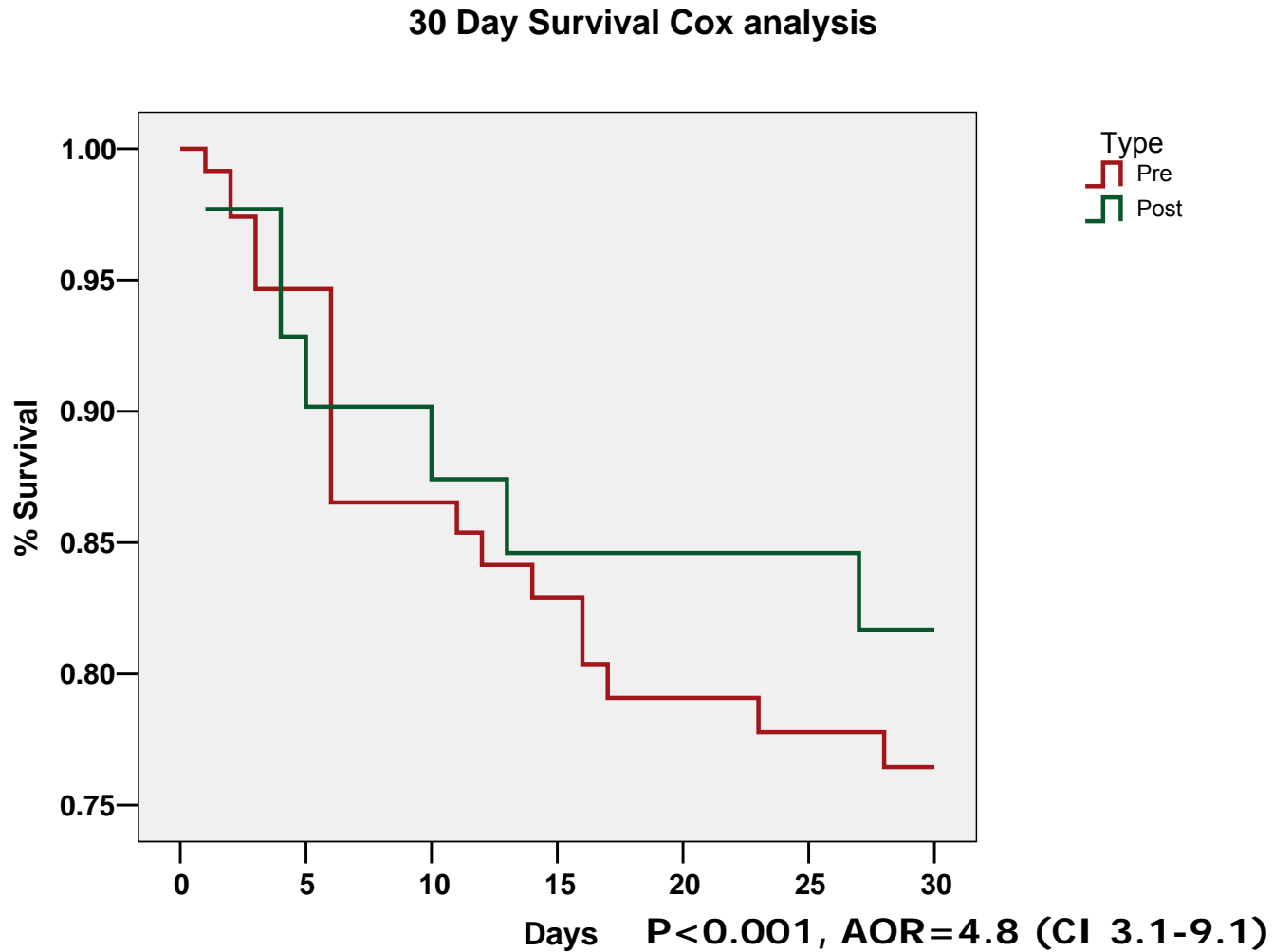
	Retrospective	Prospective	P value
Median time from GPCPC to final culture (Hrs)	53 +/- 27	57 +/- 28	0.13
Median time from GPCPC to PNA FISH report (Hrs)		7 +/- 4	<0.001
Mixed Cultures with <i>E. faecalis</i>	7	4	1
Median time to identify mixed cultures (Hrs)	109 +/- 25	96 +/- 20	1
Median time to identify mixed cultures w/ PNA FISH		5 +/- 1	<0.001
Ampicillin R (%)	100	100	1
Vancomycin R (%)	81	93	0.2
% appropriate initial antibiotic therapy	48	31	0.2

Days To Change Therapy

(*E. faecium*)



30 Day Survival Cox analysis



Limitations

- Limited cases in the intervention arm
 - Had fewer cases of Enterococcal bacteremia than predicted and have not reached our statistical power.
- Were not allowed to do a prospective randomized comparison study by the IRB
 - Limited to retrospective comparison
- There were more interventions made by antimicrobial team in the Prospective arm

Conclusion

Use of PNA FISH for Enterococci in BSI

- Allows for rapid and accurate identification of enterococcus species
- Trends toward an earlier switch to ampicillin in *E. faecalis* isolates
- Significantly reduces time to correct therapy and mortality with *E. faecium* isolates

References

- *National Nosocomial Infection Surveillance System (NNIS). Centers for Disease Control. Nosocomial enterococci resistant to vancomycin-United States, 1989-1993. MMWR Morb Mortal Wkly Rep 1993; 42:S97
- Wisplinghoff H, Bischoff T, Tallent S, et al Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE).. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004; 39:309
- Hospital Infection Control Practices Advisory Committee (HICPAC). Recommendations for preventing the spread of vancomycin resistance. Infect Control Hosp Epidemiol 1995;16:105
- Chapin K. Musgnug M. Evaluation of three rapid methods for the direct identification of Staphylococcus aureus from positive blood cultures. *Journal of Clinical Microbiology*. 41(9):4324-7, 2003 Sep.
- Harbarth S, Garbino J, Pugin J, et al. Inappropriate Initial Antimicrobial Therapy and It's Effect on Survival in a Clinical Trial of Immunomodulating Therapy for Severe Sepsis. The American Journal of Medicine. 2003 Nov;115(7):529-35.
- Ibrahim EH, Sherman G, Ward S, et al. The Influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest. 2000 Jul;118(1):146-55.
- Johnson JK, Roberts AA, Forrest GN, Lincalis DP, Venezia RA. et al Rapid Identification of Enterococcus species in Positive Blood Cultures with Therapeutic Implications. ASM (American Society of Microbiology) Meeting - 32) ASM-#C-147, Orlando 2006.