Development of a Novel Antibody-Based Assay for Simultaneous Identification of a Pathogen and Determination of its Antimicrobial Susceptibility

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ABSTRACT

BACKGROUND: Elucidation of a pathogen's antimicrobial susceptibility traditionally requires subculture after the organism is first isolated. This process takes several days, requiring patients to be treated with broad-spectrum antibiotics until a pathogen is identified. This empirically based approach has contributed to the development of bacterial resistance, and a call has been placed for the development of new methods in targeting the treatment of infectious diseases.

METHODS: By modifying a simple immunosorbent assay, we have developed a tool allowing for the simultaneous identification of a pathogen and determination of its antimicrobial susceptibility. Microtiter wells were coated with a polyclonal antibody targeting the pathogen of interest. After a specified incubation period, bacterial suspensions were added in the presence/absence of selected antibiotics. After washing, captured bacteria were detected with a horse-radish peroxidase-labeled secondary polyclonal antibody, and the optical density was read.

RESULTS: Group B streptococcus (GBS), *Enterococcus faecalis*, and *Neisseria gonorrhoeae* were each detected at 10^5 bacteria/ml following a 20-minute incubation period. Susceptibility to select antibiotics was discernable following a 6-hour incubation period (GBS and Enterococcus), and inducible resistance against clindamycin was shown when GBS was cultured in the presence of both erythromycin and clindamycin. Sensitivity was increased to 10^{-2} bacteria/ml for GBS, 10^{-1} bacteria/ml for *E. faecalis*, and 10^1 bacteria/ml for *N. gonorrhoeae* following an 18-24 hour culture.

CONCLUSIONS: This novel assay allows for the highly sensitive and specific identification of a pathogen and simultaneous determination of its antimicrobial susceptibility in a reduced time.

METHODS

Bacterial strains utilized:

GBS clinical isolates 12386 (clindamycin susceptible) and 01.12.76 (clindamycin resistant)

E. Faecalis ATCC 29212 (Vancomycin susceptible), ATCC 51299 (Vancomycin resistant)

N. Gonorrhoeae Clinical isolate 1279 (penicillin susceptible), ATCC 31426 (penicillin resistant)

E. coli, *S. aureus*, *C. albicans* and Groups A, C, F, and G streptococcus were all clinical isolates

Antibodies utilized:

All antibodies were purchased from either Virostat (Portland, ME) or ThermoFisher (Waltham, MA), and were rabbit polyclonal antibodies (#1521 anti-GBS, #1524 HRP-anti-GBS, #3711 anti-Enterococcus, #3714 HRP-anti-Enterococcus, #PA1-7233 anti-gonorrhoeae, and #PA1-73144 HRP-anti-gonorrhoeae).

Bacterial Detection and Competition Experiments:

Wells were coated with specified antibody overnight

Wells were blocked with StartingBlock™

Bacterial dilutions were prepared, starting at 0.5 McFarland, and diluted out serially from 10^8 bacteria/ml to 10^{-3} bacteria/ml

After 30 min, wells were washed with PBS-Tween

HRP-conjugated antibody was added for 20 min

Read OD_{450}

Competition Studies: Dilutions of commonly isolated bacteria were added to wells at a steady concentration of 10^8 bacteria/ml, and the amount of test bacteria was diluted out serially.

Determination of Antimicrobial Susceptibility and limit of detection studies: Bacterial suspensions were incubated in FastidiousBroth™ for specified time-points, either 6 hours (GBS and Enterococcus antibiotic susceptibility), 9 hours (inducible resistance of GBS to clindamycin), or overnight for determination of the limit of detection for all three organisms or determination of *N. gonorrhoeae* antibiotic susceptibility.

RESULTS

Figure 1 A-C. Determination of the limit of detection for the Time Zero Test

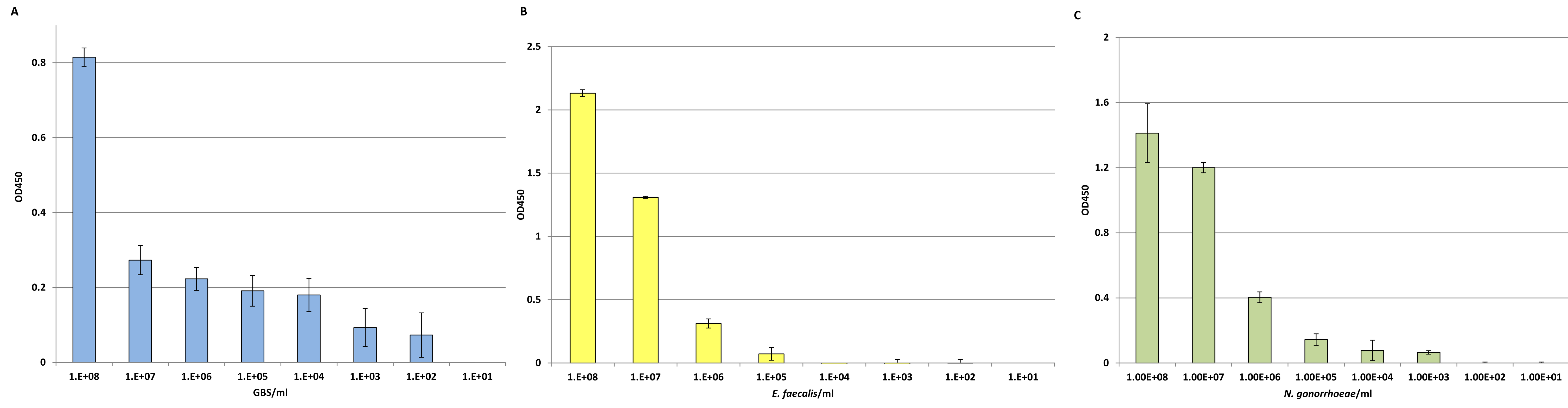


Figure 2A-C. Determination of the limit of detection following incubation at 37° Celsius

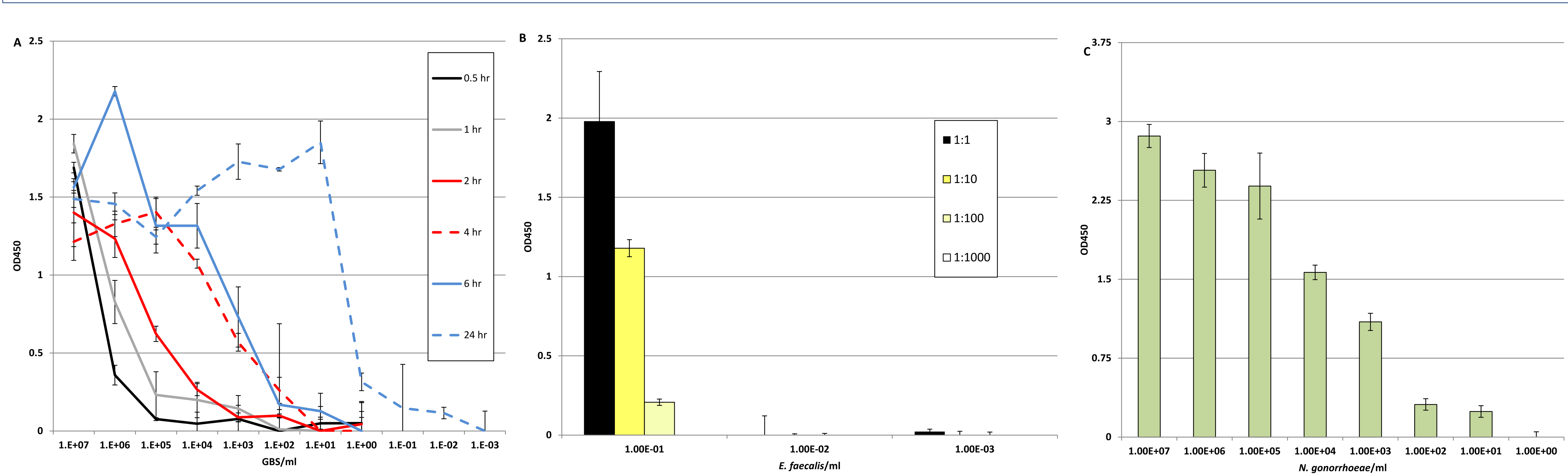
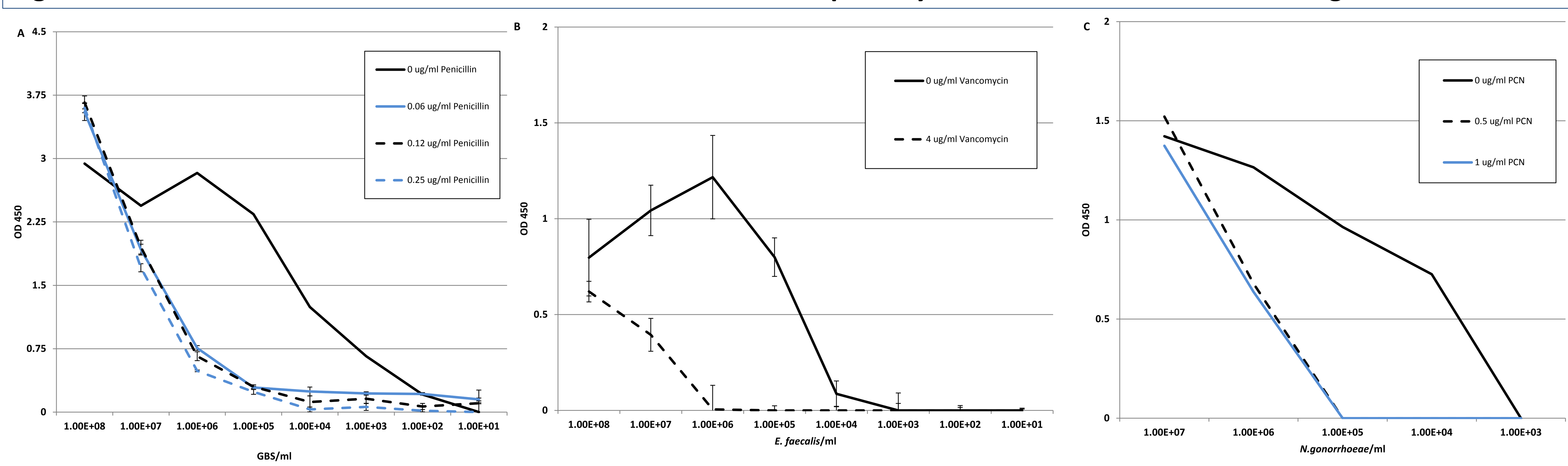


Figure 3 A-C. Bacterial identification and demonstration of susceptibility to selected antibiotics following a 6-hour culture



	10^7 bacteria/ml		10^6 bacteria/ml		10^5 bacteria/ml		10^4 bacteria/ml	
	A	B	A	B	A	B	A	B
Clindamycin 0 mg/ml Erythromycin 1.0 mg/ml	++++	++++	++++	++++	++++	++++	++++	+++
Clindamycin 0.5 mg /ml Erythromycin 1.0 mg /ml	-	++++	-	++	-	+	-	+
Clindamycin 0.05 mg /ml Erythromycin 1.0 mg /ml	-	++++	-	+++	-	+	-	+
Clindamycin 0.005 mg /ml Erythromycin 1.0 mg /ml	-	++++	-	+++	-	+	-	+

Table 1. Detection of GBS following a 9-hour incubation in the presence of erythromycin and dilutions of clindamycin. Isoate A (non-inducible resistnace to clindamycin) compared with Isolate B (inducible resistance capable). ++++ OD > 3.0. +++ OD 2.0-2.9. ++ OD 1.0-1.9. + OD 0.1-0.9. – OD 0-0.9.

CONCLUSIONS

This NOVEL assay allows for the following:

1) **Identification of a pathogen WITH SIMULTANEOUS determination of its antimicrobial susceptibility**
2) **Limit of detection RIVALS that of PCR**
3) **Unlike PCR, NO PRE-ENRICHMENT STEP NEEDED**
4) **And unlike PCR, MAY DETERMINE LIVE VS. DEAD ORGANISMS**
5) **Results are MUCH FASTER than culture**
6) **Theoretically, this test allows for detection of *de novo* antibiotic resistance, which PCR CANNOT accomplish**
7) **Potentially ANY pathogen may be targeted, as long as an antibody against it is available**

IMPLICATIONS

- **By simultaneously identifying a clinical pathogen, the physician may more rapidly utilize the appropriate antibiotic**
- **This may then decrease the use of broad-spectrum antibiotics which would decrease the development of resistance and improve outcomes**

ACKNOWLEDGEMENTS

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- **Jonathan Faro is named as a co-inventor of this technique, US20140315219 A1.**
- **Manuscript has been accepted for publication in the journal of *Infectious Diseases in Obstetrics and Gynecology***