Paper # **C-048**

Resins Do not Adsorb All Antibiotics at Peak Serum Concentrations, Especially the Newer Betalactam Antibiotics

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ABSTRACT

Background: Several publications have described the performance of resins for clinical blood samples and for seeded blood culture studies. Of interest were the studies that showed poor or discrepant results in the ability of the resins to adsorb out sufficient antibiotic to allow organism growth.
Methods: We developed a microtiter plate assay that semi-quantitatively shows the amount of antibiotic adsorption by the resins. Sixteen antibiotics, representing antibiotics that were reported as being totally adsorbed, partially adsorbed, or not adsorbed by the resins were chosen for testing. BD PLUS Aerobic/F broths (2), homemade TSB resin broths (1), and TSB broths with no resins (1) were tested. Antibiotics were added at peak serum levels to each bottle and samples were taken at 0, 60, 120, and 180 minutes. The broth sample was added to column 1 in the microtiter plate and the broth was further diluted in 2-fold dilutions to well 11. Well 12 was a growth control. Each sample was tested in duplicate. At the end of the 180 minute sampling, the bottles were inoculated with one organism and incubated overnight to verify the microtiter assay results.
Results: A total of 32 drug/bug tests were performed in the microtiter assay, of which 22 combinations had published data. The correlation between published data and microtiter results was 91%. Growth/no growth in the incubated bottles correlated with the microtiter assay. Results showed imipenem and aztreonam were not adsorbed at all. Ampicillin, cefazolin, cefepime, ceftriaxone, ciprofloxacin, ertapenem, meropenem, oxacillin, and vancomycin were partially adsorbed. Cefoxitin, cephalothin, gentamycin, penicillin, and piperacillin/tazobactam were fully adsorbed for the organisms tested. Partial or full adsorption was directly related to the organism MIC. There were no differences seen between the BD PLUS Aerobic/F broth and the home-made TSB resin broth in antibiotic adsorption performance; therefore, media has no effect on resin performance.
Conclusions: The conclusion from this study is that resins do not adsorb out several of the newer antibiotics at peak serum concentrations, thus sample timing and broth dilution should be considered to optimize recovery of organisms from blood cultures. Evaluation of adding more resin to blood culture media needs to be done to assess improvement of resin adsorption performance for antibiotics that were partially adsorbed.

INTRODUCTION

A commercially available resin bottle (Beckton Dickinson, Sparks MD; BD PLUS Aerobic/F resin), was compared to a home-made tryptic soy broth with resin (HM-TSBR, TREK Diagnostic Systems, Cleveland, OH) and tested with a semi-quantitative method for antibiotic adsorption. In this study, testing was performed in two stages. Initially, in-house antibiotic microtiter panels were prepared with HM-TSB without resin (HM-TSB) and Mueller Hinton Broth to evaluate minimal inhibitory concentration (MIC) values for each tested antibiotic/organism combination. The resulting data was compared to CLSI QC published MIC data to confirm stock antibiotic solutions were correct and provide a baseline for the microtiter adsorption testing. Following MIC confirmation, HB-TSBR and BD resin bottles were evaluated for antibiotic adsorption: at three time periods broth samples were collected from the HM-TSBR and BD resin bottles and dispensed into a microtiter panel, along with HM-TSB. Results from all three broths were compared and resulting MIC's were calculated. Antibiotic adsorption is represented by a shift in MIC of the test organism. Complete adsorption is shown by growth out to column 1 in the microtiter plate.

MATERIALS & METHODS

In-House Antibiotic Microtiter Panels for MIC Testing

- Antibiotic stock solutions were prepared and diluted in both home-made TSB (HM-TSB) and Mueller Hinton Broth (MHB) to an antibiotic concentration of 2X peak serum concentration (PSC).
- Two hundred microliters (200µl) of a 2X antibiotic solution was placed into column 1 of a 96-well microtiter panel and 100µl of appropriate broth placed into the wells of columns 2-12.
- Antibiotics were serially diluted by taking 100µl of solution from wells in column 1 and mixing with the media in column 2. Dilutions were continued from column 2 to column 11, discarding 100µl from column 11. Column 12 was used as the growth control and contained no antibiotic.
- One hundred microliters (100µl) of test organism was inoculated to obtain a final $5x10^4$ to $5x10^5$ CFU per well.
- Panels were incubated for 18-24 hours at 37°C.
- MICs for both HM-TSB and MHB were recorded and compared to CLSI published MIC values.

MATERIALS & METHODS cont.

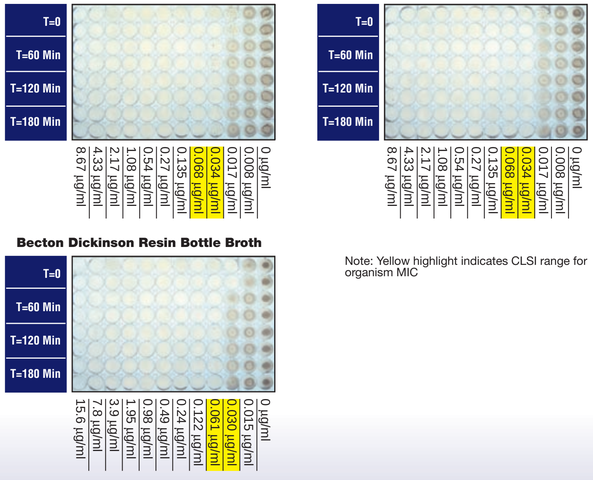
Antibiotic Adsorption by Media Containing Resin

- HM-TSB test bottles prepared with a non-ionic and cationic resin (HM-TSBR) were inoculated with PSC antibiotics and incubated with agitation. Commercially available (Becton Dickinson) resin bottles were also inoculated and incubated with agitation in the BACTEC 9240 instrument.
- At time intervals of T=0, 60, 120 and 180 minutes, 5mL broth samples were collected from each bottle type and stored at -70°C, or at 4°C if tested within 3 hours of the last sample.
- Following collection, samples were serially diluted in a 96 well microtiter panel in duplicate wells, and inoculated with specific organism/antibiotic combinations, as described above. Additionally, 10-100 CFU/ml of organism was inoculated into each test bottle and incubated overnight to verify microtiter assay results.
- Data was analyzed by comparing CLSI published MIC values to resulting MIC's from post adsorption samples. The difference between the T=0 MIC and T=180 minute MIC values represent the amount of antibiotic, in micrograms per ml, adsorbed from the test broths.

RESULTS

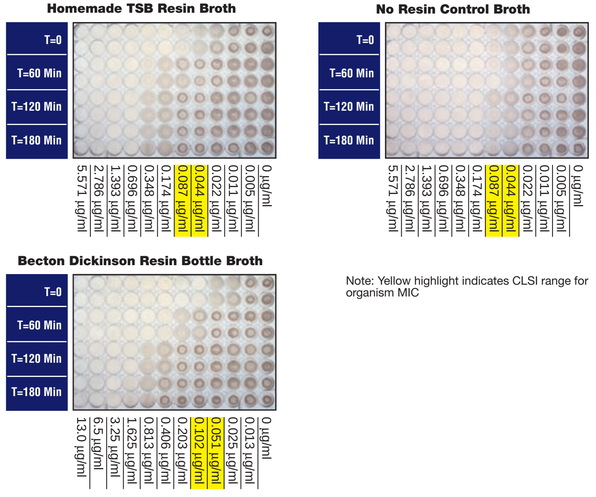
A total of 32 drug/organism tests were performed in the microtiter assay, of which 22 combinations had published data (see Table 1). The correlation between published data and microtiter results was 91% (Table 1). Growth/no growth in the incubated bottles correlated with the microtiter assay data. Results showed imipenem and aztreonam (Figure 1) were not adsorbed at all. Ampicillin, cefazolin, cefepime, ceftriaxone, ciprofloxacin, ertapenem, meropenem, oxacillin, and vancomycin were partially adsorbed (Figure 2). Cefoxitin, cephalothin, gentamicin, penicillin, and piperacillin/tazobactam were fully adsorbed for the organisms tested (Figure 3). Partial or full adsorption was directly related to the organism MIC. There were no differences seen between the BD PLUS Aerobic/F broth and the HM-TSBR in antibiotic adsorption performance; therefore, media in this study had no effect on resin performance.

Figure 1. Microtiter Showing No Antibiotic Adsorption: Imipenem and *S. aureus* ATCC 29213



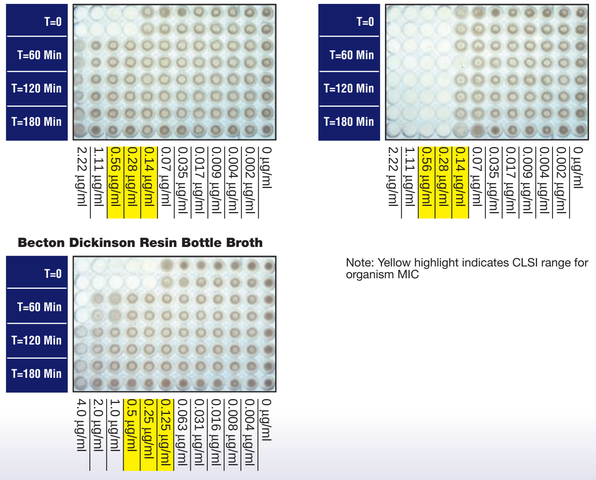
Note: Yellow highlight indicates CLSI range for organism MIC

Figure 2. Microtiter Plate Showing Partial Antibiotic Adsorption: Meropenem and *S. aureus* ATCC 29213



Note: Yellow highlight indicates CLSI range for organism MIC

Figure 3. Microtiter Plate showing Full Antibiotic Adsorption: Cephalothin and *S. aureus* ATCC 29213



Note: Yellow highlight indicates CLSI range for organism MIC

RESULTS cont.

Table 1. Comparison of Published Data With In-House Quantitative Adsorption Microtiter Test for 16 Antibiotics

Antibiotic	Organism	In house MIC (µg/ml)	Antibiotic Concentration (µg/ml)	Resin Absorbed	Published Reference	Antibiotic Completely Absorbed
			Peak	Y/N		Y/N
Ampicillin	*S. pneumoniae* 49619	0.073-0.15	47	Yes	Flayhart	No
Aztreonam	*E. coli* 25922	0.098-0.195	125	No	Gosnell	No
Aztreonam	*P. aeruginosa* 27853	1.56-6.25	125	NA	NA	No
Aztreonam	*E. coli* 25922	0.098-0.2	125	No	Pfeltz	No
Cefazolin	*E. coli* 25922	1.31-2.35	188	Yes	Bartley	Yes
Cefazolin	*S. aureus* 29213	0.16	188	NA	NA	No
Cefazolin	*S. aureus* 25923	0.36	28	Yes	Bartley	NT
Cefazolin	*S. pneumoniae* 49619	0.07-0.14	188	No	Pfeltz	No
Cefepime	*E. coli* 25922	0.04-0.08	164	No	Flayhart	No
Cefepime	*P. aeruginosa* 27853	1.28	164	Yes	Flayhart	No
Cefepime	*S. pneumoniae* 49619	0.064-0.013	164	NA	NA	No
Cefoxitin	*E. coli* 25922	1.72	110	Yes	Flayhart	Yes
Ceftriaxone	*S. pneumoniae* 49619	0.098-0.195	250	No	Flayhart	No
Ceftriaxone	*S. aureus* 29213	6.25-1.74	250	No	Gosnell	No
Cephalothin	*S. aureus* 29213	0.125-0.5	20	Yes	Flayhart	Yes
Ciprofloxacin	*S. aureus* 29213	0.2-0.4	16	Yes	Flayhart	Yes
Ciprofloxacin	*E. coli* 25922	0.0063-0.0125	16	No	Bartley	No
Ertapenem	*S. aureus* 29213	0.019-0.152	152	NA	NA	No
Gentamicin	*E. coli* 25922	0.2-0.4	8	Yes	Flayhart	Yes
Imipenem	*S. aureus* 25923	0.03	78	No	Bartley	No
Imipenem	*E. coli* 25922	0.135-0.27	48	No	Flayhart	No
Meropenem	*P. aeruginosa* 27853	0.152-1.2	48	NA	NA	No
Meropenem	*S. aureus* 29213	0.038-0.15	48	NA	NA	No
Meropenem	*E. coli* 25922	0.001-0.076	48	NA	NA	No
Oxacillin	*S. aureus* 25923	0.22-0.44	230	Yes	Flayhart	Yes
Oxacillin	*S. aureus* 29213	0.09-0.18	230	NA	NA	No
Oxacillin	*S. aureus* 43300	2.8-5.75	230	NA	NA	Yes
Penicillin	*S. pneumoniae* 49619	0.2-0.5	20	Yes	Bartley	Yes
Penicillin	*S. aureus* 25923	0.2-0.5	20	Yes	Pfeltz	NT
Penicillin	*S. aureus* 29213	0.078-0.3	20	NA	NA	Yes
Pip/Tazobactam	*E. coli* 25922	1.5-3.0	240/24	Yes	Flayhart	Yes
Pip/Tazobactam	*S. pneumoniae* 49619	0.75-1.5	240/24	Yes	Flayhart	Yes
Vancomycin	*S. aureus* 29213	0.3125-1.25	50	Yes	Flayhart	Yes
Vancomycin	*S. pneumoniae* 49619	0.15	50	33%	Flayhart	50%

Yellow highlighting indicates discrepant results between observed antibiotic absorption and published references

DISCUSSIONS AND CONCLUSION

The purpose of this study was to evaluate antibiotic adsorption for media containing resin. The results support previous blood culture studies which demonstrated variable results for antibiotic adsorption by resins.

The conclusions from this study are:

- Resin adsorption variability is due to specific organism/antibiotic MIC rather than a specific culture medium.
- Resins do not adsorb out several of the newer beta-lactam antibiotics, including 3rd and 4th generation cephalosporins and carbapenems, at peak serum concentrations.
- Sample timing and broth dilution should be considered to optimize recovery of organisms from blood cultures, even when using resin containing media.
- Evaluation of increased resin amounts or different resin materials for blood culture media needs to be performed to assess improvement of resin adsorption performance for antibiotics that were partially adsorbed.

REFERENCES

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