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Letters

Multiple Combination Bactericidal Testing

We read with interest Dr. Hibbard's letter "Combined Antimicrobial Therapy— Can We Outsmart the Microbes?" (J.S. Hibbard, *Microbe*, June 2007, p. 271). In response to Dr. Hibbard's two questions (one, "Is it time to change our infectious disease treatment strategy?," and two, "Can we outsmart the microbes?"), we would like to share our experience on the development of multiple combination bactericidal testing (MCBT) with Dr. Hibbard and readers of *Microbe*.

It all started with *Pseudomonas aeruginosa* and *Burkholderia cepacia* in cystic fibrosis (CF) patients. On routine antimicrobial susceptibility testing with single drugs, many isolates were resistant to most if not all of the antimicrobials tested. For CF lung infections, two or more antimicrobials are regularly used to treat exacerbations and to reduce bacterial loads. Without laboratory data on combination antimicrobial susceptibility testing, physicians frequently have to "best guess" a combination of two or more antimicrobials and hope for the best.

We were inspired by Dr. Yu's work on "Synergistic interaction *in vitro* with use



of three antibiotics simultaneously against *Pseudomonas* (*Stenotrophomonas maltophilia*)" (V. Yu, J. Infect. Dis. 142:602–607, 1980). Using triple combinations of antibiotics, Dr. Yu demonstrated consistent synergy against 14/14 clinical isolates of *S. maltophilia* by 3-dimensional checkerboard testing in two 96-well microtiter plates.

However, we felt that Dr. Yu's method was limited to testing of only three antimicrobial agents, and it would be also very labor intensive for routine use.

We therefore developed MCBT. Instead of using serial doubling dilution series of antimicrobials, we only used one concentration of each drug (achievable blood level) in each microtiter well. In so doing we could test a lot more antimicrobial combinations in a 96-well microtiter plate. Different panels of different antimicrobial combinations (with 1, 2, 3, or 4 drugs) were designed. Very briefly, the MCBT procedure involved inoculum preparation of the test isolate, antibiotic preparation, microtiter plate preparation, inoculation with the test isolate, incubation, reading test results of inhibition (no turbidity in test wells), subculture with a pin lid to another 96-well microtiter plate containing sterile broth, incubation of subcultured plate, and reading of results of subcultured wells for bactericidal combinations (no turbidity).

Our study group has reported MCBT findings on *B. cepacia* previously (S.D. Aaron, Am. J. Respir. Crit. Care Med. 161:1206–1212, 2000). Double antibiotic

combinations were more active than single drugs. Triple antibiotic combinations were most effective.

A similar MCBT study was reported for *P. aeruginosa* (B. J. Lang, Am. J. Respir. Crit. Care Med. 162:2241–2245, 2000). When used alone, meropenem was bactericidal against only 44% of the isolates. Double antibiotic combinations were 88 to 94% effective.

Additionally, we have tested planktonic, adherent, and biofilm-grown *P. aeruginosa* isolates (S.D. Aaron, J. Clin. Microbiol. 40:4172–4179, 2002). Biofilms were significantly less susceptible to two and three drug combinations ($P = 0.005$).

We have recently tested *Staphylococcus epidermidis* and *S. aureus* retrieved from device-associated infections (R. Saginur, Antimicrob. Agents Chemother. 50:55–61, 2006). Biofilm cultures were more resistant to single and combination antibiotics than planktonic counterparts ($p < 0.001$). MCBT reveals new options for combination antibiotic therapy.

We also determined biofilm and planktonic susceptibilities of *Haemophilus influenzae* isolates from otitis media with effusion (OME). Biofilm cultures were more resistant. MCBT suggests novel antibiotic regimens that could be studied for treatment of OME (R. Slinger, Diagn. Microbiol. Infect. Dis. 56:247–253, 2006).

We started doing MCBT 20 years ago. Presently we have more than 140 medical centers (mainly in the U.S. and Canada) sending their resistant isolates to

our laboratory for MCBT. We received 332 isolates from the U.S. alone in 2006. While the major isolates referred are still *P. aeruginosa* and *B. cepacia* from CF patients, we are seeing other organisms (e.g., *Acinetobacter* sp. and methicillin-resistant *Staphylococcus aureus*), the origins of which are not limited to patients suffering from CF. These trends indicate the problems of resistant organisms, not only in CF, but also in other infectious diseases.

With all these problems at hand, and also to prevent similar situations that Dr. Hibbard mentioned in the treatment of tuberculosis ("Unfortunately, due to the fact that only one antibiotic was frequently added to the treatment regimen at a time and combination therapy was not used, a few strains of TB eventually became resistant to all TB therapies"), it seems logical to consider treatment of other infectious diseases with combination therapy.

Dr. Hibbard asked: Combined antimicrobial therapy—can we outsmart the microbes? We hope it does, especially if we have learned the lesson with TB therapy and use it wisely. We also hope that MCBT-guided therapy can help to solve some of the problem cases.

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