Susceptibility Testing of Bordetella pertussis

Korgenski and Daly recommend charcoal horse blood agar (Regan-Lowe agar) for the detection of erythromycin resistance in *Bordetella pertussis* by agar dilution, Etest methodology, or agar diffusion. MICs for most isolates were ≤ 0.06 to 0.12 µg/ml by agar dilution and the Etest, while the MIC for the only resistant strain was 16 to 64 µg/ml (4).

Charcoal agar may be suitable for discrimination between erythromycin susceptibility and resistance in B. pertussis, but I would like to caution against the use of charcoal agar for susceptibility testing of B. pertussis in general. A previous study by our group showed that agar dilution MICs of erythromycin for B. pertussis were several dilutions higher on charcoal agar than those determined on Mueller-Hinton agar and that the MIC for Staphylococcus aureus ATCC 29213 on charcoal agar exceeded the recommended reference range (2). Similar results were obtained with antimicrobial agents other than erythromycin (3). A number of studies relating to Legionella susceptibility testing on the charcoal-containing BCYE- α agar have stressed the inactivation by charcoal of many antimicrobial agents, including erythromycin (1, 5, 6). Mueller-Hinton agar supplemented with 5% horse blood is the most suitable medium for susceptibility testing of *B. pertussis* by agar dilution (2). Appropriate media for the Etest and the role of the agar diffusion method for testing a slow-growing fastidious organism like B. pertussis should be evaluated in larger studies with different antimicrobial agents.

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Authors' Reply

Emergence of erythromycin resistance in clinical isolates of Bordetella pertussis has only recently been described. To the best of our knowledge, only one other study detecting erythromycin resistance in a clinical isolate of B. pertussis (1) has been published. Standardized susceptibility testing methodology for *B. pertussis* isolates has not been established. The purpose of our publication was to (i) communicate the possible emergence of erythromycin resistance in B. pertussis isolates, (ii) report on the isolation of an apparent erythromycin-resistant strain in Utah, and (iii) propose a screening tool for the detection of potential erythromycin-resistant strains that could be easily performed in most clinical laboratories. This screening test utilized a commercially prepared medium being used by many laboratories for the primary isolation of B. pertussis isolates (Regan-Lowe charcoal agar without cephalexin) and a 15-µg erythromycin disk. This screening test easily discriminated between erythromycin-resistant strains (both the Utah and Arizona isolates) (personal communication) and erythromycin-susceptible strains. We recommended that any strain suspected of being erythromycin resistant be sent to the Centers for Disease Control and Prevention or a referral lab for further testing. We appreciate the comments Dr. Hoppe has made concerning the problems incurred by utilizing charcoal agar when testing erythromycin as well as some other antimicrobial agents, and would not argue with his suggestion to utilize a different agar medium that has not demonstrated these problems. We have not performed studies that would allow us to make a medium recommendation at this time. However, in our limited study, the erythromycin disk screen on Regan-Lowe agar without cephalexin was adequate in detecting potentially resistant strains. If the emergence of erythromycin-resistant B. pertussis strains is widespread, then a more standardized, reproducible protocol with interpretative guidelines, taking into account both in vitro methodology parameters and antimicrobial agent pharmokinetic properties, needs to be studied and recommended.

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