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Abstract (poster session)

Reduced susceptibility of Clostridium difficile to metronidazole detected by agar incorporation but not Etest

C.H. Chilton*, G.S Crowther, J. Freeman, S.L. Todhunter, S. Nicholson, M.H Wilcox (Leeds, UK)

Objectives: To re-evaluate the susceptibility of clinical isolates of C. difficile (ribotype 001) to metronidazole in order to determine the stability of previously detected reduced susceptibility phenotype. To determine the extent of variation in MICs according to agar dilution, E-test and basal media. Methods: C. difficile isolates previously identified as showing reduced susceptibility to metronidazole had been stored at -80°C with no selective pressure for >=2 years. Isolates were reconstituted onto CCEYL agar, and inoculated into pre-reduced Schaedler broth to achieve a 0.5 McFarland solution for overnight incubation. Metronidazole MICs of these isolate broth cultures were determined in duplicate using both agar incorporation and E-test methods on both Wilkins-Chalgren and Brucella agars. MICs were read after 48 hours. Results: Metronidazole MICs determined by E-test were lower than by agar incorporation regardless of agar used (Table). MICs determined by both methods were lower on Brucella agar than Wilkins-Chalgren agar. All 7 isolates showed reduced susceptibility to metronidazole (MIC>= 4 mg/L) by agar incorporation (both agars) and E-test (Wilkins-Chalgren agar); however, only 1 strain (ribotype 010 control) showed reduced susceptibility by E-test on the recommended Brucella agar. Conclusions: Reduced susceptibility of these C. difficile isolates to metronidazole is stable over time. MIC values are affected by agar type, with Brucella agar consistently giving lower MIC values than Wilkins-Chalgren agar. MICs determined by E-test were typically 4-8-fold lower than those determined by agar incorporation (Brucella agar), which thus has the potential to misclassify the susceptibility result. As active gut metronidazole concentrations have been shown to be ~9mg/L, this could also have implications in the efficacy of metronidazole as a therapeutic option for CDI. The results reinforce the importance of using standardised methods to determine MICs for C difficile, particularly when investigating reduced susceptibility to metronidazole.

Strain	Ribotype	Agar incorporation Wilkins-Chalgren agar (mg/L)	Agar incorporation Brucella agar (mg/L)	E-test Wilkins-Chalgren agar (mg/L)	E-test Brulcella agar (mg/L)
11/11	001	16	8	8	2
5/7	001	8	8	4	2
4/12	001	8	8	6	2
15/81	001	8	4	4	1.5
11/81	001	16	8	16	2
7356014	001	8	8	4	2
E4 (control)	010	16	16	8	8