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Abstract (poster session)

Development of an agar dilution susceptibility testing method for Actinomyces species

M. Wootton*, V. Hall, V.E. Daniel, R. A. Howe (Cardiff, UK)

Objectives: Actinomycosis is a chronic disease characterized by abscess formation, tissue fibrosis and draining sinuses, caused by Actinomyces species. These bacteria are normal colonising organisms of the oropharynx, gastrointestinal tract and female genital tract, requiring a break in the mucous membranes to invade deeper body structures and cause illness. Infections often develop in tissue adjacent to mucous membranes; oral and cervicofacial infections are most common but any body-site can be infected and, rarely, disseminated spread can occur. Susceptibility testing of anaerobes in general, and in actinomycetes in particular, is problematic due to growth requirements and slow growth. Currently, only CLSI have described an anaerobe susceptibility testing method. This study aims to compare combinations of media, inoculum size, blood and additives on growth & quality of MIC cut off to inform the development of a EUCAST method. Methods: 45 Actinomyces species of varied morphology were used; A. israelii, A. gerencseriae, A graevenitzii, A. meyeri, A. naeslundii, A. odontolyticus, A. urogeneitalis, A turecensis, A. cardiffensis, A. funkei, A. europaeus. Agar dilution (AD) (CLSI) with Penicillin was performed using McFarland 1, 2 & 4 inoculum densities. Combinations of media, blood and additives used were: Brucella agar (BA) + 5% laked sheep blood (LSB) + haemin (h) + vitamin K (vk) (CLSI), Mueller Hinton agar (MHA) + 5% defibrinated horse blood (DHB) + NAD (EUCAST-F), plus CLSI and EUCAST method variants; 1- BA + DHB + h + vk, 2- BA + 5% LSB + NAD + h + vk, 3- BA + 5% DHB + h + vk, 4- MHA + LSB + NAD + H + vk, 5- MHA + DHB + NAD + h + vk. Quality of growth and cut off were compared. MICs for each combination were compared to CLSI method. Results: Quality of growth and cut offs were compared (Table 1). Growth and cut off quality was good to poor when using MHA but good to excellent for BA. It was considered that CLSI method resulted in accurate MICs. Percentage agreement to CLSI MICs was best (98%) in combination 3 and worst in EUCAST-F (66%). Conclusions: The CLSI method using Brucella agar and 5% laked sheep blood gives the best quality growth and cut offs for Actinomyces species using penicillin. However DHB can be substituted with little loss to growth and cut off quality or MIC accuracy.

	Quality of growth	Quality of cut off	% MIC agreement with CLSI	% MIC disagreement with CLSI
CLSI	Excellent	Excellent	N/A	N/A
EUCAST	Good	Fair	66	34
1	Good	Good	96	4
2	Good	Good	97.8	2.2
3	Very good	Very good	98	2
4	Poor	Very poor	90.1	9.1
5	Poor	Poor	74	26