Development of an agar dilution susceptibility testing method for Actinomyces species.

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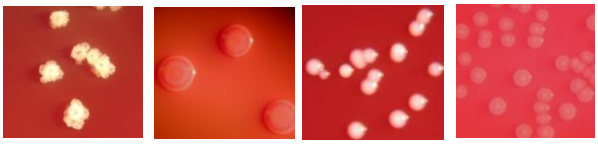
Introduction

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.

Figure 1: Morphology of *Actinomyces* species



A. graevenitzii *A. urogenitalis* *A. cardiffensis* *A. funkei*

Table 1: Combination of media

	Media	Blood type (5%)	Haemin (5mg/L)	Vitamin K (10mg/L)	NAD (20mg/L)
CLSI	Brucella	Laked sheep	✓	✓	
EUCAST	Mueller Hinton	Defibrinated horse			✓
1	Mueller Hinton	Laked sheep	✓	✓	✓
2	Mueller Hinton	Defibrinated horse	✓	✓	✓
3	Brucella (Sigma)	Defibrinated horse	✓	✓	✓
4	Brucella (BD)	Defibrinated horse	✓	✓	
5	Brucella (BD)	Laked sheep	✓	✓	✓

Methods

45 *Actinomyces* species of varied morphology (Figure 1) were used; *A. israelii, A. gerencseriae, A graevenitzii, A. meyeri, A. naeslundii, A. odontolyticus, A. urogenitalis, A turicensis, 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 are detailed in Table 1. Quality of growth and cut off were compared. MICs for each combination were compared to CLSI method

Figure 2: Quality of growth per media combination.

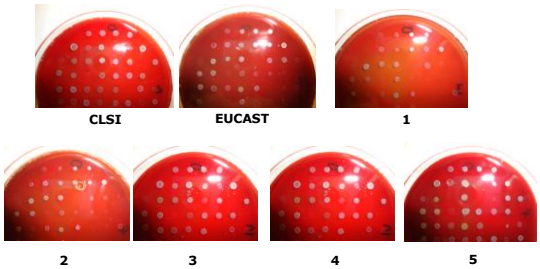


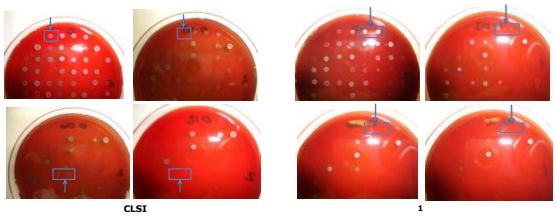
Table 2: Growth curves in AST broths by method.

	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	Poor	Very poor	90.9	9.1
2	Poor	Poor	74	26
3	Good	Good	96	4
4	Very good	Very good	98	2
5	Good	Good	97.8	2.2

Results

Inocula at McFarland 2 were most consistent. Quality of growth and cut offs were compared (Table 2). Growth quality was very poor to good when using MHA but good to excellent for BA (Figure 2 & 3). Quality of growth for more exacting species such as *A graevenitzii* was particularly affected by the media combination. Cut off quality was very poor to fair when using MHA but good to excellent for BA. Trailing end points were seen over 5 $\log_2$ dilutions. It was considered that CLSI method resulted in accurate MICs. Percentage agreement to CLSI MICs was best (98%) in combination 4 and worst in EUCAST (66%).

Figure 3: Cut off quality



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.

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