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Development of an agar dilution susceptibility testing method for Fusobacterium mecrophorum

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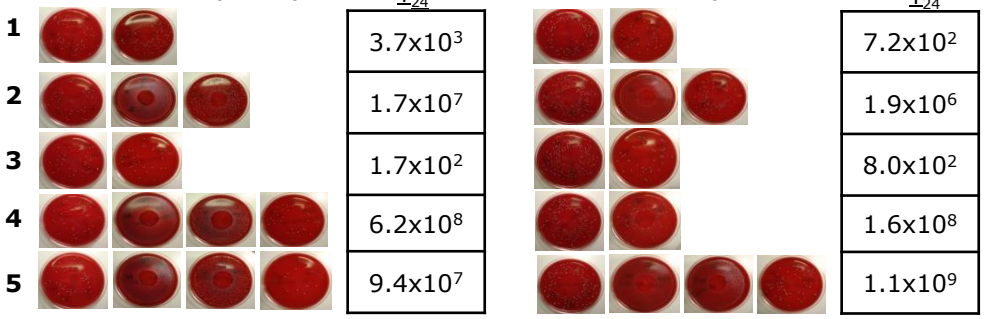
Introduction

Fusobacterium necrophorum is a Gram-negative, non-spore-forming anaerobe and is a normal inhabitant of the alimentary tract of animals and humans. As an opportunistic pathogen, it causes numerous necrotic conditions and is the bacterial cause of pharyngitis, the latter with the potential to develop into the sometimes fatal (5%) Lemierre's syndrome. Susceptibility testing of anaerobes in general, and in *fusobacterium* in particular, is problematic due to growth requirements. Currently, only CLSI have described an anaerobe susceptibility testing method. This study aims to compare combinations of media, inoculum size, blood type and additives on growth & quality of MIC cut off to inform the development of a EUCAST method. MIC were then performed to a number of potential therapy options

Fig 1: Media/supplement comparison in 2 *F. necrophorum* isolates



Methods

Growth curves were performed on 2 *F. necrophorum* isolates using 5 combinations of media: 1. Brucella broth (BB) + 5% laked sheep blood (LSB) + haemin (h) + vitamin K (vk) (CLSI), 2. BB + 5% LSB + h + vk + 20mg/L L-cysteine (C), 3. Mueller Hinton broth (MHB) + 5% lysed horse blood (LHB), 4. MHB + 5% LHB + 20mg/L C. 5. Fastidious anaerobe broth (FAB). 160 *F. necrophorum* isolates were

Methods cont.

tested using agar dilution (AD) with Brucella agar + 5% LSB + h + vk + 20mg/L C against penicillin (PEN), erythromycin (ERY), metronidazole (MET), levofloxacin (LEV), clindamycin (CLD), co-amoxiclav (CoA), clarithromycin (CLA), pip/tazobactam (P/T), rifampicin (RIF), meropenem (MER), cefalexin (CEF) & tetracycline (TET). *B. fragilis* 25285 and *S. aureus* 29213 controls were also tested.

Table 1: Susceptibilities of *F. necrophorum*

	Range	MIC_{50}	MIC_{90}	Breakpoint	% Suscept
PEN	0.004-0.12	0.015	0.03	≤0.5/1/≥2	100
ERY	1-32	4	8	No BP	N/A
MET	0.12-2	1	1	≤8/16/≥32	100
LEV	2->128	4	4	No BP	N/A
CLD	0.004-0.06	0.015	0.03	≤2/4/≥8	100
CoA	0.004-0.12	0.06	0.06	≤4/8/≥16	100
CLA	1-16	2	4	No BP	N/A
P/T	0.004-0.12	0.015	0.03	≤32/64/≥128	100
RIF	0.25-4	1	2	No BP	N/A
MER	0.002-0.008	0.004	0.004	≤4/8/≥16	100
CEF	0.03-2	0.5	1	No BP	N/A
TET	0.03-4	0.25	0.5	≤4/8/≥16	100

Results

Best growth was with cysteine containing media (2, 4 & 5). Control strain MICs were within targets for all media combinations. L-cysteine was considered essential for susceptibility testing of *F. necrophorum*. Susceptibilities to all antimicrobials were performed using media 2 and results can be seen in Table 1.

Conclusions

L-cycsteine is an essential growth factor for susceptibility testing of *F. necrophorum*. The CLSI and EUCAST media supplemented with L-cysteine gave the best quality growth for *F. necrophorum*. 100% susceptibility was found with all antimicrobials tested where a breakpoint was available.

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