Population dynamics and sporulation of quinolone resistant and sensitive *Clostridium difficile* strains

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Introduction

C. difficile (CD) is anaerobic spore forming Gram-positive bacillus responsible for hospital acquired diarrhoea. The sporulation is important for pathogen to survive in unfavourable conditions (e.g aerobic atmosphere or presence of intestinal indigenous bacteria such as lactobacilli). Quinolone (especially moxifloxacin) resistance has been associated to higher virulence of CD and used for respective screening.

Objectives

The aim of our study was to evaluate the differences between moxifloxacin resistant and sensitive strains of CD concerning population dynamics and sporulation in different environmental conditions.

Methods

Dynamics in growth and sporulation of 30 CD non-027 ribotype strains (9 moxifloxacin resistant, 21 moxifloxacin sensitive) were studied in brain-heart infusion broth using serial dilution and plating at 0, 10, 24 and 48 h. Initial bacterial concentration was $5x10^5$ CFU/ml. Environmental conditions compared were anaerobic at 37°C *vs*. ventilated tubes at 22°C and for 12 CD strains anaerobic at 37°C with or without presence of 5 lactobacillus strains of human origin in equal amounts. All five lactobacillus strains were tested separately against 12 CD strains.

Results

Median growth of CD at 48 h was 4.0 log10 CFU/ml higher (7.0 log10 CFU/ml *vs.* 3.0 log10 CFU/ml, respectively; p<0.001) and median sporulation 2.8 log10 CFU/ml higher (5.3 log10 CFU/ml *vs*. 2.5 log10 CFU/ml, respectively; p<0.001) at 37°C compared to 22°C.

Growth at 22°C (Fig. 1) influenced more moxifloxacin resistant strains compared to moxifloxacin sensitive ones (median -2,8 log10 CFU/ml *vs.* median -1.4 log10 CFU/ml, respectively, p=0.044, at 48 h).

The spore amount at the starting point (0 h) was similar in moxifloxacin resistant and sensitive strains (Fig. 1).

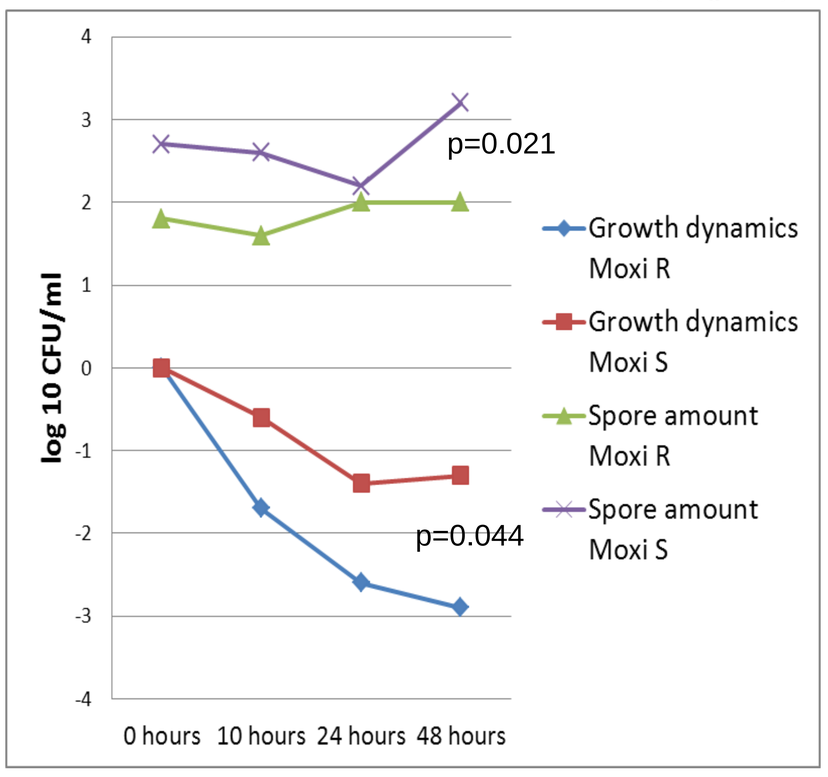

Figure 1. Population dynamics and sporulation of moxifloxacin resistant (Moxi R) and sensitive (Moxi S) *C. difficile* strains at 22°C

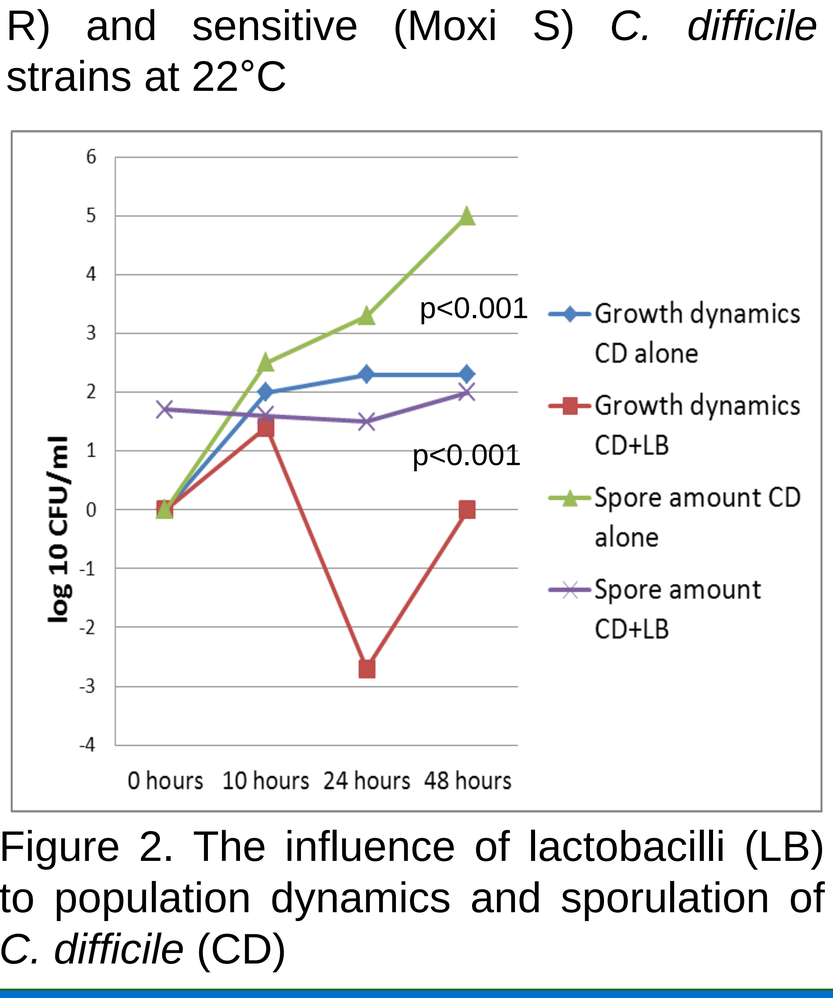

Figure 2. The influence of lactobacilli (LB) to population dynamics and sporulation of *C. difficile* (CD)

After 48 h at 22°C significantly less spores were detected in moxifloxacin resistant compared to moxifloxacin sensitive strains (median 2.0 log10 CFU/ml *vs*. 3.2 log10 CFU/ml, respectively; p=0.021).

The growth and sporulation activity of moxifloxacin resistant and sensitive strains was similar at 37°C.

Co-culturing of CD strains with lactobacillus strains significantly lowered the growth rate of CD (median 2.3 log10 CFU/ml *vs*. 0 log10 CFU/ml, respectively; p<0.001) and amount of detected CD spores (5.0 log10 CFU/ml *vs*. 2.0 log10 CFU/ml, respectively; p<0.001) at 48 h (Fig. 2) when compared to CD alone. At the same time the growth of lactobacilli was similar in presence or absence of CD in culture (median 7.0 log10 CFU/ml *vs*. median 7.0 log10 CFU/ml, respectively; p=0.539) at 48 h of growth.

The influence of lactobacilli to growth of CD was similar regardless of moxifloxacin susceptibility, however the amount of detected spores was significantly lower in moxifloxacin resistant compared to moxifloxacin sensitive strains (median 1.3 log10 CFU/ml *vs*. 2.3 log10 CFU/ml, respectively; p=0.012) at 48 h co-growth with lactobacilli.

Conclusions

The population dynamics and sporulation of moxifloxacin resistant CD strains is more influenced by unfavourable environmental conditions e.g. lower temperature and aerobic atmosphere or presence of lactobacilli.

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