The Effect of Probiotics on in Vitro Growth of Carbapenemase Producing *Klebsiella Pneumoniae* (KPC-Kp)

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INTRODUCTION

Carbapenemase producing *Klebsiella pneumoniae* (KPC-Kp) is a public health concern and increasing importance worldwide. Having a healthy microbiota could prevent the expansion and persistence of exogenously acquired bacterial species, but this mechanism can be impaired by antibiotic treatment. Research suggests that when probiotics, such as *Lactobacillus rhamnosus* GG (LGG) and *E.coli* Nissle (EcN), are regularly ingested as part of the diet, may boost the body's immune system and mantain intestinal balance through providing healthy microflora. So far no data are available regarding the role of probiotics on KPC-Kp growth.

In a series of *in vitro* studies we evaluate the impact of LGG and EcN on the growth of KPC-Kp.

MATERIALS & METHODS

From June 2015, we conducted a series of *in vitro* studies at Tufts Medical Center, Boston, MA, on the *in vitro* effect of LGG and EcN on KPC-Kp using and co-culture and conditioned fresh media. *K. pneumoniae* KPC- producing (ATCC BAA 1705), *E. coli* Nissle, *L. rhamnosus GG* (ATCC 53103) and *E. coli* ATCC 25922 (control strain) were used in this study.

Co-Culture Overnight starter broth cultures were generated by inoculating a single colony from a fresh plate culture into 20 mL of Luria Bertani (LB) broth (BD Difco 244610). For LGG deMann Ragosa Sharpe (MRS) broth (BD Difco 288130) was used. The cultures were incubated at 35-37^{0}C in an anaerobic chamber (10%H_2 5%CO_2 85%N_2) The following morning working broth cultures of each organism were prepared by inoculating 20 Ml+ of fresh broth, 9:1 LB:MRS, with 0.2 mL of the overnight starter culture. This was incubated in the anaerobic chamber until the cultures reached an OD600 of approximately 1.0 ODU. Co-cultures were created by combining 5 mL of each probiotic culture with 10 mL of the KPC culture and 5 mL of 9:1 LB:MRS broth. Single culture of each strain was performed as a growth control. Incubation was under anaerobic conditions. To evaluate growth samples were serially diluted in PBS and plated in duplicate on MacConkey agar (Remel R01552) and for LGG containing cultures MRS agar (Oxoid CM03618) at time= 0, 4, 24 and 48 hours. MacConkey plates were incubated overnight at 35^{0}C in ambient air, MRS under anaerobic conditions for 48 hours. Colony forming unit counts (CFU) were determined from averaging the counts obtained from plates growing between 25 and 250 CFU.

MATERIALS & METHODS (1)

Conditioned Media Overnight starter cultures were prepared as above for the EcN, LGG, and *E.coli* control strains. 1 mL of each culture was singly inoculated into 40 mL of fresh LB broth and incubated for 24 hours under the same anaerobic conditions. The conditioned cultures were then centrifuged for 30 min at 6,100 × g and the supernatants were filtered through 0.2-μm filters. Nutrients were adjusted by the addition of 20X LB medium to a final concentration of 0.5X. pH was adjusted to 7, and the medium was then filtered. 20 mL of each conditioned medium and an unconditioned control were inoculated with 0.5 mL of KPC-Kp working culture and bacterial growth was evaluated at T=0, 4, 24 and 48h as described above.

Effect of pH. To evaluate the effect of pH on the growth and expression of the KPC-Kp LB:MRS broth were prepared at varying pH values: 4.5, 5.0, 5.5. Hydrochloric acid was used to adjust the pH of the media and each was then filter sterilized. Working cultures of the KPC-Kp were inoculated into 20 mL of each broth and an unaltered broth control (pH 6.7), incubated and enumerated as described above.

The results were confirmed with at least three independent experiments. Each sample was assayed in triplicate and the mean activity and standard deviation are presented. Student't test was performed.

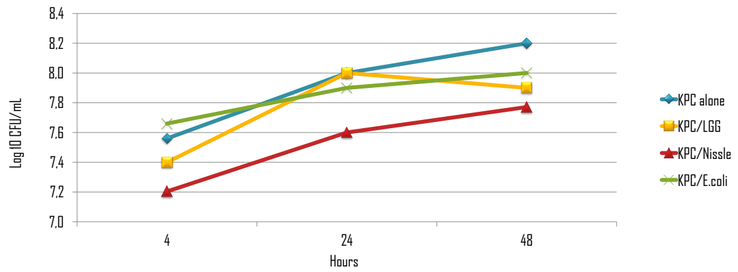
RESULTS

Our data showed a decrease in KPC-Kp *in vitro* growth with EcN using fresh conditioned media (**Table 1 & Figure 1**), with a significant decrease at 4h and 48h after co-incubation (p= 0.011 and p=0.02, respectively), but not with LGG.

Table 1 . Fresh conditioned media: KPC-Kp and EcN

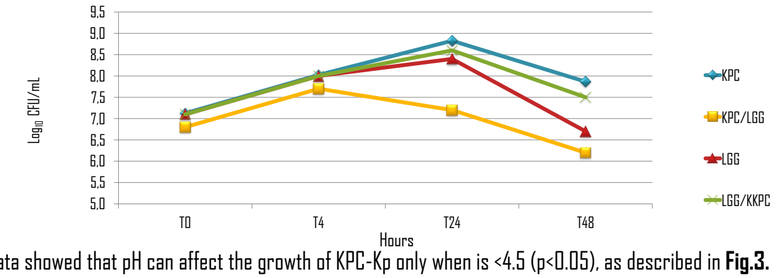
Time	KPC BAA1705 Log_{10} CFU/mL Mean (SD)	KPC BAA1705/EcN Log_{10} CFU/mL Mean (SD)	P value
T4	7.6 (±0.11)	7.2 (±0.06)	**0.011**
T24	8 (±0.25)	7.7 (±0.37)	0.42
T48	8.2 (±0.11)	7.8(±0.05)	**0.02**

Figure 1 . Fresh conditioned media: KPC-Kp and EcN

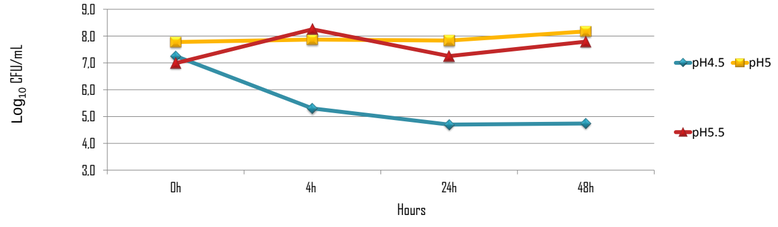


In co-culture, a decrease was observed only when KPC-Kp was co-cultured with LGG (**Table 2 & Figure 2**), with a significant decrease in growth at 48h (p=0.03).

Time	KPC BAA1705 Log_{10} CFU/mL Mean (SD)	KPC BAA1705/LGG Log_{10} CFU/mL Mean (SD)	P value
T0	7.125 (±0.22)	6.8 (±0.15)	0.11
T4	8.025 (±0.76)	7.73 (±0.49)	0.53
T24	8.825 (±0.41)	7.23 (±0.81)	**0.05**
T48	7.867 (±0.59)	6.2 (±0.66)	**0.03**



Our data showed that pH can affect the growth of KPC-Kp only when is <4.5 (p<0.05), as described in **Fig.3.**



CONCLUSION

Our data showed a decrease in KPC-Kp growth with LGG and EcN. These data may be support the idea of a randomized controlled pilot study of a nutritional supplement in KPC-Kp colonized subjects, examining the effect of LGG or EcN on KPC-Kp gut colonization.