Antagonistic activities of Kéfigel and Kéfigel products against *Staphylococcus aureus* isolated from diabetic foot ulcers

Soraia Zenão[1]; Alfredo Aires[2]; Carla Dias[3]; Maria José Saavedra[3]; Conceição Fernandes[1]

[1] CIMO-Mountain Research Centre, ESA-Polytechnic Institute of Bragança, Bragança, Portugal;
[2] CITAB-Centre for the Research and Technology of Agro-Environmental and Biological Sciences University of Trás-os-Montes e Alto Douro, Vila Real, Portugal;
[3]CECAV-Veterinary and Animal Science Research Center, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

Kéfigel® is a commercial gel used for cosmetic purposes. Main constituents are kefir grains, nettle and lavender extracts. Diabetic foot ulcers are often complicated by *Staphylococcus aureus* [1] with high prevalence of methicillin-resistant [2].

Thus, the purpose of this study was to evaluate antibacterial properties of Kefigel®, against isolates from diabetic foot ulcers.

Material and Methods

- Antibacterial effect was assessed for Kefigel® (DMSO 10% to final concentration of 40 $mg.mL^{-1}$), kefir grains (DMSO 10% to final concentration of 100 $mg.mL^{-1}$), as well as for nettle (*Urtica dioica* L) and lavender (*Lavandula angustifolia* Mill) extracts (extracts in DMSO 10%, to final concentration of 100 $mg.mL^{-1}$).
- Here we used 20 *S. aureus* isolates (10 MSSA and 10 MRSA) collected from several diabetic foot ulcers.
- Growth screening was assessed by disk diffusion assay method [3], 15 µL of tested suspension, DMSO 10% as negative control, gentamicin (CN_{10} - Oxoid CT0024B) as positive control, 24h/37ºC. Relative efficacy was assessed according [4].
- Minimum Inhibitory Concentration (MIC) was assessed by broth microdilution method with resazurin [5], and minimum bacterial activity was assessed by solid medium growth, of bacterial suspension from microplate well, immediately after well considered MIC.
- Effects of Kefigel® on specific growth rate, were assessed by broth growth.
- Differences were tested by Wilcoxon test.

Results and Discussion

Fig 1. Example of inhibition halos obtained by diffusion method.

Only nettle and lavender etanolic extracts showed antibacterial activity.

These extracts showed an efficacy between 50 and 100% relative to gentamicin.

Generally, the MRSA isolates sowed higher inhibition halos comparing MSSA isolates.

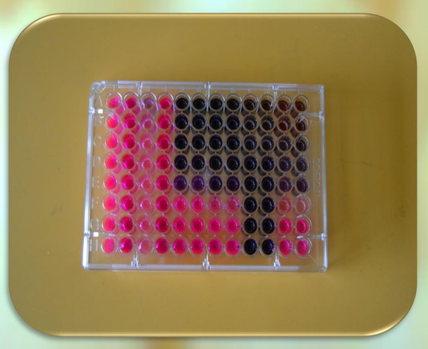

Fig 2. Example of microplate MICs determination.

There were no differences in MICs between strains, for plants extracts: nettle extract range between 0.188-0.500 $mg.mL^{-1}$ for MSSA strains and 0.063-0.500 $mg.mL^{-1}$ for MRSA strains, lavender extract range between 0.063-1.000 $mg.mL^{-1}$ for MSSA strains and 0.125-0.250 $mg.mL^{-1}$ for MRSA strains.

Minimum bacterial activity showed that effects were mainly bacteriostatic.

Kefigel® affects mostly the strains of *S. aureus* MSSA, presenting mainly bacteriostatic effect (6/10), after 3-6 h of incubation (Fig 3 and Fig 4). A bactericid effect was also observed in two strains *S. aureus* MSSA (Fig 5).

For S. aureus MRSA strains, Kefigel® showed a bacteriostatic effect only in three strains (Fig 6) and a bactericid effect in one strain (Fig 7).

These results suggest that the presence of Kefigel® has an effect on *S. aureus*, most pronounced in strains MSSA, compared to strains MRSA, probably resulting of sinergic/adittive effects of their components.

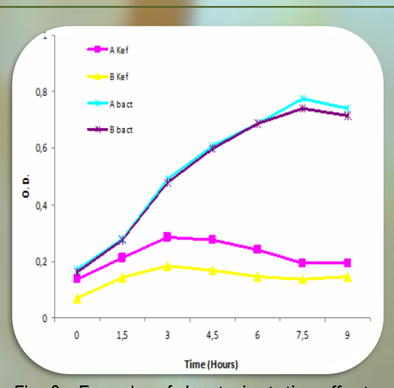

Fig 3. Exemple of bacteriostatic effect on MSSA strains

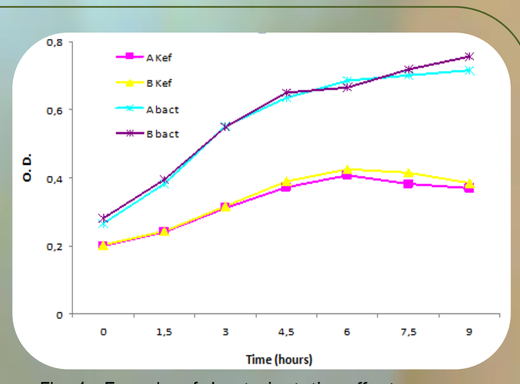

Fig 4. Exemple of bacteriostatic effect on MSSA strains

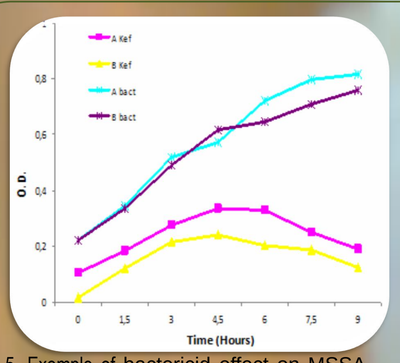

Fig 5. Exemple of bactericid effect on MSSA strains

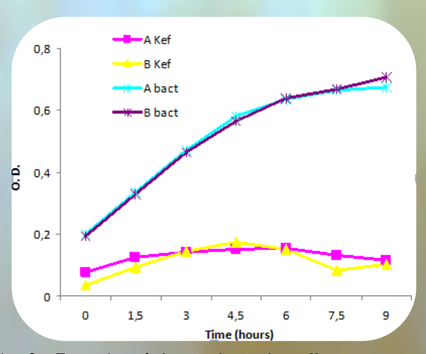

Fig 6. Exemple of bacteriostatic effect on MRSA strains

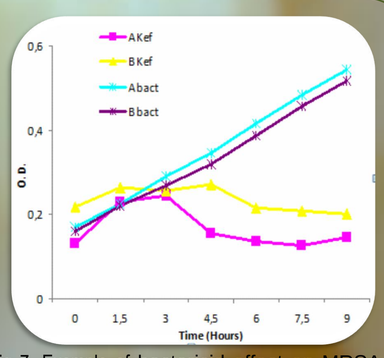

Fig 7. Exemple of bactericid effect on MRSA strains

Conclusions

- ✓Kéfigel® is a natural product with potential antibacterial action, probably due to sinergic/adittive effects of their components;
- ✓Generally, it was observed an effective bacteriostatic effect against *Staphylococus aureus;*
- ✓Effects on *S. aureus* were most pronounced in MSSA strains compared to MRSA strains;
- ✓ Topical use of Kefigel® for prevention of diabetic foot ulcers infections can be useful, however further investigations were needed.

References

(1) Powlson, S.A., Coll, A.P. (2010) The treatment of diabetic foot infections. *J Antimicrob Chemother*, 65, 3-9.
(2) Green, M.F., Aliabadi, Z., Green, B.T. (2002). Diabetic Foot: Evaluation and Management. *Southern Medical Journal*, 95, 95-101.
(3) Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard-Ninth Edition, M07-A9, Vol. 32 No. 2. CSLI (Ed.), Wayne, PA 19087, USA, 1-88 (2012).
(4) Aires, A., Mota, V.R., Saavedra, M.J., Rosa, E.A.S., Bennett, R.N. (2009).*J Applied Microbiol* 106: 2086-2095.
(5) Sarker, S.D., Nahar, L. and Kumarasamy, Y. (2007). Methods 42, 321–324.