P2621 Efficacy of chlorhexidine alone and combined with calcium hydroxide against *Staphylococcus aureus* and *Enterococcus faecalis*

Maria Isabel Millán-Lou1,2, Victoria Millán-Lázaro2, Santiago Poc3, Oscar Alonso3, Concepción López1, Antonio Rezusta*1,2,4

1 Servicio de Microbiología, Hospital Universitario Miguel Servet, Zaragoza, Spain, 2 Instituto de Investigación Sanitaria de Aragón, Zaragoza, Spain, 3 Facultad de Ciencias y de la Salud y el Deporte, Huesca, Spain, 4 Universidad de Zaragoza, Zaragoza, Spain

**Background:** Calcium hydroxide (Ca(OH)\(_2\)) has been used as an intracanal medication and chlorhexidine (CHX) has antimicrobial activity against Gram-positive and Gram-negative bacteria. *Enterococcus faecalis* is the most frequently isolated strain in failed endodontic therapy cases since it is resistant to Ca(OH)\(_2\). The aim of this study was to investigate time-kill curves with these substances at different concentrations in order to establish the bactericidal effect with a lethality of \(10^{-6}\) (6-log) against *Staphylococcus aureus* and *Enterococcus faecalis*.

**Materials/methods:** Three replicates were made for each microorganism and experiment. *S. aureus* (ATCC 29213) and *E. faecalis* (ATCC 29212) were grown overnight and inoculum suspensions were adjusted at 0.5 McFarland. The inhibitory activity of different concentrations ranged from 0.06% to 0.00023% of CHX alone against *S. aureus* and from 0.06% to 0.00047% against *E. faecalis* were tested. In addition, two of the lowest CHX concentrations that killed 6-log in the two bacteria were selected to add different concentrations of Ca(OH)\(_2\) ranged from 0.03g to 0.00047g. The plates were incubated at 35°C for 24h. The number of living bacteria (CFU/mL) of each plate using the agar plate count method was used to analyse the bactericidal effect.

**Results:** The growth of *S. aureus* was completely inhibited by chlorhexidine ranged from 0.06% to 0.0018%. CHX at 0.0018% plus Ca(OH)\(_2\) is more effective than CHX at 0.0037% plus Ca(OH)\(_2\) ranged from 0.03g to 0.0037g.

In the case of *E. faecalis*, all the bacteria were killed by chlorhexidine ranged from 0.06% to 0.00094%. CHX at 0.0075% plus Ca(OH)\(_2\) at 0.03g is more effective than CHX at 0.0037% plus Ca(OH)\(_2\) at 0.03g of Ca(OH)\(_2\) but not in the rest of concentrations studied.

**Conclusions:** In conclusion, it seems that mixing CHX with Ca(OH)\(_2\) does not improve its antibacterial property against *S. aureus* and *E. faecalis*. Only the combinations at 0.03g of Ca(OH)\(_2\) with 0.0037% or 0.0018% CHX were efficient in eliminating *S. aureus*, and 0.03g of Ca(OH)\(_2\) with 0.0075% or 0.0037% CHX in *E. faecalis*.