



# **Book of Abstracts**

## **III International Conference on Antimicrobial Research**

**Madrid, Spain, 1-3 October 2014**

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III International Conference on Antimicrobial Research - ICAR2014

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## Chitosan effect upon biofilm formation of multiresistant *Staphylococcus aureus* strains

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Antibiotic resistance within biofilms is higher than in planktonic cells with antibiotic concentrations around 1000-fold higher than those registered for planktonic growth. This higher resistance is thought to be the underlying reason as to why treatment with antimicrobial agents fail and it is estimated that *ca* 65-80% of all infections are biofilm related. Furthermore, antibiotic development pipelines rarely test the susceptibility of recalcitrant biofilm cells or utilize animal models in which bacteria form biofilm infections.

In later years, in what was thought would be the answer to the growing antimicrobial resistance problems, new sources of antimicrobials were sought with natural compounds being the preferred answer. Among the explored compounds was chitosan, a polysaccharide with confirmed antimicrobial activity against planktonic cells, who has gained a particular interest due to its biocompatibility and wide spectrum of activity.

In order to properly assess the potential effect of chitosan upon multiresistant microorganisms, three *Staphylococcus aureus* strains – two multiresistant clinical isolates, one MRSA and one MSSA, and a control MSSA strain (ATCC25923) were used in the present study. To fully comprehend chitosan's effect upon these microorganisms a two pronged approach was undertaken: first the effect of two chitosan molecular weights (MW) (624 kDa and 107 kDa) in a planktonic setting was assessed via determination of MICs, MBCs. Having established this baseline, the effect of the same chitosans upon biofilms was assessed via determination of Minimal Biofilm Inhibition Concentrations (MBIC), inhibition at sub-MIC concentrations, of biofilm formation and mature biofilms, and through the effect upon cellular metabolism via the XTT assay.

The results showed that in the planktonic phase, chitosan was active at low concentrations, however no significant differences were found between the tested strains, with both chitosans presenting an average MIC of 0.5 mg/ml. On the other hand, when analysing the results obtained for biofilms, several differences were observed. First when analysing the MBIC results it was possible to see that, contrary to most antimicrobials, chitosan was still effective at relatively low concentrations with MBICs varying between 0.6 mg/ml (MRSA) and 1 mg/ml (both MSSA strains). A similar behaviour was observed for biofilm formation and mature biofilm assays at sub-MIC concentrations, presenting inhibition percentages between 50 and 75% and higher inhibition percentages being observed for both multiresistant isolates, particularly MRSA. Lastly, the results observed in the XTT assay showed a similar trend with the multiresistant clinical isolates reflecting higher levels of cellular metabolism impairment, particularly for the 107 kDa chitosan.

In conclusion, chitosan displayed an evident strong effect against the tested *S. aureus* strains in planktonic and sessile state. While the results observed showed no differences between strains in planktonic phase the biofilm results showed that the multiresistant microorganisms were more sensitive to chitosan than the control strain. In all biofilm related assays both chitosans at sub-MIC concentrations, exhibited high inhibition percentages preventing biofilm formation, disrupting mature biofilms and cellular metabolism. Furthermore, and contrary to traditional antimicrobials, chitosan's MBIC values were not 10 to 1000 fold superior to those registered for the MICs, with only a 2 fold increase (0.5 to 1 mg/ml) being registered in the worst case scenario. Overall, these results show the potential of chitosan as a means to control the rapid growth of antibiotic resistances among microorganisms and as a possible treatment to multiresistant bacterial infections.

**Keywords:** chitosan; *S. aureus*; MRSA; multiresistant microorganism