High hydrostatic pressure effect associated with disinfectant to inactivate pathogenic bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*)

Marriam Yamin, M.Sc.1 ; Ancelmo Rubelo de Souza, M Sc.1; Dr. Prof. Carlos Francisco Sampaio Bonafe,Ph.D1
[1] Laboratory Of Thermodynamics Of Proteins, Functional And Molecular Biology, State University Of Campinas (UNICAMP), Campinas, Sao Paulo, Brazil.

ABSTRACT

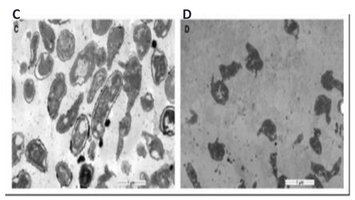
In the hospital environment, genus *Klebsiella pneumoniae & Pseudomonas aeruginosa* are highly resistant bacterial to several antibiotics, often causing infections in immunocompromised patients, as well as post-surgical infections. More includes surgical site, wound, catheter, blood stream, & ventilator associated infections, in part due to the inadequate sterilization of some medical surgical materials and biopharmaceutical solutions. There is a great concern for efficiently sterilize these materials, so the use of an alternative technique called as high hydrostatic pressure (HHP) to inactivate microorganisms could be very effective, especially for the sterilization of temperature sensitive materials. Although the mechanisms of inactivation of microorganisms involved are not yet well established, hence with the indication of significant damage in the cell wall, ribosomes, cellular content and plasma membrane.

Keyword: Sterilization, *Pseudomonas aeruginosa & Klebsiella pneumoniae,* High hydrostatic pressure Induced Inactivation, Glutaraldehyde, Synergistic effect, Biofilm.

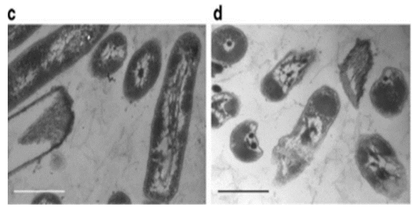
BACKGROUND

As biofilm formation by pathogenic bacteria in health settings is becoming a major risk for deaths, high cost treatment for patients (9.8 billion $ annually, USA report,2012), and incidence rate 5-8% every year due to improper disinfection of surfaces, surgical materials, materials for transplantation etc. Disinfection of such objects when Implemented by disinfectants that can be hazardous to human health also turned to be as big problem for the patients, like use of glutaraldehyde 2% which has toxic effects. so in such cases the use of alternative technique like high hydrostatic pressure can be the good alternative, even for the temperature sensitive materials sterilization, as this is cold sterilization method, which doesn't cause damage to material and can be very applicable. As per these bacterial strains are highly resistant to variety of antibiotics administered for treatment.

Previous studies in our lab with high pressure treatment at 350 MPa performed by our group have shown that the *Aeromonas hydrophila* can be inactivated in milk (Durães *et al,* 2012), and *Mycobacterium abscesses* present in contaminated PVC pipe was previously inactivated at a moderate temperature (60 °C) (Souza *et al*, 2013).



TEM of *A.hydrophila* strain AH 191, untreated **(C)** and **(D)** HHP treated at 350 MPa, 25ºC for 1hr. (Duraes et al, 2012)



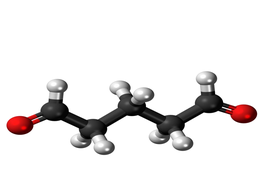
TEM of *M.abscessus* strain 19977 (60ºC at 0.1 MPa for 45 min) in fig. **C,** (250 MPa) in fig. **D.** (Souza et al,2013)

OBJECTIVES

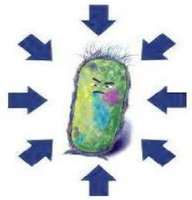
The main objectives of this study is to evaluate the inactivation pattern of pathogenic bacterial strains of *Klebsiella pneumoniae* (ATCC BAA 1705 & ATCC 4352) & *Pseudomonas aeruginosa* (ATCC 27853 & NM 31) by high hydrostatic pressure technique with or without chemical and physical conditions like disinfectant glutaraldehyde, N-acetylcysteine (NAC) and temperature respectively to implement for sterilization of medical and biopharmaceuticals which are sensitive to elevated temperature conditions.

MATERIALS & METHODS

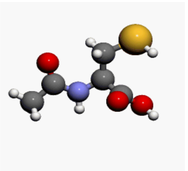
- **Experimental approach includes two different strains of *Klebsiella pneumoniae* (ATCC BAA 1705 & ATCC 4352) & *Pseudomonas aeruginosa* (NM 31 & ATCC 27853).**
- **Inactivation pattern analysis of bacteria by treated all strains with different concentration of glutaraldehyde, various temperatures like 10°C, 25°C & 45°C and HHP (250 & 350 MPa) separate or in combinations of all these variables to check synergism.**
- **In *vitro* biofilm formation and inactivation by using biofilm assays in 96- well plates by N-acetylcysteine (NAC) with or without HHP treatment.**
- **Structural analysis of HHP treated or untreated Biofilm & bacterial cells by Atomic force microscopy (AFM) & Transmission electron microscopy (TEM)**
- **Statistical analysis made by mean and standard deviation using Origin 8 Pro software.**



Glutaraldehyde



HHP effect



N- acetylcysteine

RESULTS & DISCUSSIONS

- **In this study, we observed that even though for some bacterial strains high hydrostatic pressure did not able to give significant inactivation individually but in combination with lower concentrations of glutaraldehyde i.e. 1.8 mM at 45°C temperature, we acheived total inactivation of NM 31 strain of *Pseudomonas aeruginosa*, in figure 4.**
- **On the other hand, our recent preliminary results with HHP treatment of *Klebsiella pneumoniae* strain ATCC 4352 shows slight inactivation of 3 log CFU/mL which is not significant & ATCC BAA 1705 strain expressed almost total resistance to HHP (250 Mpa) in figure. 2 . Resistance with pressure is comprises with the possession of some genetically acquired "operons" which codes for barotolerance property of bacteria for example: deep-sea bacteria (Koki Horikoshi, 1998).**
- **HHP exclusively effective for only one strain of Pseudomonas aeruginosa (ATCC 27853) at both 250 and 350 MPa. In figure 1. that appeared to be most sensitive strain to HHP even at 5 min of exposure.**
- **To make synergistic strategies, we performed several test to see the effect of other variables in combination like different glutaraldehyde concentrations and temperature (25°C & 55°C), can be observed in figure 3. which summarized that on 55°C when we treat our pseudomonas strain ATCC 27853, the bacteria inactivated 4 CFU/mL as compared to room temperature.**
- **In figure 5 & 6, illustrated the treatment for the inactivation of different strains of *Staphylococcus aureus (ATCC 25923 & BEC 9393)* by glutaraldehyde, HHP and temperature with or without combination of all variables to show process of synergism between them and observed significant inactivation of both strains with 0.16mM of glutaraldehyde on 350 MPa & at 50°C. on the other hand figure 7. presenting the bacterial growth curve on different temperatures. (this is our another work that we will submit very soon for possible publication).**

RESULTS

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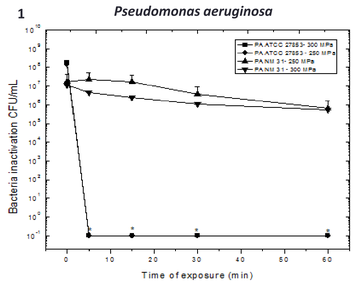

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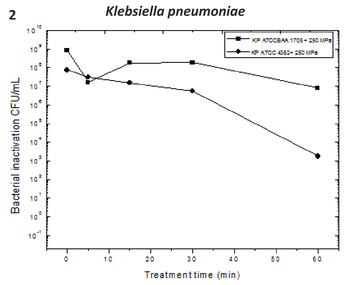

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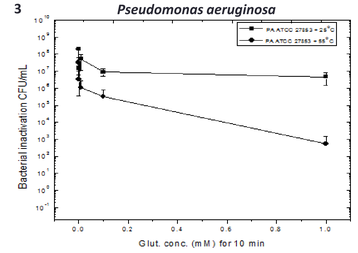

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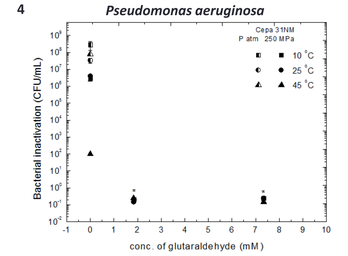

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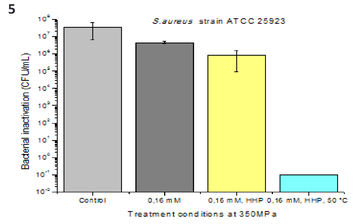

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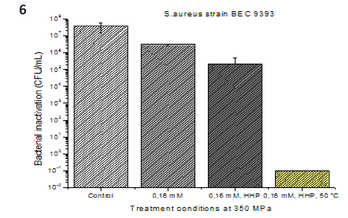

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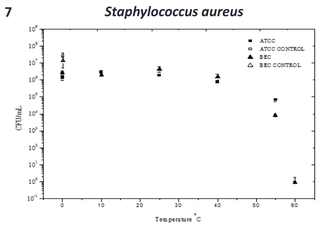

CONCLUSIONS

- **Elimination of large number of bacteria from medical and biopharmaceutical materials reveals that, under relatively moderate conditions, indicates that HHP can be good alternative for sterilization.**
- **The ability of HHP in conjunction with temperature and glutaraldehyde, to eliminate significant number of bacteria suggests that HHP could be particularly useful for sterilization in hospital settings.**