

Comparison between biological and chemical methods to kill multi-drug resistant *Staphylococcus aureus*

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ABSTRACT

Infections with multi-drug resistant strains become a big problem specially the nosocomial infections, which can cause death especially for the debilitated older adults. The aim of this study is to find a better way to kill multi-drug resistant *staphylococcus aureus* instead of the orthodox medicine, which become scarcely effective. The multi-drug resistant organisms are undergo progressing due to the continuous evolution of the antimicrobial patterns. This problem had directed the researchers to use alternative therapy. In this work ozonated water and *Bacillus lentus* have been chosen to study their activity and the merits of each in comparison with the traditional antibiotics.

Keywords: multi-drug resistant, *Bacillus lentus*, Ozone solution, alternative therapy, traditional antibiotics.

1. INTRODUCTION

Staphylococcus aureus spreads by airborne and via the hands of health-care workers [1] it causes infection most commonly at sites of lowered host resistance. The genetic plasticity of *S. aureus* has resulted in the emergence of varying degrees of antibiotic resistance and virulence patterns [2,3]. Hospital and clinical environments are fertile grounds for microorganism proliferation, often breeding bacterial species highly resistant to mainstream antibiotics. This problem had directed the researchers to use alternative treatments to deal with the resistant microorganisms, by using the ozone or bacterial secretion [4,5]. Ozone is one of the best disinfectants that capable of preventing infection outbreaks [6], the ozone solutions could be used to disinfect medical instruments and similar equipment and they are useful in reducing the number of bacterial infections caused by inadequate disinfection against new resistant strains [7]. Some strains of *Bacillus* can produce bactericidal molecules against the resistant strains [8]. By using different possible methods, one may examine the results to show the best way to kill the resistant strain, which caused problems in

community and hospitals. Therefore the object in this work is to estimate the best way to kill resistant strains of *S. aureus* with least disadvantage.

2. MATERIALS AND METHODS

2.1 Sample collection

S. aureus were obtained from diabetic foot patients admitted in Al-Kindy teaching hospital. While *B. lentus* was obtained from food contamination research center, Ministry of Science and Technology, Baghdad- Iraq.

2.2 Testing *S. aureus* against traditional antibiotics

S. aureus was tested for 15 antibiotic including (Amoxicillin, AX, 25mcg; Amikacin, AK, 30 mcg; Cephalothin, KF, 30 mcg; Ciprofloxacin, Cip, 5mcg; Chloramphenicol, C, 30 mcg; Cefotaxime, CTX, 30mcg; Doxycyclin, Do, 30mcg; Erythromycin, E, 10mcg; Gentamycin, CN, 10mcg; Oxacillin, OX, 1mcg; Penicillin, P, 10U; Rifampin, RA, 5mcg; Tetracyclin, TE, 10mcg; Trimethoprim, Tmp, 10mcg; Vancomycin, V, 30mcg) by using Kirby-Bauer disk assay.

2.3 Testing the activity of *B. lentus*

B. lentus was cultured in nutrient broth overnight at 37°C, after centrifugation at 14,000 rpm for 10 min. the supernatant was collected and applied on multi- drug resistant *S. aureus* by agar- well diffusion method.

2.4 Testing the activity of Ozone

Ozonated water was obtained by generating ozone gas using ozone generator FQM-B05. Then it was tested against the pathogenic bacteria. The effect of ozone was noticed after mixing 1 ml of ozonated water with 1 ml of 10^8 cfu/ml of *S.aureus* and 0.1 ml was plated on nutrient agar.

2.5 Testing the side effect of ozone

The ozone generator FM-B05 was used in the experiment in a room (high= 3.8 m; length= 7.5m; width= 3.6m). Albino mice (age: 6-8 weeks; weight: 25-28g) were used to observe the effect of breathing in the same room of ozone generator.

3. RESULTS AND DISCUSSION

S.aureus from diabetic foot was multi- drug resistant strain according to the results of disc diffusion test (fig 1). The isolate were susceptible only to vancomycin (18 mm) while the results obtained from *B. lentus* supernatant (23 mm). The Ozone was applied to this isolate and the effective concentration was 22.75ppm to eradicate the multi- drug resistant bacteria. Mice lung section (Fig. 2a, 2b) showed thickening of alveolar wall with focal chronic inflammatory cells when they breathed the air of the room in which ozone was generated.

It is understood that the antimicrobial activity of ozonated water depends on the ability of ozone to attack the cell envelop, which is followed by the breakdown of the bacterial cell, and therefore its death. This mode of action means that it is not selective in contrast to ordinarily antimicrobial agents including the antimicrobial molecules produced by *B. lentus* which are almost selective to a specific microorganism group. It should further be indicated that the action of ozonated water on different species depends on the concentration of ozone together with environmental conditions.

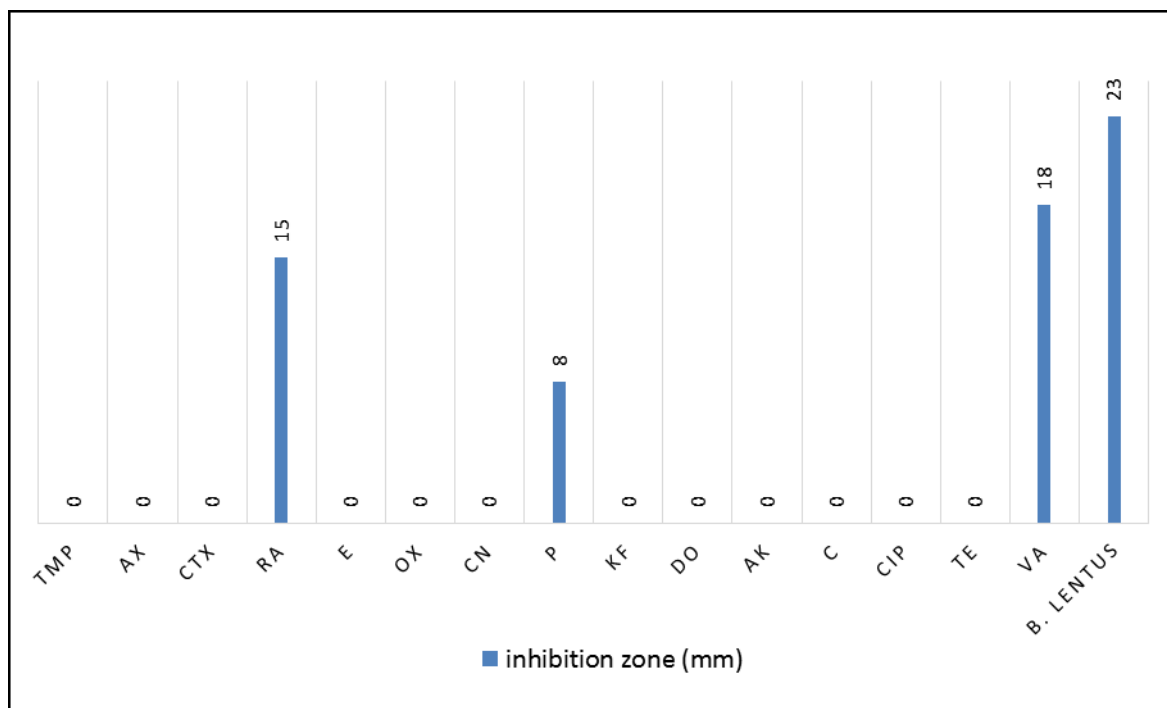


Figure 1: The susceptibility of multi-drug resistant *S. aureus* to different antimicrobial agents.

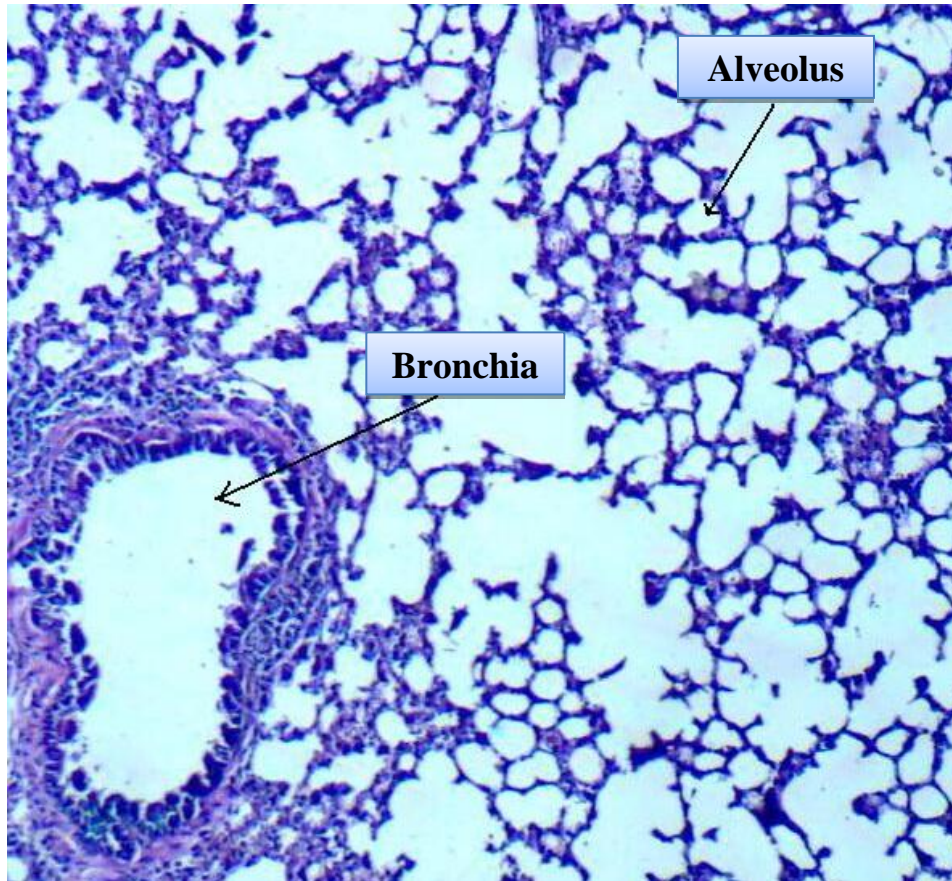


Figure 2a: Section of lung without exposing to gaseous ozone, showing normal structure of alveoli with lymphoid cells aggregate.

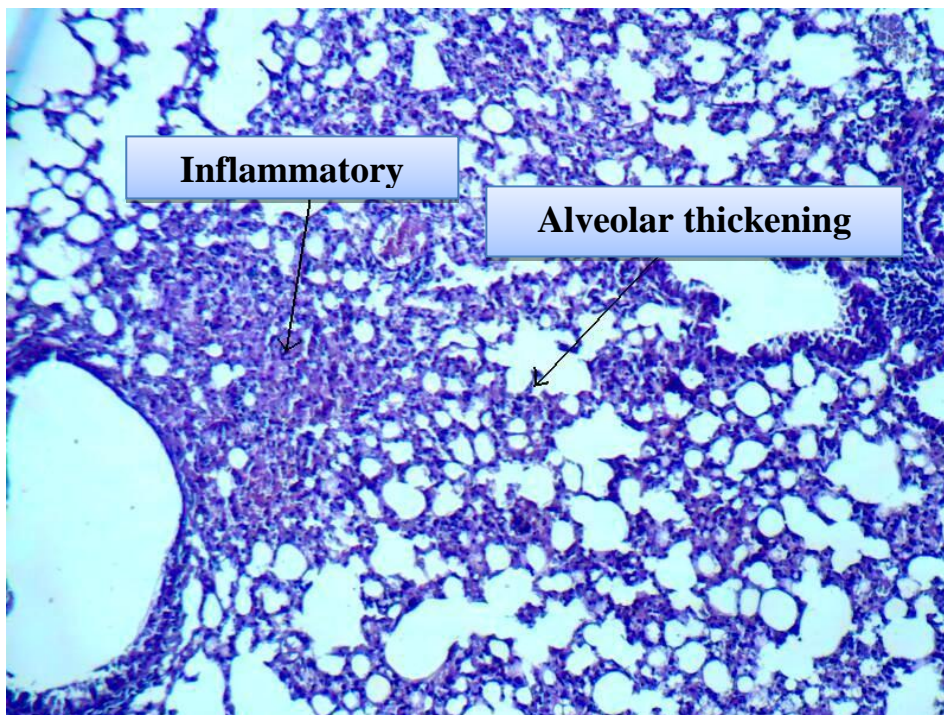


Figure 2b: Section of lung exposed to gaseous ozone, showing slight thickening of alveolar wall with focal chronic inflammatory cells

4. CONCLUSION

The activity of both *Bacillus lentus* and ozonated water, have been tested and compared with traditional antibiotics. Further, the merits of each of them have

been explored. It may easily be concluded that the use of ozonated water may be considered to be a better antimicrobial agent but caution is needed not to inhale gas during the application.

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