

Double Blind Study of the Bactericidal Properties of Nano solution of Silver colloid.

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The structure of water has been described by Prof. Rustom Roy some years ago. This is the solution of nano-silver embedded in water. We have carried out a double blind study of the silver nano-solution vis-à-vis its bactericidal properties. We are not aware of any such study done in the past. The double blinding was done by the one of us (SA**) and the microbiological studies were done in the Kasturba Medical College microbiology laboratories in India. The American Biotech Labs provided the solution.

Materials and methods

Microorganisms

One strain each of methicillin resistant *Staphylococcus aureus*, Vancomycin resistant *Enterococcus faecalis*, drug resistant *Escherichia coli*, *Klebsiella pneumoniae* (Extended β -lactamase producer), *Salmonella typhi*, *Shigella flexneri*, *Pseudomonas aeruginosa* were used in the study. Standard methods were used for the isolation and identification of bacteria¹. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as controls. A clinical isolate of *Candida albicans* was also used in the suspension test.

Preparation of bacterial inoculum

The bacterial colonies were inoculated into 3 ml peptone water and incubated at 37°C for 4 to 6 h. The turbidity was adjusted with McFarland 0.5 standard (bacterial concentration 1.5×10^5 cfu/ml)

Minimum inhibitory concentration test

The minimum inhibitory concentration (MIC) of Aquasol was determined using macrobroth dilution test². Aquasol was diluted two-folds in 2 ml Mueller-Hinton broth (Hi Media, Mumbai, India) in sterile test tubes. Each tube was inoculated with 0.02 ml of bacterial inoculum to get an initial bacterial concentration of approximately 1.5×10^5 cfu/ml. Bacterial growth control was included along with each test. The tubes were incubated at 37°C for 18 h and examined. The lowest concentration of Aquasol that inhibited bacterial growth was considered MIC. Each experiment was repeated three times.

Suspension test

The contact time required to kill the microorganisms was determined by the suspension test³. A 0.02 mL of bacterial of bacterial inoculum and fungal inoculum was added to 2 mL of Aquasol and left for 4 h. Then a 0.1 mL aliquot of the mixture was removed at 15

min intervals, diluted and inoculated on blood agar and into tryptic soy broth to detect surviving microorganisms. The experiments were repeated three times.

Results and Discussion

We studied the inhibitory effect on bacteria of Aquasol by determining the MIC. The results indicate that Aquasol inhibits both gram positive and gram-negative bacteria. (Table 1) The results were reproducible. This indicates that the bacterial structure does not influence the effect of Aquasol. Similar susceptibility of both drug resistant and susceptible bacteria to Aquasol is noteworthy. The suspension test revealed that the contact time required to kill bacteria is shorter than that for *C. albicans*. The differences in the cell wall structure of bacteria and fungi may be responsible for this. In recent years, drug resistance has increased considerably in many bacterial pathogens. Treatment of such infections is quite difficult. We need safe and more effective drugs for the treatment of drug resistant infections. Aquasol may prove its worth in treatment of resistant infections. However, further in vivo studies in animals and patients are needed to know about the safety and efficacy of Aquasol.

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References

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Macrobroth dilution and Time-kill assay were used to study the antibacterial effect of Aquasol.

Table 1. Minimum inhibitory concentration of aquasol (solution A)

Bacteria	Dilution of Aquasol inhibiting bacterial growth
<i>S. aureus</i> ATCC 25923	1 in 8

Methicillin Resistant <i>S.aureus</i>	1 in 4
<i>E. coli</i> ATCC 25922	1 in 4
<i>E. coli</i> (Drug resistant)	1 in 2
<i>K. pneumoniae</i> (ESBL positive)	1 in 2
<i>P. aeruginosa</i> (drug resistant)	1 in 4
Vancomycin resistant <i>E. faecalis</i>	1 in 4
<i>S. typhi</i>	1 in 4
<i>S. flexneri</i>	1 in 4

Table 2. Time required to kill bacteria and candida

Bacteria	Time (min) required to kill
<i>S aureus</i> ATCC 25923	30
Methicillin resistant <i>S.aureus</i>	60
<i>E.coli</i> ATCC 25922	90
<i>E. coli</i>	90
<i>K. pneumoniae</i> (ESBL producer)	45
<i>P. aeruginosa</i>	90
<i>S. typhi</i>	30
<i>S. flexneri</i>	30
Vancomycin resistant <i>E. faecalis</i>	45
<i>C. albicans</i>	120

Aquasol (2 ml) was taken in test tube and inoculated with suspension of microorganisms to achieve initial concentration of 10^5 cfu/ml. The tubes were incubated at 37 C and at 15 min intervals 0.01 volume of the material was inoculated on agar plate to detect viable

bacteria. The time mentioned is the minimum contact time required to kill microorganisms.

Solution B had no inhibitory effect at all. Solution A was the Aquasol and solution B was just distilled water. They look alike.