

Bactericidal activity of combinations of Silver–Water Dispersion™ with 19 antibiotics against seven microbial strains

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The recent increase in the incidence of infections due to bacterial resistance to antibiotics has been recognized as an alarming problem, especially in the hospital environment with probability of cross-infection. Silver–Water Dispersion™ solution as an antibacterial is claimed to have no bacterial resistance. Nineteen antibiotics were checked in combination with Silver–Water Dispersion™ solution against seven microbial organisms for synergism. First, minimal inhibitory concentrations were determined for the individual antibiotics and Silver–Water Dispersion™ solution individually. Those combinations of individual antibiotics with Silver–Water Dispersion™ that displayed synergism were further evaluated through the checkerboard method. Synergistic activity of Silver–Water Dispersion™ solution in combination with nineteen antibiotics was tested against seven bacterial strains, except where an organism was known to be resistant to the antibiotic. Out of 96 tests, five were synergistic, 89 additive, and two antagonistic.

Keywords: Antibiotics, bacterial resistance, Silver–Water Dispersion™, synergism.

SERIOUS infections, particularly antibiotic-resistant, often result in therapeutic failure when treated with seemingly appropriate single-drug antibiotic regimens, despite readily achievable minimum inhibitory concentrations (MICs). The mutations responsible for antibiotic resistance in bacteria do not arise as a result of the ‘need’ of the organism. Futuyma¹ has noted that: ‘... The adaptive needs of the species do not increase the likelihood that an adaptive mutation will occur; mutations are not directed towards the adaptive need of the moment. . . .’ Mutations have causes, but the species need to adapt isn’t one of them’. Alternatives must therefore be sought to overcome infections carrying highly resistant strains.

Silver–Water–Dispersion™ solution has been shown as an effective antibiotic against many Methicillin-resistant *Staphylococcus aureus* (MRSA) and multiple drug-resistant (MDR) strains (*Escherichia coli*, *Pseudomonas aeruginosa*).

As high level acquired resistance to conventional antibiotics is frequent, it seems reasonable to use combination therapy in order to achieve bactericidal synergism. Active silver solutions have shown marked activity against proven bacterial-resistant strains. Hence, a range of antibiotics were tested with Silver–Water Dispersion™ solution to determine antagonism, additive and synergistic effects against a panel of microbial strains.

Antibiotic discs used in this investigation are standardized discs by Pathoteq Biological Laboratories, India: Amoxicillin (AX, 30 mcg), Carbenicillin (CN, 100 mcg), Cefoperazone (CP, 75 mcg), Ceftizidime (FG, 30 mcg), Ciprofloxacin (RC, 5 mcg), Clindamycin (CD, 2 mcg), Doxycycline (DX, 30 mcg), Erythromycin (ER, 15 mcg), Gentamycin (GM, 10 mcg), Kanamycin (KA, 30 mcg), Nalidixic Acid (NA, 30 mcg), Oxacillin (OC, 1 mcg), Penicillin-G (PG, 10 units), Rifampin (RF, 5 mcg), Streptomycin (SM, 10 mcg), Tetracycline (TE, 5 mcg) Tobramycin (TB, 10 mcg) and Trimethoprim (TP, 5 mcg).

The antibiotic solutions used in the study are KANAMAC-500 (Kanamycin injection, Macleods Pharmaceuticals Ltd, Daman); FORTUM* (Ceftizidime injection, GlaxoSmithKline Pharmaceutical Ltd, India); MIKACIN (Amikacin injection, Aristo Laboratories Ltd, Daman). MAGNAMYCIN* (Cefoperazone injection, Astral Pharmaceutical Industries, India).

The 32 ppm silver in distilled water (S–W D™) was obtained from American Biotech Laboratories, Utah, USA.

The organisms used are: *E. coli* (MDR) strain from stool sample; *Ps. aeruginosa* (multiple-drug resistant) strain from sputum. These two strains were obtained from P.D. Hinduja Hospital (Mumbai); Methicillin-resistant *S. aureus* was obtained from Lokmanya Tilak Municipal Hospital. *Shigella flexneri*, *Salmonella typhi*, *S. aureus* 6538 P, *Bacillus subtilis* and *Candida albicans* are in-house laboratory strains.

Nutrient agar (Hi-Media, Mumbai) used in the antibiotic spectrum studies and Silver–Water Dispersion™ and antibiotic combination studies contains peptic digest of animal tissue 50.00 g/l; yeast extract 1.5 g/l; beef extract 1.5 g/l; sodium chloride 5.00 g/l; agar type I 25 g/l; pH 7.4 ± 0.2. Nutrient broth (Hi-Media, Mumbai) used in the macrodilution method (MIC) contains peptic digest of animal tissue 50.00 g/l; yeast extract 1.5 g/l; beef extract 1.5 g/l; sodium chloride 5.00 g/l; glucose 5 g/l; pH 7.4 ± 0.2.

Actively growing 16-h-old culture was surface-spread using sterile cotton swabs onto the nutrient agar surface (Hi-Media). The plates were kept aside for absorption for 15 min. The antibiotic discs were then placed onto the agar surface and the plates were incubated at 37°C for 24 h. All plates were examined for any zones of inhibition around the antibiotic discs that would indicate sensitivity of the organism. Zone diameters were recorded in millimetres using a zone reader (Hi-Media) and interpreted according to the standard charts provided by the National Committee for Cultural and Laboratory Standards².

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Table 1. MICs of eight bacterial strains with Silver–Water Dispersion®

Antibiotic discs (mcg)	<i>E. coli</i> (MDR)	<i>Ps. aeruginosa</i> (MDR)	MRSA	<i>S. aureus</i> 6538 P	<i>S. typhi</i>	<i>Sh. flexneri</i>	<i>B. subtilis</i>
Amikacin	R	R	S	R	R	R	R
Amoxicillin	S	R	S	S	S	S	R
Carbenicillin	R	R	PR	S	S	S	R
Cefoperazone	R	R	S	R	S	S	R
Ceftizidime	R	R	R	S	S	S	R
Ciprofloxacin	R	R	PR	S	S	S	PR
Clindamycin	R	R	PR	R	R	S	S
Doxycycline	R	R	S	S	S	S	S
Erythromycin	R	R	S	R	R	PR	S
Gentamycin	S	R	S	S	S	S	S
Kanamycin	PR	R	S	S	S	S	S
Nalidixic acid	R	R	R	PR	S	S	S
Oxacillin	R	R	S	S	R	R	PR
Penicillin-G	R	R	R	S	R	S	S
Rifampin	PR	R	R	S	S	S	S
Streptomycin	S	R	S	S	S	S	S
Tetracycline	R	R	S	S	S	S	S
Tobramycin	R	R	S	S	S	S	S
Trimethoprim	R	R	S	S	S	S	S

R, Resistant; S, Sensitive; PR, Partially resistant.

Organism	MIC (ppm)
<i>E. coli</i> (MDR)	3
<i>Ps. aeruginosa</i> (MDR)	4
MRSA	8
<i>S. aureus</i> 6538 P	10
<i>Sh. flexneri</i>	2
<i>S. typhi</i>	2
<i>B. subtilis</i>	17
<i>C. albicans</i>	15

Actively growing 16-h-old culture was surface-spread using sterile cotton swabs onto the nutrient agar surface (Hi-Media). The plates were laid aside for absorption for 15 min. Wells were punched into the agar surface using a 10 mm diameter cork-borer aseptically. Next 100 ml of Silver–Water Dispersion™ was introduced into the wells. The plates were incubated at 37°C for 24 h. Sensitivity was indicated by the zone of inhibition around the well. Zone diameter was recorded in millimetres using the zone reader (Hi-Media).

Drug interaction studies were carried out using the agar well diffusion method. Actively growing 16-h-old culture was surface-spread using sterile cotton swabs onto the nutrient agar surface (Hi-Media). The plates were laid aside for absorption for 15 min. Wells were punched into the agar surface for adding Silver–Water Dispersion™ solution (as above). The antibiotic discs were placed at a distance which was the average of their zone diameters obtained individually. The plates were incubated at 37°C for 24 h. The inhibition pattern obtained was recorded as synergy, additive or antagonistic according to the criteria stated by Konmen *et al.*².

MICs of the Silver–Water Dispersion™ and antibiotic solutions for the seven microbes were determined using the macrodilution broth susceptibility test. A standardized suspension of approximately 10⁵–10⁶ CFU/ml density was obtained by inoculating the culture in nutrient broth (Hi-Media) and incubating the tubes at 37°C for 4 to 6 h. A serial dilution of Silver–Water Dispersion™ was prepared within a desired range. Similarly, the antibiotic solutions are also diluted. 100 ml of the standardized culture suspension was then inoculated and tubes were incubated at 37°C for 24 h. MIC was defined as the lowest concentration of the inhibiting agent that completely inhibited bacterial growth. MIC can be visually examined by checking the turbidity of the tubes.

The study of combined antimicrobial activity of Silver–Water Dispersion™ and antibiotics against seven microbes was carried out using the checkerboard assay method. In a final volume of 5 ml in each tube antibiotic solution and Silver–Water Dispersion™ solution diluted from appropriate stock solution were added. Standardized culture suspension (10⁵–10⁶ CFU/ml) was prepared by inoculating the organisms in nutrient broth incubated at 37°C for 4–6 h.

Table 2. Synergism of Silver–Water Dispersion™ with antibiotics

Antibiotic discs (mcg)	<i>E. coli</i> (MDR)	<i>Ps. aeruginosa</i> (MDR)	MRSA	<i>S. aureus</i> 6538 P	<i>S. typhi</i>	<i>Sh. flexneri</i>	<i>B. subtilis</i>
Amikacin	–	–	Syn.	Add.	–	–	Add.
Amoxicillin	–	–	Ant.	Add.	Add.	Add.	Add.
Carbenicillin	–	–	Add.	Add.	Add.	Add.	Add.
Cefoperazone	–	–	Syn.	Add.	Add.	Add.	Add.
Ceftizidime	Syn.	Syn.	Add.	Add.	Add.	Add.	Add.
Ciprofloxacin	–	–	Add.	Add.	Add.	Add.	Add.
Clindamycin	–	–	Add.	Add.	–	Add.	Add.
Doxycycline	–	–	Add.	Add.	Add.	Add.	Add.
Erythromycin	–	–	Add.	Add.	–	Add.	Add.
Gentamycin	Add.	–	Add.	Add.	Add.	Add.	Add.
Kanamycin	Syn.	–	Add.	Add.	Add.	Add.	Add.
Nalidixic Acid	–	–	–	Add.	Add.	Add.	Add.
Oxacillin	–	–	Ant.	Add.	–	Add.	Add.
Penicillin-G	–	–	Add.	Add.	Add.	Add.	Add.
Rifampin	Add.	Add.	Add.	Add.	Add.	Add.	Add.
Streptomycin	Add.	–	Add.	Add.	Add.	Add.	Add.
Tetracycline	–	–	Add.	Add.	Add.	Add.	Add.
Tobramycin	Add.	–	Add.	Add.	Add.	Add.	Add.
Trimethoprim	–	–	Add.	Add.	Add.	Add.	–

Syn., synergistic; Add., Additive.

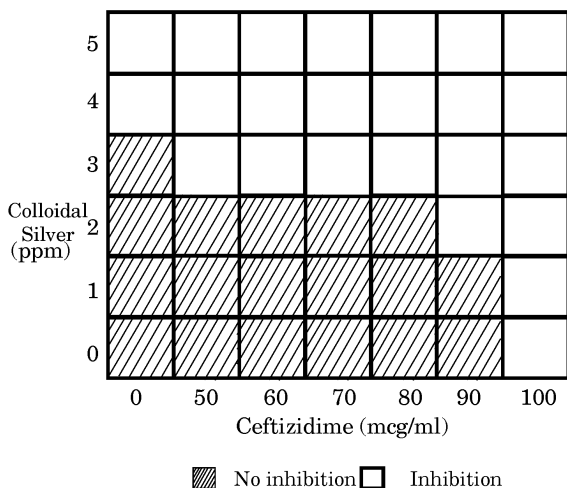


Figure 1. Checkerboard inhibition result of *Ps. aeruginosa* with S–W D™ and Ceftizidime combination.

100 ml of this suspension was then inoculated in the tubes and incubated at 37°C for 24 h. The tubes were observed for turbidity to determine inhibition and compared with the positive and negative controls.

Konman *et al.*³ described the method for quantifying MIC results obtained in terms of Fractional Inhibitory Concentration (FIC) index, defined as the sum of FIC values of two drugs in combination: FIC index = FIC of drug A + FIC of drug B,

$$\text{FIC of drug A} = \frac{\text{MIC of drug A in combination with drug B}}{\text{MIC of drug A}}$$

An index of less than 0.5 is considered as evidence of Synergism; an index of > 2.0 is evidence of antagonism.

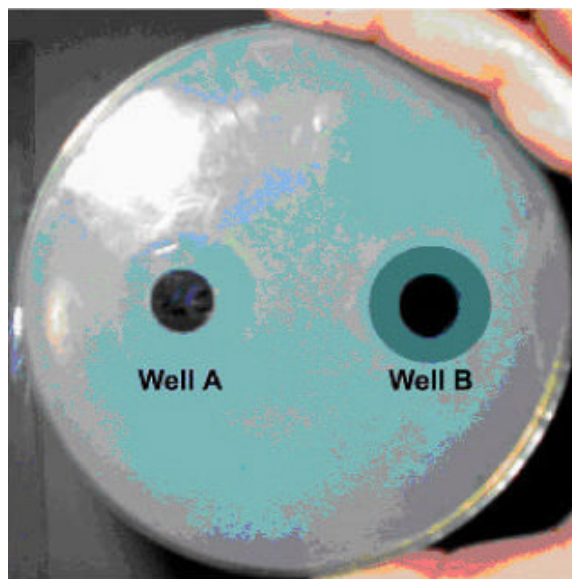
A panel of bacterial strains was characterized using the 19 antibiotics under study. Results are shown in Table 1. The yeast *C. albicans* shows resistance to all the antibiotics under study, but does show sensitivity to Silver–Water Dispersion™. The two hospital isolates *E. coli* and *Ps. aeruginosa* were found to be largely resistant to the antibiotics under study, with *Ps. aeruginosa* showing resistance to all antibiotics being studied except for Ceftizidime and Rifampin. Though MRSA was reported by the hospital to show multiple resistance, laboratory sub-culturing has probably lowered its resistance. All the eight isolates studied showed susceptibility to Silver–Water Dispersion™ solution when tested both by the agar cup method as well as by the MIC macrodilution test. Their MIC values are also given in Table 1.

Table 2 shows synergism results of Silver–Water Dispersion™ in combination with various antibiotics when evaluated by the disc/cup combination. These tests were not performed when the organism was resistant to the antibiotic. However, it may be noted that no organism was resistant to Silver–Water Dispersion™. Thus the number of tests completed was 96 out of the possible 133 between 19 antibiotics and seven organisms. The tests showed that five combinations were synergistic, 89 additive and two antagonistic.

Five synergistic combinations in the agar diffusion method (see Table 2), when checked by macro dilution showed only two combinations to be synergistic. We chose the two synergistic and two additive combinations among the five and determined MICs of the antibiotics and Silver–Water Dispersion[®], and the checkerboard

Table 3. MICs of S-W D™, four antibiotics and combinations thereof

Bacterial strain	Antibiotic	MIC (µg/ml)	Colloidal silver MIC (ppm)	FIC of colloidal silver and antibiotics combination
<i>E. coli</i> (MDR)	Kamaycin	110	3	1.23
<i>Ps. aeruginosa</i> (MDR)	Ceftizidime	100	4	1.4
MRSA	Amikacin	0.8	0.5	0.31
MRSA	Cefoperazone	10	0.5	0.18

**Figure 2.** MRSA inhibition with Penicillin-G alone (Well A), and in combination with Silver-Water Dispersion® (Well B).

combinations of the four as reported. The FIC index reported in Table 3 is calculated favouring a minimum value of Silver-Water Dispersion®. When the inhibition is seen from the antibiotic side, the FIC index may be different. As seen in Figure 1, using an antibiotic of 50 mg/ml and Silver-Water Dispersion® of 3 ppm for the inhibitory combination, the FIC index is 1.25 and not 1.4 as reported in Table 3.

Table 2 shows that silver is mostly additive to the antibiotic efficacy. In the combinations examined, it is independent in its bacterial activity except for five out of 96 synergistic combinations. It is also shown to have two combinations which appeared antagonistic. These two combinations were further examined.

To validate the results of Amoxicillin antagonism, 30 mcg dilution of Amoxicillin was prepared in Silver-Water Dispersion™ (32 ppm) solution. 100 ml of this combination was added to a single well, and kept for diffusion followed by incubation. No antagonistic effects were noted under these conditions as the zone of inhibition observed was 19 mm, comparable to 20 mm with a Silver-Water Dispersion™ and 21 mm with Amoxicillin.

Many a times, antibiotics may cause symptoms in patients to temporarily disappear and yet the antibiotics may leave

behind a host of resistant organisms in the system. These resistant organisms reappear at a later date straining the immune system. Figure 2 shows Penicillin-G (10 units) (well A) and Penicillin-G (10 units) in combination with Silver-Water Dispersion™ solution (32 ppm) (well B). Well A inhibition zone is not clear and displays the presence of resistance to Penicillin-G. Whereas well B having a combination of Penicillin-G and Silver-Water Dispersion™ demonstrates the powerful clearing ability of Silver-Water Dispersion™.

It is clear that the combination will allow a more complete clearing of the pathological organism.

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