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A unique Silver Sol with broad antimicrobial properties

Abstract

This research reports the antibacterial activity of a Silver Sol (ASAP Solution®) and compares it with representatives from five classes of antibiotics–the penicillins, macrolides, cephalosporins, fluorinated quinolones, and tetracyclines. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the antibiotics and the Silver Sol were determined for fifteen strains of pathogenic bacteria. Determination of silver particle localization within treated bacteria and partial physical characterization of the silver particles were accomplished using electron microscopy (EM) in conjunction with photoelectron spectroscopic imaging (PSI). The Silver Sol was found to have a broad spectrum of antibacterial activity when compared with the other antibiotics. With the exception of two, all of the bacterial strains tested exhibited MICs of 2.5 ppm silver or less.

Silver particle sizes, measured with an electron microscope (EM), ranged from 0.45 nm to 85 nm in diameter with the average being 10.6 nm. Silver particles were found in the interior of both silver treated Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) cells using the EM; no visible silver particles could be seen in any cells of the other treated strains. However, Photoelectron Spectroscopic Imaging (PSI) showed that all treated strains of bacteria, whether or not particles were visible in the EM, contained silver.

A Perkin-Elmer's Atomic Adsorption instrument was used to measure the concentration of ionic silver in each batch of 44 Silver Sols produced, and this demonstrated that the Silver Sol averaged less than four percent ionic silver. An additional test, conducted by an independent analytical chemistry laboratory[1] concluded that the 22 ppm Silver Sol was "primarily metallic silver" in content. Several studies are presented to demonstrate that metallic silver is NOT toxic - and this is certainly true at the concentrations of silver present in any of the sols included under Silver Sol.

Introduction

Silver has been used for numerous medicinal purposes[2-8]. Some of the more notable uses have been the application of silver nitrate to the eyes of newborns to reduce the incidence of ophthalmic neonatorum[3] and silver in the form of silver sulphadiazine for the prevention and treatment of burn wound infections[9]. To date, a number of biomaterials that would potentially benefit the medical community have been impregnated with silver, including biomaterials impregnated to reduce bioburden. The list of silver-impregnated medical materials includes, but is not restricted to, bioactive-glass doped with silver oxide for use in prosthetic device[10], silver-coated endotracheal tubes to combat ventilator-associated pneumonia[11], silver-impregnated wound dressings for use on burn wound patients[12,13], and silver in sewing cuffs used for mechanical heart valves to prevent prosthetic valve endocarditis[4].

Question of Toxicity

Ionic silver can form compounds with biologically active chemical groups, including sulfhydryl, carboxyl, phosphate, hydroxyl, and amino groups, which are present in the cell in membranes, proteins, nucleic acids, and other cellular components. Thus it is important to point out that there is a wide difference in the toxicity of silver as an ion and silver as a metal. A document produced in 1990 by the Agency for Toxic Substances and Disease Registry, an agency of the U. S. Public Health Service, notes that "these silver compounds (i.e. ionic silver) will be the main thrust of this profile "and the main cause of the toxicity discussed in their document". Silver Sol contains silver in its metallic form. This has been shown to be non-toxic and non-cytotoxic by a number of sources. In the same spirit, the Merck Manual of Diagnosis and Therapy does not list metallic silver as a heavy metal poison or as a metal that causes nephrotoxicity[14].

Cellular cytotoxicity was tested on Vero cells and HEP2 cells by Viridis Biopharma Pvt. Ltd. of Mumbai, India. No cytotoxicity was found with either the Vero cells or the HEP2 cells tested with the ASAP 10 ppm and the ASAP 22 ppm products.

In addition, Silver Sol was tested by an independent laboratory in an LD-50 test at the maximum amount specified by the Federal Hazardous Substance Act (FHSA), 16 CFR 1500. In this test, 5g of Silver Sol per kilogram of rat body weight was given to both male and female test rats. The NAMSA lab drew the following conclusion: "Under the conditions of this study, there was no mortality or significant evidence of toxicity observed in the rats. The test article (ASAP Solution®) would not be considered toxic at a dose of 5g/kg by oral route in the rat".

Finally, the ASAP Solution® was tested for toxicity by the USP Systemic injection test in the mouse model. This test was carried out by the Shri C. B. Patel Research Centre for Chemistry and Biological Sciences in Mumbai, India. The conclusion of the test[15] was that there was no toxicity from the injection of 50 ml of ASAP Solution® per kg weight of animal. The animals were observed immediately after injection, and at 4, 24, 48 and 72 hours following injection. At this point the animals were put to sleep and a gross necropsy was done, showing no toxic effects.

Background

The medicinal use of silver is not new; it was used to treat a variety of diseases and conditions in the early 20th century. With the advent of antibiotics, the use of silver declined. Recently, large pharmaceuticals (such as Roche) are again investigating silver as an antimicrobial.

No studies on the antimicrobial effects of the Silver Sol have been reported in the scientific literature. Given the properties of other silver compounds, it is plausible that the Silver Sol would have broad spectrum antimicrobial properties. The purposes of this study were to determine the in vitro antibacterial activity of the Silver Sol and to compare that activity with the activities of representative antibiotics from five classes of antibiotics—the tetracyclines, the fluorinated quinolones, the penicillins, the cephalosporins, and the macrolides. A further characterization of the Silver Sol was made by electron microscopy and photoelectron spectroscopy, and the localization of silver particles within treated bacteria was determined.

Materials and Methods

Antimicrobials

Erythromycin (Westwood Pharmaceuticals, New York), Ofloxacin (Sigma, St. Louis, Missouri), Tetracycline (Sigma), Penicillin G (Sigma), and Cefaperazone (Sigma) were used. Antibiotics were diluted to a concentration of 10 ppm (µg/ml) and used immediately for each test. The Silver Sol was obtained from American Biotech Labs (Alpine, UT). Concentrations of silver in the Silver Sol were determined by American Biotech Labs using an atomic absorption spectrometer (Perkin Elmer) and were determined to be either 10 ppm or 20 ppm (µg/ml).

Microorganisms

The following bacterial strains were used: Streptococcus gordonii [S.gordonii] (ATCC 10558), Streptococcus mutans [S. mutans] (ATCC 25175), Streptococcus pyogenes [S. pyogenes] (ATCC 19615), Escherichia coli O157:H7 [E. coli 0157:H7] (ATCC 43895) all of which were obtained directly from the American Type Culture Collection (ATCC). Streptococcus pneumoniae [S. pneumoniae] (ATCC 6303), Klebsiella pneumoniae [K. pneumoniae] (ATCC 13883), S. typhimurium (ATCC 14028), E. coli (S.E. Luria Strain B ATCC 11303), Enterobacter aerogenes [E. aerogenes] (ATCC 13048), P. aeruginosa (ATCC 27853), Enterococcus faecalis (E. faecalis), Shigella boydii (S. boydii) [Utah Valley Regional Medical Centre clinical isolate, Provo, Utah]. Staphylococcus aureus (S. aureus) [non-haemolytic Utah Valley Regional Medical Centre clinical isolate, Provo, Utah] were obtained from the Brigham Young University, Department of Microbiology, Clinical Laboratory Science bacterial collection. Klebsiella oxytoca (K. oxytoca) [Provo River, Utah isolate], Salmonella arizonae (S. arizonae) [Provo River, Utah isolate], Enterobacter cloacae (E. cloacae) [Provo River, Utah isolate] were gifts from the Central Utah Water Conservancy District.

Broth Macrodilution Susceptibility Testing

The broth macrodilution susceptibility test[16] was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the Silver Sol and antibiotic preparations and to compare the activity of the Silver Sol with that of each of the antibiotics.

Streptococcal cultures were prepared by inoculating the bacteria into tryptic soy broth, (TSB), (Difco) and incubating at 37°C in a 5% CO_2 atmosphere until mid-log phase growth was reached. All other bacteria were prepared by inoculating the bacteria in Mueller-Hinton Broth, MHB, (Difco) and incubating at 37°C until mid-log phase was reached. The bacterial suspensions were then adjusted to a 0.5 McFarland Standard using a spectrometer (Spectronic 301, Milton Roy) and diluted one thousand-fold to approximately 105 CFU/ml.

One millilitre of culture was then added to each of 10 two-fold serial dilutions in either TSB (streptococcal species) or MHB (all other species) containing the antimicrobial to be

tested. This made the concentration of antimicrobial in the first tube 5ppm, 2.5ppm in the second tube, with each subsequent tube containing half the concentration of the previous tube. Cultures were incubated for 24 hours and the MIC was defined as the lowest concentration of the Silver Sol or antibiotic that prevented growth as determined visually by the presence or absence of turbidity in the tube after incubation. The MBCs were demonstrated by removing a 0.1-ml aliquot from the non-turbid tubes and plating it immediately onto tryptic soy agar (enriched with 5% sheep blood) for streptococcal species, or Mueller-Hinton agar for all other bacteria. The MBC was defined as the lowest concentration of the Silver Sol or antibiotic allowing the growth of fewer than 10 colonies.

Particle Size Estimation

To estimate silver particle size, a drop of the Silver Sol was placed onto a Formvar-coated copper sample grid and let dry 24 hours. TEM micrographs of the silver particles were taken at a magnification of 100,000 times at 100kV. The micrographs were scanned into Adobe Photoshop and analyzed using Image Processing Toolkit software for Adobe Photoshop. Particle sizes were imported into Microsoft Excel where the data was analyzed.

Localization of Silver Particles

To determine the localization of silver particles in bacteria treated with the Silver Sol, S. aureus and E. coli were each grown in Luria broth for 24 hours at 37°C with shaking. After incubation, both strains of bacteria were inoculated into Luria broth and grown to a 0.5 McFarland standard. Silver Sol was added to make an 18 ppm final concentration. The cultures were incubated for 24 hours at 37°C.

Bacteria kill was determined by spreading 100 µl aliquots of the treated culture onto Luria agar (Difco) and incubating 24 hours at 37°C, after which, the plates were observed for the presence or absence of growth. After incubation with the Silver Sol, the bacteria were harvested by centrifuging at 830Xg for 15 minutes in the SLA-1500 rotor in a Sorvall RC5C Plus high speed centrifuge. Bacterial pellets were resuspended, concentrated into a 1.5 ml microcentrifuge tube and centrifuged at 1530Xg for 10 minutes. The supernatants were withdrawn and the pellets were fixed at 4°C with 2% glutaraldehyde buffered with 0.06M sodium cocadylate, for 24 hours. After the fixation with glutaraldehyde, the fixative was withdrawn and samples were fixed with fresh buffered 2% glutaraldehyde for one hour. The pellets were washed with 0.06M sodium cocadylate three times for eight minutes each, to remove the fixative. They were removed from the 1.5-ml microcentrifuge tubes and put into glass vials. Samples were dehydrated in consecutive washes of 10%, 30%, 50%, 70%, 95% ethanol/water and 100% ethanol (three washes) for 80 seconds each. The pellets were washed three times with 100% acetone. Samples were infiltrated with 25%/75%/100% Spurr's resin/Acetone and allowed to polymerize for 24 hours at 70°C in 100% Spurr's resin. All washes and infiltrations were performed in a Pelco microwave on the low setting (100 to 200 watts). Ultra thin sections were cut using an RMC MTX ultramicrotome with a Microstar 2mm diamond knife. The samples were observed in the Phillip's EM201 transmission electron microscope at 100 KV.

E. coli had to be prepared differently, as it would not form a hard pellet. After concentration into a 1.5 ml microcentrifuge tube the bacteria were resuspended in 2% glutaraldehyde, buffered in 0.06 M sodium cocadylate, fixed for 20 minutes at 25°C, and pelletized by centrifugation for 10 minutes at 830Xg. Bacteria were resuspended in 1 ml 0.06M sodium cocadylate for 10 minutes, centrifuged, and all the supernatant was withdrawn. Bacteria were resuspended in FMC Sea Plaque GTG low melt agarose and immediately centrifuged at 760Xg for eight minutes in a non-refrigerated Eppendorf microcentrifuge. The tip of the microcentrifuge tube was severed and the solidified agarose/bacteria pellet was extracted. The pellet was trimmed of excess agarose and cut into smaller blocks for dehydration. Sample preparation was then carried out as with S. aureus.

Preparation for photoelectron spectroscopic imaging (PSI) was performed by sectioning embedded pellets of Silver Sol-treated and non-treated bacteria at 25-45 nm thick sections using a 2 mm Microstar diamond edged knife in the RMC MTX microtome. Staining was performed on post-embedded samples with Reynold's lead citrate and 5% uranyl acetate in 50% ethanol. Samples were first stained with Reynold's lead citrate for 15 minutes, rinsed with distilled water, and dried. Samples were stained with uranyl acetate for 10 minutes, rinsed with 50% ethanol, and dried for 24 hours at 25°C.

Results

Broth Macrodilution Susceptibility Testing

The Silver Sol was bacteriostatic and bactericidal at 10 ppm for all organisms tested. For most of the organisms the Silver Sol was bacteriostatic and bactericidal at lower levels. The data is presented in Tables 1 and 2. Comparable data for the antibiotics is also presented in these tables.

Particle Size Estimation

The average particle size of the silver in Silver Sol was found to be 10.6 nm in diameter (Figure 1). The particles have a wide range of diameters, from 0.45 nm to 85 nm in diameter.

Localization of Silver Particles in Treated Bacteria

S. aureus and E. coli were chosen as representative bacteria for gram positive and gram negative organisms (respectively) to study cellular localization of the silver particles. Samples of Silver Sol-treated S. aureus were observed with and without post-staining to determine if staining would interfere with the ability to determine the location of the silver particles. The samples that were not post-stained, (Figure 2 a, b), as well as those that were, (Figures 3a-d), showed discernible electron dense particles on both the interior and exterior of the S. aureus cells. The particles ranged in size from 5 to 76 nm in diameter. These appeared to be due to treatment with the Silver Sol, as similar particles were not apparent in post-stained and unstained controls that were not treated with the Silver Sol (Appendix A, Figures 1, 2, 3, and 4). The sizes of the silver particles observed were consistent with those observed in the particle size estimation study.

Silver particles were found throughout the S. aureus cells and were not generally associated with one type of cellular structure (i.e. membranes, cell wall, DNA, etc.). Particles observed on the interior of the cell were found to be localized within the cytoplasm as opposed to the periplasm. Particles observed in the interior of the cells exhibited three distinct shapes: amorphous particles were most numerous, followed by hexagonal particles and then bitrigonal particles, which were rarely observed.

E. coli did not contain any large electron-dense particles characteristic of the treated S. aureus cells and contained only the amorphous particles. Those treated E. coli cells that contained discernible silver particles only contained very small silver particles (Figure 4a). At higher magnifications the particles were on the order of 6 to 15 nm in diameter (Figure 4b) as estimated from micrographs. When post-staining was performed on the Silver Sol-treated E. coli, localization of the silver particles was easily observable (Figure 4b, 4c). There was a visible increase in the number of particles present in those treated samples that were post-stained over those that were not (Figure 4c compared to 4a).

Photoelectron Spectroscopic Imaging (PSI) was utilized to determine the presence or absence of silver on the interior of the cells in the Silver Sol-treated bacteria. The intensity and change of colour denotes both the presence and concentration of silver. Yellow denotes the lowest concentrations of silver and deep purple denotes the highest concentrations of silver. The colour black outside the cells denotes a lack of silver. PSI images showed that both E. coli and S. aureus cells contained silver (Figures 5a and b). The smaller arrows indicate large silver particles located on the exterior of the cells and the large arrows indicate cells. It can be seen in Figure 5a that an E. coli cell with no discernible silver particles still has silver throughout the cell. As with E. coli, silver is distributed throughout the bacteria in the treated S. aureus cells (Figure 5b). This is different from the control PSI images of S. aureus and E. coli that were not treated with the Silver Sol (Appendix A, Figures 9 and 10).

Discussion

The Silver Sol tested in this study was found to be both bacteriostatic and bactericidal for all organisms tested (Tables 1 and 2). Broth Macrodilution Susceptibility Testing Results and Discussion The most interesting observation was the broad spectrum of antibacterial activity the Silver Sol demonstrated.

Antibacterial activity was observed at low concentrations, independent of the organism tested. Considering how the MICs were performed (i.e.two-fold serial dilutions), it was interesting that the MICs were within two-fold dilutions of each other for all but two organisms—S. faecalis and S. aureus (which had MIC values of 10 ppm and 5 ppm, respectively). MIC values ranged between 1.25 ppm and 2.5 ppm for both gram positive and gram negative organisms (Table 1 and 2). The MBC values gave similar results (Table 1 and 2) with values ranging from 1.25 ppm to 5 ppm with the exception of S. mutans, S. gordonii, and S. faecalis (which all had MBC values of 10 ppm). E. coli O157:H7 was also tested in an MIC test and the Silver Sol was found to inhibit growth at 2.5 ppm and kill at 5 ppm . The data suggest that the Silver Sol exhibited a broader spectrum of activity than the other antibiotics tested at a concentration of 5 ppm. It was effective against both gram positive and gram negative organisms, inhibiting the growth of and killing the bacteria at the concentrations tested.

The particle size estimation study showed particles from the Silver Sol to range from 0.45 nm to 85 nm in diameter, with an average particle size of 10.6 nm (Figure 1). Due to striking differences in cellular morphology between the types of bacteria, we investigated how the gram negative bacteria processed the silver compared with the gram positive bacteria.

Transmission Electron Microscopy (TEM) determined the localization of silver particles after challenge in two representative bacteria—S. aureus and E. coli. The micrographs suggested that the silver particles penetrated the S. aureus cell wall and membrane.

It is possible in all treated bacteria to determine where silver particles were localized. Silver particles were observed both on the interior and the exterior of the bacteria. In all micrographs of S. aureus, challenged with silver, the silver particles have a wide range in size, 5 to 76 nm, similar to that in Figure 1. Silver particles were found throughout the interior of the bacteria and not consistently associated with any one cellular structure. In control micrographs of untreated S. aureus with post-staining (Appendix A, Figure 4) no silver-like particles are present, so any observance of

particles upon treatment with the Silver Sol is due to the treatment and not the post-stain.

The hexagonal and bitrigonal crystals observed in the micrographs (Figures 3b and 3c) strongly suggest that silver was accumulated by S. aureus in crystalline form, since such crystals were not observed in the micrographs used to estimate particle size and not all bacteria in which there were particles had discernible crystals. Sequestering silver into a more innocuous state may be one way the cells attempt to detoxify the silver. These shapes are consistent with shapes of silver crystals found in resistant bacteria that survive challenge with ionic silver by accumulating silver into metallic and sulphide forms[17]. However, the silver in the Silver Sol is not ionic, but rather metallic, and no resistance to Silver Sol has ever been demonstrated.

The results from the TEM studies with E. coli gave unexpected results. E. coli did not contain large electron-dense particles like those found in S. aureus. When E. coli was challenged with the Silver Sol (Figure 4a), a higher degree of detail was observed over that of controls with no post-staining (Appendix A, Figures 5 and 6). When post-staining was performed on the Silver Sol-treated E. coli, localization of the silver particles was easily observable, and the number of discernible particles increased (Figure 4b and c). E. coli also contained a narrower size range of particle sizes (6 to 15 nm) in contrast to S. aureus (5 to 76 nm). The increase in the number of particles visible in post-stained samples may be due to a reaction similar to that in autometallography, in which silver metal is used as a catalytic agent to induce the reduction of silver ions. By coating the surface of the atoms with silver[18,19], this would make visible as few as 6 to 10 atoms of silver.

There may be fractions in the silver, other than easily discernible particles, that have antimicrobial activity. Not all bacteria observed in the micrographs of treated cells contained particles, but all bacteria were killed. In addition, the number of visible particles increased upon application of the post stain.

Photoelectron Spectroscopic Imaging (PSI) determined the presence or absence of silver within the cells of treated bacteria in which no visible particles were present. PSI showed that both E. coli (Figure 5a) and S. aureus (Figure 5b) cells contained silver, but not the characteristic silver particles observed in the size determination study. E. coli (Figure 5a) with no discernible silver particles still had silver throughout the cell. Silver was also distributed throughout the bacteria in S. aureus cells.

This suggests that there may be multiple targets distributed throughout the cell. PSI showed the presence of silver within bacteria even after the multiple washes required for TEM preparation. This suggests some barrier retaining the silver inside the cells.

Later Data Distinguishing Silver Sol from Other Silver Colloids:

The Silver Sol was further examined at the Materials Research Laboratory at the Pennsylvania State University using Energy Dispersive Spectroscopy, Environmental Scanning Electron Microscopy, Inductively Coupled Plasma Spectroscopy, Near Infrared Spectroscopy, Scanning Electron Microscopy, Transmission Electron Microscopy, and Raman Spectroscopy. This research was conducted by late Prof. Rustum Roy and Dr. Rick Hoover. They compared the Silver Sol to a number of commercially available "silver colloids" that were available at that time, as well as to High Performance Liquid Chromatography (HPLC) water, and normal de-ionized water.

The conclusions they shared with us are a small portion of paper just published [20](Rustum Roy, W.A. Tiller, Iris Bell, M.R. Hoover 2005 The Structure Of Liquid Water; Novel Insights From Materials Research; Potential Relevance to Homeopathy Materials Research Innovations 9-4:577-608)

The silver particles in Silver Sol were pure, metallic silver, mostly 20-30 nm in size. There may have been silver oxide present, but if so, it was a tiny amount. Using Raman Spectroscopy the Silver Sol could clearly be distinguished from any of the "silver colloids," as well as from the de-ionized water and the HPLC water. Using the Raman spectroscope, it was possible to obtain a "unique" footprint for the Silver Sol. It is possible the water may have experienced a greater epitaxial effect, explaining the effectiveness of the Silver Sol. (Permission given to use this data by late Prof. Rustum Roy, Feb. 16, 2006)

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The authors also thank late Prof. Rustum Roy for making available to them the data he obtained on the Silver Sol.

Table 1

Organism	Tetracycline	Ofloxacin	Penicillin G	Cefaperazone	Erythromycin	Silver
P.aeruginosa ATCC 27853	0.078/5.0	0.156/0.313	0.13/>5.0	2.5/5.0	2.5/>5.0	1.67/5.0
E. coli B S.E. Luria Strain B 11303	1.67/>5.0	0.104/0.156	>5.0/>5.0	0.625/>5.0	5.0/>5.0	2.5/2.5
E. aerogenes ATCC 13048	>5.0/>5.0	0.078/0.156	>5.0/>5.0	2.92/>5.0	>5.0/>5.0	2.5/2.5
E. cloacae clinical isolate	1.67/>5.0	0.156/0.156	>5.0/>5.0	>5.0/>5.0	>5.0/>5.0	2.5/5.0
S. typhimurium ATCC 14028	1.25/>5.0	0.078/0.156	>5.0/>5.0	1.25/2.5	5.0/>5.0	2.5/5.0
S. arizonae Provo River isolate	0.625/>5.0	0.078/0.078	>5.0/>5.0	0.833/>5.0	4.17/>5.0	2.5/5.0
S. boydii clinical isolate	1.25/>5.0	0.078/0.156	>5.0/>5.0	0.625/0.625	5.0/>5.0	1.25/1.25
K. pneumoniae ATCC 13883	2.5/>5.0	0.4170.625	>5.0/>5.0	>5.0/>5.0	>5.0/>5.0	2.5/2.5
K. oxytoca clinical isolate	1.25/>5.0	0.104/0.156	>5.0/>5.0	1.25/>5.0	>5.0/>5.0	1.25/1.25

Table 1: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) results for gram negative organisms tested. Data are presented as the average of three replicates for both the MIC and MBC. The ">" denotes that the concentration needed to obtain the MIC/MBC was higher than the parameters set for the test. The antibiotics were diluted to a concentration of 10 ppm(ug/ml)

Table 2

Organism	Tetracycline	Ofloxacin	Penicillin G	Cefaperazone	Erythromycin	Silver
S. pyogenes ATCC 19615	0.625/>5.0	1.25/2.5	>5.0/>5.0	0.313/1.25	0.003/0.019	2.5/5.0
S. mutans ATCC 25175	0.625/>5.0	2.5/>5.0	0.521/>5.0	1.25/>5.0	0.009/0.019	2.5/>5.0
S. gordonii ATCC 10558	0.156/0.625	2.5/5.0	0.009/0.039	1.25/1.25	0.005/0.019	2.5/>5.0
S. pneumoniae ATCC 6303	0.078/0.625	2.5/2.5	0.019/0.019	0.313/0.313	0.002/0.004	2.5/2.5
E. faecalis 2-54	0.313/>5.0	1.25/5.0	5.0/>5.0	>5.0/>5.0	0.009/1.25	>5.0/>5.0
S. aureus clinical isolate	0.313/>5.0	0.417/0.625	2.5/>5.0	5.0/5.0	0.039/>5.0	5.0/5.0

Table 2: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) results for gram positive organisms tested. Data are presented as the average of three replicates for both the MIC and MBC. The ">" denotes that the concentration needed to obtain the MIC/MBC was higher than the parameters set for the test. The antiobiotics were diluted to a concentration of 10 ppm (ug/ml)

Percent of Silver Sol Silver Particles Falling into a Specific Size Category

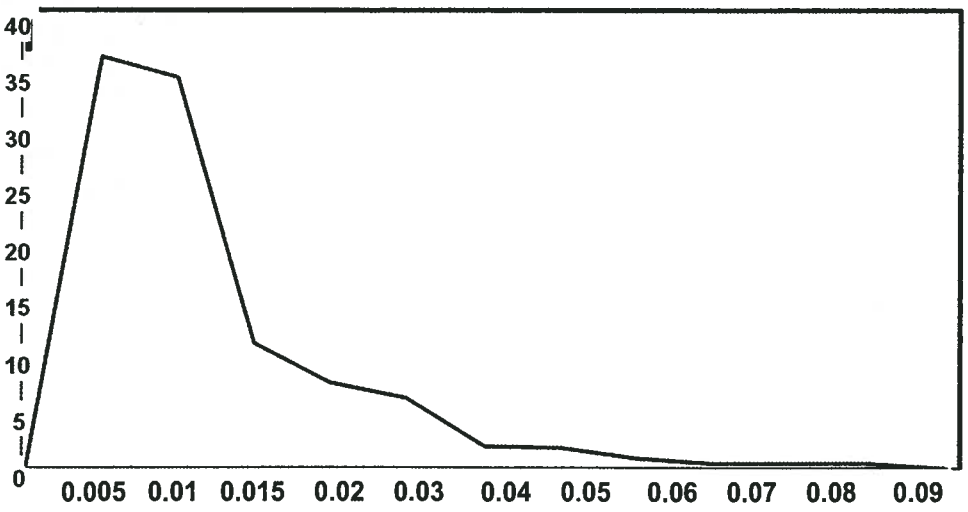

Figure 1: Silver particle size distribution. The particle size estimation study showed particles from the Silver Sol (ASAP Solutions®) with a wide range of sizes, from 0.45 nm to 85 nm in diameter, with an average size of 10.6 nm.

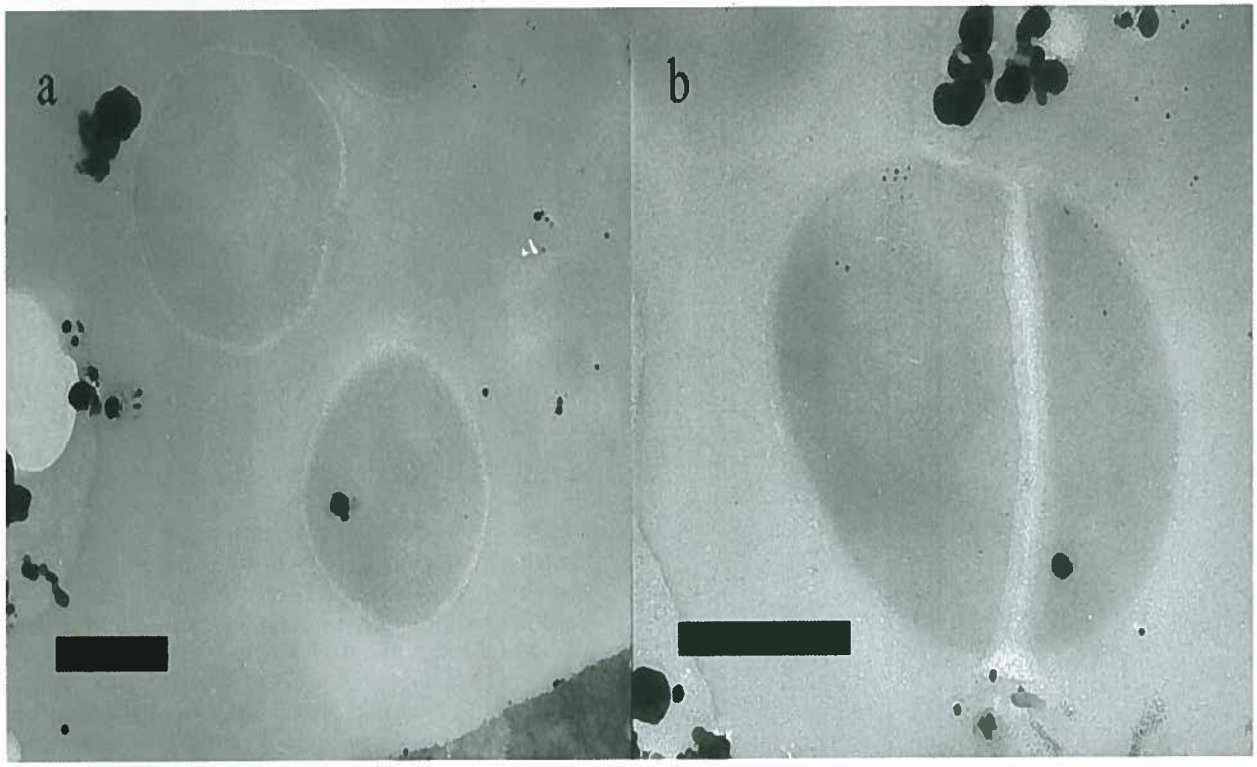

Figure 2: TEM micrographs of silver-treated S. aureus without post staining. (a) After the treatment of the bacteria with Silver Sol (ASAP Solutions®), electron dense particles are observed. There are silver particles within, as well as outside, the bacteria. Bar equals 1 µm. (b) At higher magnifications it can be discerned that there are a range of sizes of silver particles within the bacteria after treatment with Silver Sol (ASAP Solutions®). Bar equals 0.5 µm.

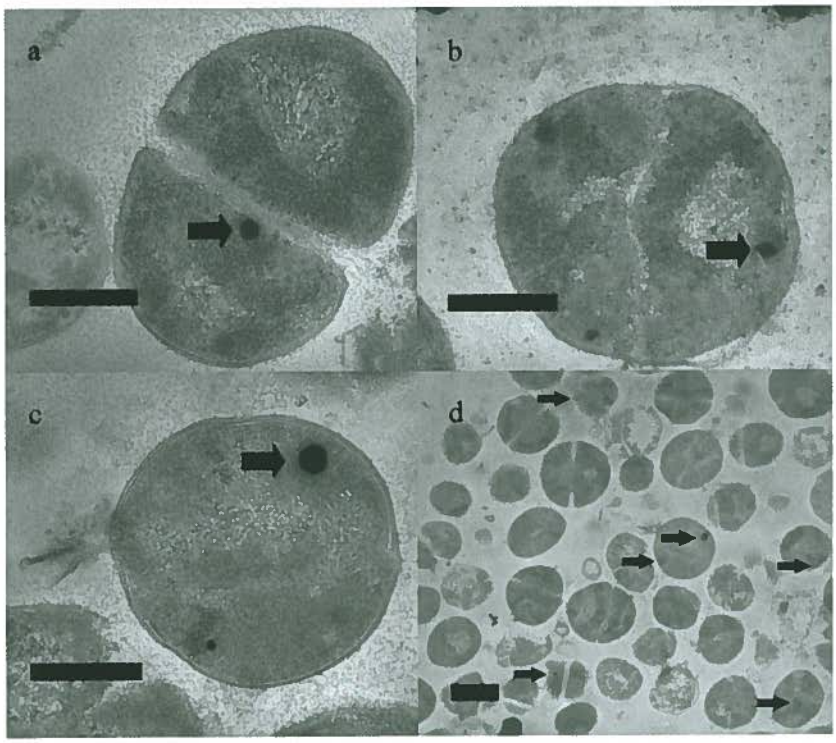

Figure 3: TEM micrographs of HVAC silver-treated S. aureus with post-staining. (a) A silver particle is clearly discernible inside the bacterium, suggesting that the Silver Sol (ASAP Solutions®) may enter bacteria to effect antimicrobial activity. Bar equals 0.5 µm. (b) Along with the irregular, globular silver particles a more ordered bitrigonal crystal of silver has formed inside the cell. Since these highly ordered silver particles were not observed in the size estimation study it is assumed the silver crystals formed after treatment with Silver Sol (ASAP Solutions®). Bar equals 0.5 µm. (c) Hexagonal crystals of silver were observed inside cells. The bar indicates 0.5 µm. (d) Treated cells at a lower magnification show a range of sizes of particles within cells as indicated by the arrows. Bar equals 1 µm.

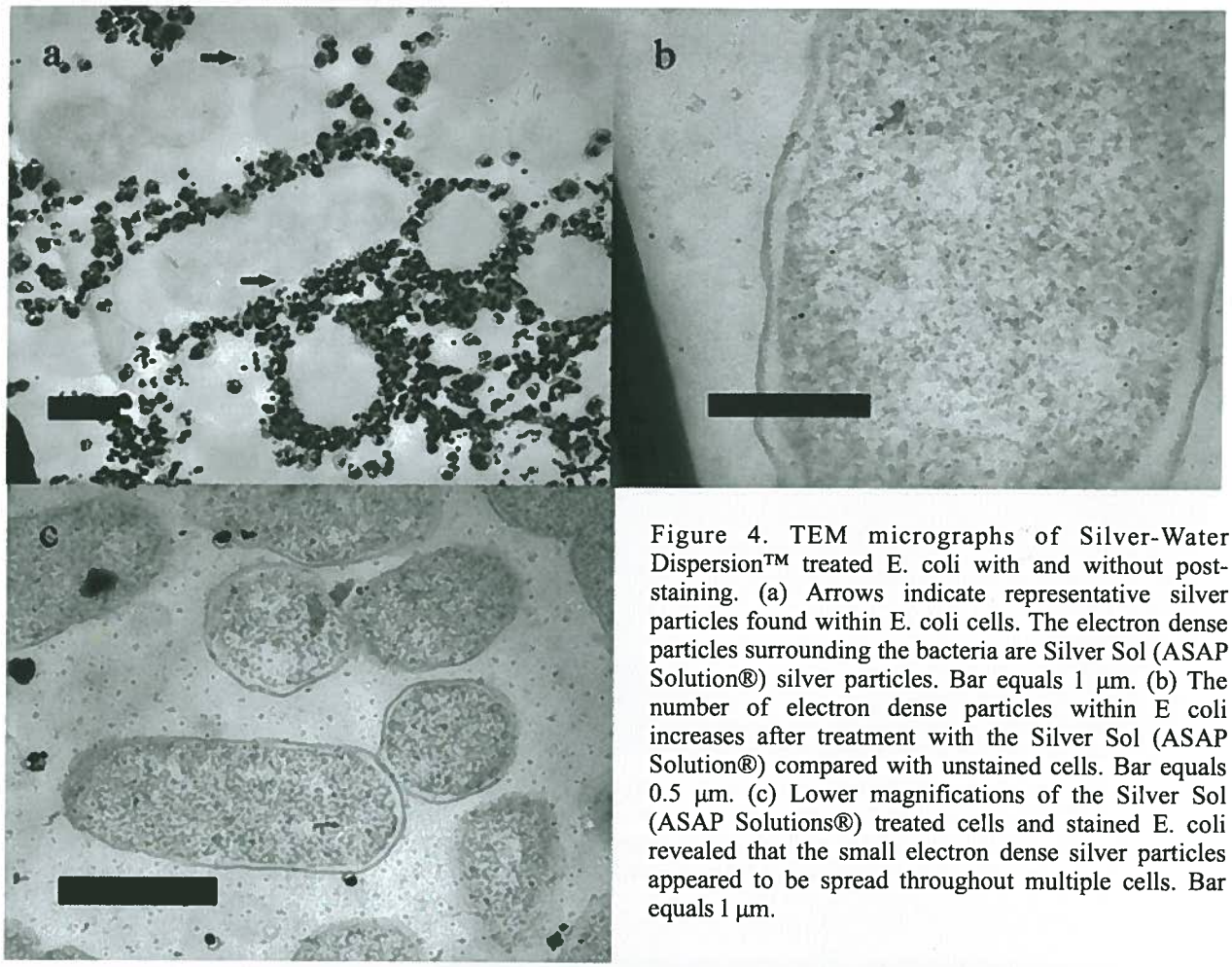

Figure 4. TEM micrographs of Silver-Water Dispersion™ treated E. coli with and without post-staining. (a) Arrows indicate representative silver particles found within E. coli cells. The electron dense particles surrounding the bacteria are Silver Sol (ASAP Solution®) silver particles. Bar equals 1 μm. (b) The number of electron dense particles within E coli increases after treatment with the Silver Sol (ASAP Solution®) compared with unstained cells. Bar equals 0.5 μm. (c) Lower magnifications of the Silver Sol (ASAP Solutions®) treated cells and stained E. coli revealed that the small electron dense silver particles appeared to be spread throughout multiple cells. Bar equals 1 μm.

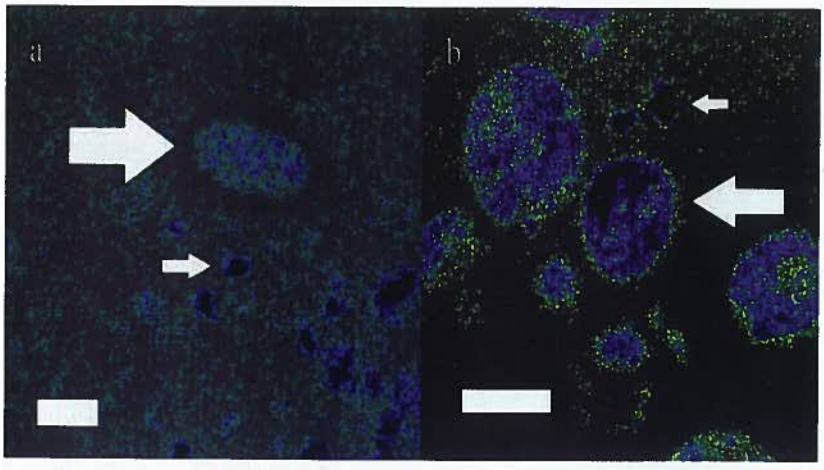

Figure 5: ESI image of Silver Sol (ASAP Solutions®) -treated E. coli and S. aureus. (a) The large arrow indicates an E. coli cell without silver particles. The small arrow indicates a particle of Silver Sol (ASAP Solutions®) silver. ESI imaging shows that treated cells contain silver even when no characteristic Silver Sol (ASAP Solutions®) -silver particle is present inside the cells. Bar equals 1 μm. (b) The large arrow indicates a S. aureus cell. The small arrow indicates a Silver Sol (ASAP Solutions®) silver particle. As with E. coli, cells of S. aureus not containing visible silver particles after treatment with Silver Sol (ASAP Solutions®) silver are shown to contain silver after ESI imaging. Purple, blue, green, and yellow denote the presence of silver in decreasing concentrations in that order, respectively. Bar equals 1 μm.

Appendix A

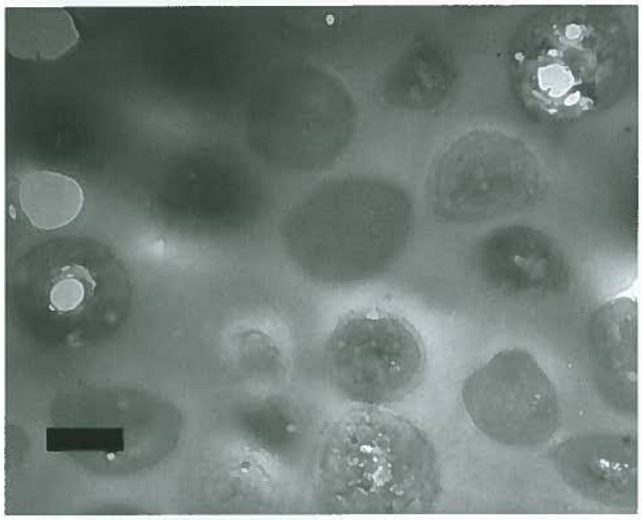

Figure 1: TEM micrograph of untreated S. aureus without post staining. The interior may be distinguished from the exterior of the bacteria. However, more detail is not readily distinguished. Bar equals 0.5 µm. Post staining. The micrograph shows that post staining brings out more detail than without post staining, as is expected.

Figure 2: TEM micrograph of untreated S. aureus without post staining. At higher magnifications, no more fine structure was apparent than at lower magnification. Bar equals 0.5 µm.

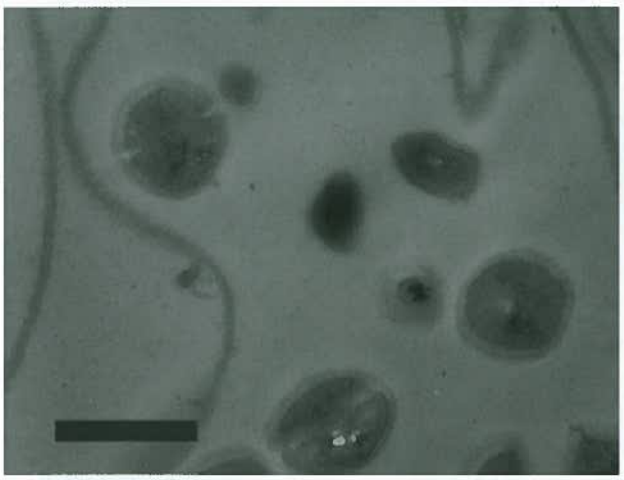

Figure 3: TEM micrograph of untreated S. aureus with post-staining. As shown, post staining brings out more detail than without post staining. The artifacts are due to the post staining. Bar equals 1µm.

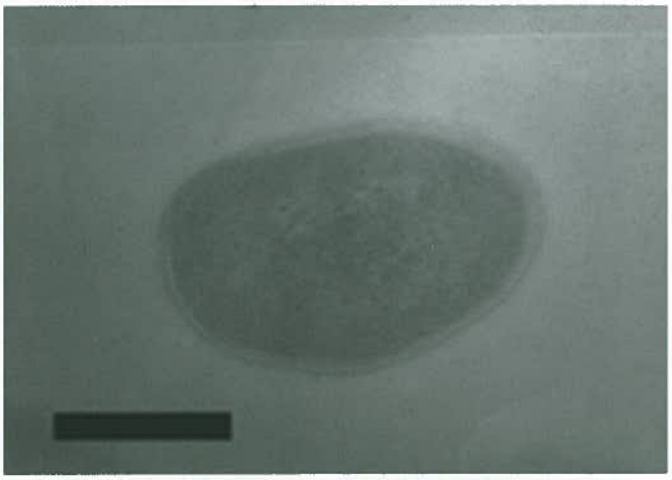

Figure 4: TEM micrograph of untreated S. aureus with post staining. At higher magnifications more structures may be observed with post staining. Cell wall, membranes DNA may be discerned in the bacterial cell. Bar equals 0.5 µm

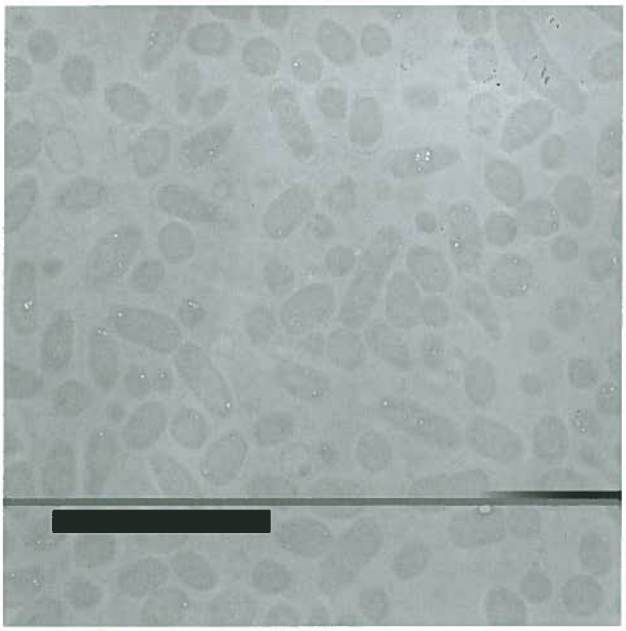

Figure 5: Untreated E. coli without post staining. As with S. aureus without staining or treatment, little more than interior versus exterior of the cell may be distinguished, which is to be expected without staining. Bar equals 10 µm.

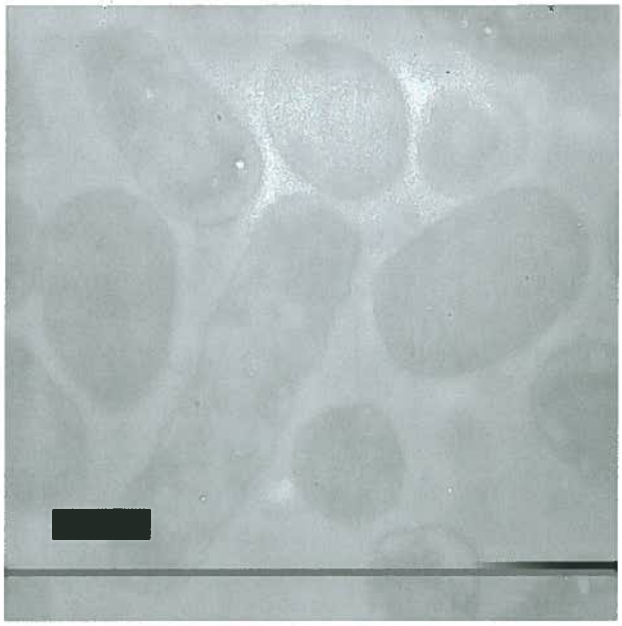

Figure 6: Untreated E. coli without post staining. Higher magnifications yield little more information than the micrographs taken at lower magnifications. Bar equals 1 µm.

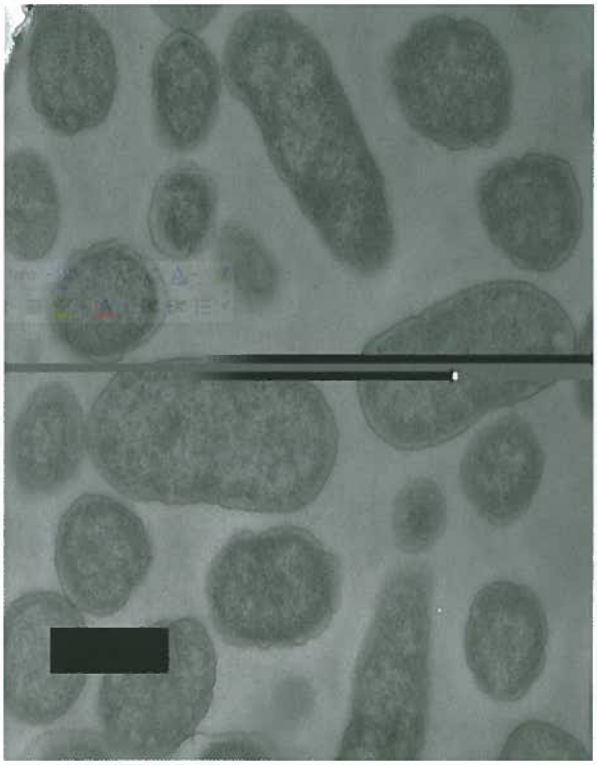

Figure 7: Untreated E. coli with post staining. As can be expected with staining a high amount of structural detail may be discerned. The cell wall as well as the membranes and nucleic acid are also distinguishable. Bar equals 1 µm.

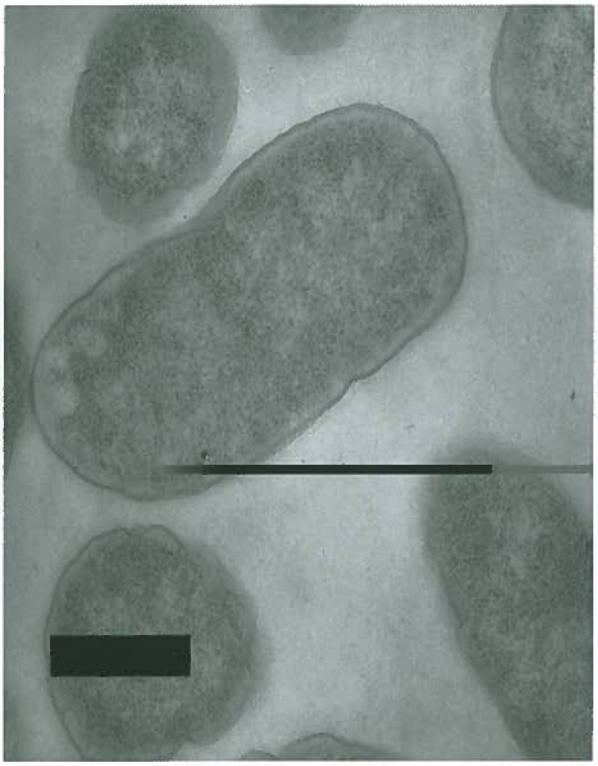

Figure 8: Untreated E. coli with post staining. As with the lower magnification more detail may be discerned with staining than without staining.

Figure 9: ESI control micrograph for untreated E. coli. This micrograph shows background in a sample of unstained, untreated E. coli.

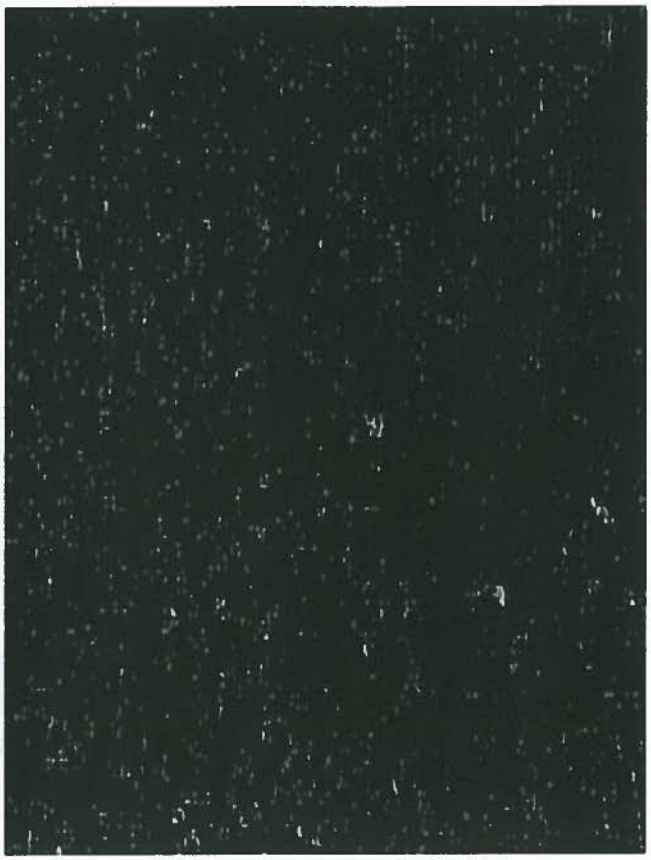

Figure 10: ESI control micrograph for untreated S. aureus. This micrograph shows background for untreated, unstained S. aureus.

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Reductionism on the way out in medworld

Reductionists believe that one theory or phenomenon is reducible to some other theory or phenomenon. In Mathematics, for instance, a reductionist would take any given mathematical theory as reducible to logic or set theory. In Biology, a reductionist would take biological entities like cells as reducible to collections of physio-chemical entities like atoms and molecules. Reductionist philosophers and metaphysicists believe that all phenomena, including mental phenomena like consciousness are identical to physical phenomena. Unfortunately, reductionism in healthcare has resulted in treating suffering humans as 'cases' and making treatment worse than suffering from illness.

A linear equation, on the other hand, assumes that there are one or more variables as in quantum mechanics. Applied to medical science, this underlines the need to respect the capacity of the human body and its in-built protective mechanism. It involves restraint from excessive interference in the name of treatment with powerful drugs and modern gadgets.