

MICROBIOLOGY DEPARTMENT
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(801) 378-2889 / FAX (801) 378-9197

12/8/98



To: American Silver LLC

From: David A. Revelli
Brigham Young University

I was able to test the American Silver's ASAP product against a strain of *E. coli* last week in a Minimum Inhibitory Concentration test (MIC). A MIC is a test designed to measure the growth of bacteria in the presence of a particular concentration of an antibiotic. The test ran over a period of 48-hours and American Silver's ASAP product was tested at various concentrations (i.e. 10ppm, 5ppm, 2.5ppm, 1.25ppm, .625ppm, .313ppm, .156ppm, .078ppm, .039ppm, .019ppm, .010ppm). A small amount of *E. coli* was placed into each test tube with the above written concentrations of American Silver's product and placed in a special incubator that lets the bacteria grow. The test tubes with the different concentrations of American Silver's ASAP product and *E. coli* were then checked at multiple times (i.e. 12 hrs, 24 hrs, 36 hrs, 48 hrs, and 60hrs.). After 12 hours, growth of *E. coli* was stopped at a concentration of 1.25ppm and higher. At 24 hours nothing had changed--growth was still stopped at a concentration of 1.25ppm. When the test tubes were checked at 36 hours, growth was seen in the test tube with a concentration of 2.5ppm. At 48 hours, no change had occurred--the growth of the bacteria was still halted at 2.5ppm and no growth was seen in later times in the test tubes with the concentrations of 5 and 10ppm--these remained free from growth. So from this preliminary data it can be suggested that American Silver's product does, in fact, have anti-bacterial properties. This may mean that if somebody, who is taking American Silver's product and is exposed to a bacteria that causes food poisoning, such as *E. coli*, they may not get sick or if they do, the period of time they are sick could be drastically reduced.

Here is the short list of pathogens; the ones with an asterisk are the ones that we have here and that I will be starting on:

- Porphyromonas gingivalis* (implicated in gingivitis)
- Streptococcus mutans* (tooth decay)
- Streptococcus gordonii* (tooth decay)
- **Streptococcus pneumoniae* (pneumonia, meningitis)
- **Staphylococcus Aureus* (toxic shock syndrome, meningitis, impetigo, and many more)
- Streptococcus pyogenes* (sore throats, skin infections, etc.)
- **Neisseria gonorrhoeae* (gonorrhoeae)
- Campylobacter pylori* (ulcers)
- **Salmonella arizona* (food poisoning)
- **E. coli* (food poisoning)
- **Klebsiella spp.* (food poisoning)
- **Pseudomonas aeruginosa* (wound and nosocomial infections)

If you have any questions, please call me at the lab 378-4517 or at home 489-5877.

David Revelli

To: American Silver LLC

From: David A. Revelli

Last week I ran an MIC, as described before, on *Staphylococcus aureus* and again on *E. coli* B. *Staph. aureus* is responsible for illnesses like toxic shock syndrome, meningitis, impetigo, etc. The test was ran as before except that the antibiotic, tetracycline, was run at 10ppm against colloidal silver for comparison. One thing is to be noted, tetracycline is a very potent broad spectrum antibiotic-- broad spectrum means that it is able to be used against a wide variety of bacteria. In the MIC with *Staph. aureus*, colloidal silver stopped the growth of the bacteria down to a concentration of 5ppm. Tetracycline stopped growth down to a concentration of .625ppm. The MIC with *E. coli* had the same results with colloidal silver stopping growth down to a concentration of 5ppm and tetracycline stopping growth down to a concentration of .625ppm.

What can we conclude from these observations? Well we can say that against *E. coli* and *Staph. aureus* tetracycline is more effective than colloidal silver when taken at doses lower than 5ppm. But we can also say that at 5 to 10ppm, which is the concentration that American Silver LLC makes for sale, that colloidal silver does as good of a job in stopping the growth of these major pathogens. Furthermore, there is no record that I can find of silver resistant bacteria as there are of tetracycline resistant bacteria and colloidal silver is a non-prescription anti-bacterial agent unlike tetracycline, which you have to have a doctor's prescription to buy it at the local pharmacy.

This is all that I have to report at this time. This week I am finishing with the bacterial pathogens that we have here at BYU.

Have a Happy New Year,

David A. Revelli

1/5/99

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To: American Silver LLC

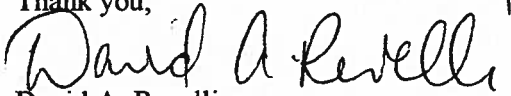
From: David A. Revelli
Ron W. Leavitt, PhD
Department of Microbiology
Brigham Young University

This week brought some surprising results. The MICs were performed as described before using both American Silver's ASAP product and tetracycline. The organisms tested were *Streptococcus pyogenes* (throat infections, skin infections, etc.), *Salmonella arizona* (food poisoning), *Klebsiella pneumoniae* (pneumonia, meningitis, nosocomial infections— infections spread in hospitals), *Shigella boydii* (violent food poisoning), *Pseudomonas aeruginosa* (diphtheria-like illnesses, burn and cut infections, meningitis, pneumonia, etc), and *Streptococcus faecalis* (urinary tract infections and endocarditis).

The results are as follows: *Strep. pyogenes* was inhibited at a concentration of 2.5ppm of American Silver's ASAP product, and at a concentration of .313ppm of tetracycline(tet). *Salmonella arizona* was inhibited at a concentration of 5ppm of American Silver's product and a concentration of 5ppm of tetracycline. *K. pneumoniae* was inhibited at a concentration of 5ppm of American Silver's product, and a concentration of 10ppm of tetracycline. *Shigella boydii* was inhibited at a concentration of 2.5ppm of American Silver's product, and a concentration of 5ppm of tetracycline. *Pseudomonas aeruginosa* was inhibited at a concentration of 5ppm, and the maximal concentration of tetracycline(10ppm) that was used did not inhibit the growth of *P. aeruginosa*. *Strep. faecalis* was inhibited by a concentration of 5ppm of American Silver's product and a tetracycline concentration of 5ppm.

What do these results show us? Basically, what the results suggest is that, like tetracycline, American Silver's ASAP product is a broad spectrum antimicrobial agent--it is able to effectively stop the growth of a large variety of organisms. And, in the case of *K. pneumonia*, *Shigella boydii*, *Pseudomonas aeruginosa*, American Silver's ASAP product was able to inhibit growth better than tetracycline. As for *Salmonella arizona* and *Strep. faecalis*, American Silver's product worked as well as tetracycline. In fact, the only bacteria for which tetracycline did a better job than American Silver's product was *Strep. pyogenes*.

Thank you,


David A. Revelli

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January 22, 1999

To: American Silver, LLC ASAP

From: David A. Revelli
Department of Microbiology
Brigham Young University

It turns out that American Silver's ASAP product works even better than previously reported. A re-evaluation of the data from the previous experiments showed that American Silver's product inhibited the growth of bacteria at lower concentrations. Colloidal silver inhibited the growth of *E. coli* at a concentration of 5ppm, what it should read is that it inhibited growth at a concentration of 2.5ppm and so on.

This week two types of tests were performed. One MIC was performed on *Haemophilus influenzae*. Growth of *H. influenzae* was inhibited at a concentration of 1.25ppm. Also, another type of experiment was run—a Minimum Bacteriocidal Concentration (MBC). Basically, a MBC tests whether an antibiotic is bacteriostatic (inhibits growth) or bacteriocidal (actually kills the bacteria). A MBC is done by taking .1ml from those test tubes of a MIC that have incubated for 24 hours and still remained clear (clearness denotes that the American Silver's product has inhibited the growth in those test tubes) and spreading that amount onto a petri dish filled with a nutrient agar that allows the growth of the bacteria. After incubation for 24 hours the plate is observed for growth. If the antibiotic is bacteriocidal no growth will be observed, which denotes the ability of the antibiotic to kill the bacteria. If the antibiotic is bacteriostatic then growth will be observed as tiny dots on the surface of the agar; these "dots" represent individual bacteria that have grown to form a larger colony of bacteria.

The results of the MBCs are as follows: *Streptococcus pyogenes*—no growth was observed on any of the agar plates, *Shigella boydii*—no growth was observed on any of the plates, *Haemophilus influenzae*—no growth was observed on any of the plates, *Pseudomonas aeruginosa*—no growth was observed on the 5ppm plate, but, on the 2.5ppm plate the bacterial colonies were too numerous to count, *Streptococcus faecalis*—no growth was observed on the 5ppm plate and on the 2.5ppm plate the colonies were too many to count, *Staphylococcus aureus*—no growth was observed on the 5ppm plate, but, the colonies on the 2.5ppm plate were too numerous to count.

The results of these preliminary tests suggest that American Silver's ASAP product has bacteriocidal (bacteria-killing) capabilities at concentrations above 5ppm and that it does not simply stop the growth of the bacteria.

All testing was accomplished using product obtained from American Silver LLC ASAP at a concentration of 10ppm.

Thank you,

David A. Revelli

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January 28, 1999

To: American Silver LLC ASAC 007

From: David A. Revelli
Department of Microbiology
Brigham Young University

Last week we did a variety of things. Minimum Bacteriocidal Concentration (MBC) tests were run on *Klebsiella pneumonia* (the causative agent in some forms of food poisoning) and also on *Salmonella arizona* (another cause of food poisoning). Two concentrations of the *Klebsiella pneumonia* MIC were plated out, 5ppm and 2.5ppm, and upon both no growth was observed. Only one concentration from the *Salmonella arizona* was plated out, 5ppm, and no growth was observed. So again, it can be suggested that American Silver LLC's colloidal silver has a bacteriocidal effect at the concentration at which it is sold. American Silver LLC's product adds two more disease-causing bacteria to its "hit list."

Also, time was spent on the transmission electron microscope (TEM), which is a microscope capable of magnifying objects by 100,000 times their normal size. This was done to measure the particle size of American Silver LLC's product. Digital analysis of the various concentrations have to be performed but the preliminary results are as follows. The average particle size of the 10ppm AC product was approximately 0.010 microns. The average particle size of the 30ppm AC product was approximately 0.015 microns. And the average size of the 21ppm DC product was approximately 0.010 microns.

A digital analysis done through the use of a computer was performed on the 21ppm DC product to measure exact particle sizes. Digital analysis is performed by taking a picture of the colloidal silver with the TEM and then feeding that picture into a computer, which then measures literally hundreds of particles and gives an average particle size. The average size of the 21ppm product was .010 microns. A digital analysis of the 10ppm AC and the 30ppm AC products will be performed next week.

Also, the shipment of the organisms that cause tooth decay (*Streptococcus mutans* and *Streptococcus gordonii*) and gingivitis (*Porphyromonas gingivalis*) has arrived and testing has begun on those.

Thank you.

A handwritten signature in cursive script that reads "David A. Revelli".
David A. Revelli

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February 3, 1999

To: American Silver, LLC ASAP

From: David A. Revelli
Department of Microbiology
Brigham Young University

The results of last week's Transmission Electron Microscope measurements were sent last week. The only other results to report this week are the MIC and the MBC that were performed upon *Streptococcus mutans*, a major player in tooth decay. The MIC results are as follows: growth was inhibited at a concentration of 5ppm by American Silver's product—that concentration was plated out and no growth was observed. Growth was also inhibited by tetracycline at a concentration of 5ppm—that concentration was plated out and no growth was observed.

Basically, as can be suggested by the results, American Silver's ASAP product kills one of the major constituents of tooth decay and it does it as well as the prescription antibiotic tetracycline.

Thank you,

A handwritten signature in black ink that reads "David A. Revelli". The signature is written in a cursive style with a large initial "D".

David A. Revelli

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February 12, 1999

To: American Silver, LLC

From: David A. Revelli
Department of Microbiology
Brigham Young University

This week's work revolved around one organism—*Streptococcus gordonii*. *Streptococcus gordonii* is a bacterium that can be routinely found in the mouth. Upon performing dental surgery or tooth extractions, *S. gordonii* may enter the blood stream and has been implicated in infective endocarditis, which is an infection of the heart valves.

A MIC was performed with *S. gordonii* and the results are as follows: *S. gordonii* was inhibited at a concentration of 3.75ppm of Americana Silver's ASAP product. The results of the MBC are still pending.

A handwritten signature in cursive script that reads "David A. Revelli".

David A. Revelli

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To: American Silver LLC

Bill,

I was able to test the colloidal silver against a strain of *E. coli* last week in a Minimum Inhibitory Concentration test (MIC). A MIC is a test designed to measure the growth of bacteria in the presence of a particular concentration of an antibiotic. The test ran over a period of 48-hours and the colloidal silver was tested at various concentrations (i.e. 10ppm, 5ppm, 2.5ppm, 1.25ppm, .625ppm, .313ppm, .156ppm, .078ppm, .039ppm, .019ppm, .010ppm). A small amount of *E. coli* was placed into each test tube with the above written concentrations of colloidal silver and placed in a special incubator that lets the bacteria grow. The test tubes with the different concentrations of colloidal silver and *E. coli* were then checked at multiple times (i.e. 12 hrs., 24 hrs., 36 hrs., 48 hrs., and 60hrs.). After 12 hours, growth of *E. coli* was stopped at a concentration of 1.25ppm and higher. At 24 hours nothing had changed--growth was still stopped at a concentration of 1.25ppm. When the test tubes were checked at 36 hours, growth was seen in the test tube with a concentration of 2.5ppm. At 48 hours, no change had occurred--the growth of the bacteria was still halted at 2.5ppm and no growth was seen in later times in the test tubes with the concentrations of 5 and 10ppm--these remained free from growth. So from this we can scientifically conclude that colloidal silver does, in fact, have anti-bacterial properties. This may mean that if somebody, who is taking colloidal silver and is exposed to a bacteria that causes food poisoning, such as *E. coli*, they may not get sick or if they do, the period of time they are sick could be drastically reduced.

Here is the short list of pathogens, the ones with an asterick are that ones we have here and that I will be starting on:

- Porphyromonas gingivalis (implicated in gingivitis)
- Strep. mutans (tooth decay)
- Strep. gordonii (tooth decay)
- *Strep. pneumoniae (pneumonia, meningitis)
- *Staph. Aureus (toxic shock syndrome, meningitis, impetigo, and many more)
- Strep. pyogenes (sore throats, skin infections, etc.)
- *Neisseria gonorrhoeae (gonorrhoeae)
- Campylobacter pylori (ulcers)
- *Salmonella (food poisoning)
- *E. coli (food poisoning)
- *Klebsiella spp. (food poisoning)
- *Pseudomonas aeruginosa

If you have any questions, please call me at the lab 378-4517 or at home 489-5877.

A handwritten signature in cursive script that reads "David Revelli".

David Revelli

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April 21, 1999

To: American Silver, LLC ASAP

From: David A. Revelli
Brigham Young University

MICs were performed with *Streptococcus pneumoniae* using American Silver's ASAP product and American Silver's AC/DC product. Also a 21ppm DC product, a 40ppm DC product, and a 10ppm DC product—these last three products are not made by American Silver, LLC. The results of the MICs are as follows: American Silver's ASAP product inhibited growth of *S. pneumoniae* at a concentration of 2.5ppm. American Silver's AC/DC product inhibited the growth of *S. pneumoniae* at a concentration of 3ppm. The 21ppm DC product inhibited growth at a concentration of 10.5ppm. The 40ppm DC product inhibited growth at a concentration of 5ppm. And the 10ppm DC product inhibited growth at a concentration of 2.5ppm.

MBCs were performed to determine bacteriocidal (bacteria-killing) activity of each silver product. The results are as follows: American Silver's ASAP product killed *S. pneumoniae* at 5ppm. The AC/DC product from American Silver, LLC killed *S. pneumoniae* at a concentration of 3ppm. The 21ppm DC product killed *S. pneumoniae* at a concentration of 10.5ppm. The 10ppm DC product did not kill *S. pneumoniae* at any concentration tested. And the 40ppm DC killed *S. pneumoniae* at a concentration of 5ppm.

These results suggest that American Silver's ASAP and AC/DC products have greater antimicrobial activity than the other silver products tested. American Silver's products are more effective at lower concentrations than any of the DC products that have been tested.


David A. Revelli

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May 4, 1999

To: American Silver, LLC

From: David A. Revelli
Brigham Young University

MICs and MBCs were performed using American Silver's ASAP II product against the following bacteria: *E. coli*, *Klebsiella pneumoniae*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus gordonii*. The results are as follows: *E. coli* was inhibited at a concentration of 3ppm and killed at the same concentration. *K. pneumoniae* was inhibited at a concentration of 6ppm and killed at the same concentration. *S. faecalis* was inhibited at a concentration of 12ppm and killed at the same concentration. *S. aureus* was inhibited at a concentration of 6ppm and killed at a concentration of 12ppm. *S. pyogenes* was inhibited at a concentration of 0.18ppm and was killed at a concentration of 0.38ppm. And *S. gordonii* was inhibited at a concentration of 3ppm and killed at the same concentration.

These results suggest that American Silver's ASAP II product is at least as effective as American Silver's ASAP product at killing the bacteria tested. As always, these tests are run three times each so that it can be shown that these results are reproducible. These are the first tests with the ASAP II product so two more tests will have to be performed on each bacteria to give more concrete evidence.


David A. Revelli



May 13, 1999

American Silver's Antibacterial Product (ASAP Solution) Testing Results Summary

American Silver's ASAP Silver Supplement has been tested against the following organisms.

Staphylococcus aureus (Pneumonia, eye infections, skin infections (boils, impetigo, cellulitis, and post-operative wound infections), toxic shock syndrome, meningitis, food poisoning, osteomyelitis, and many others) inhibited @ 2.5 ppm and killed @ 5 ppm. 1/22/99 BYU Report.

Shigella boydii (Bacillary dysentery—characterized by severe cramping abdominal pain and bloody diarrhea) inhibited @ 1.25 ppm and killed @ 2.5 ppm. 1/22/99 BYU Report.

Salmonella arizona (Food poisoning, etc.) inhibited @ 2.5 ppm and killed @ 5 ppm. 1/28/99 BYU Report.

E. coli (Food poisoning, urinary tract infections, traveler's diarrhea, diarrhea in infants, respiratory tract infections, and wound infections) inhibited and killed @ 2.5 ppm. 1/22/99 BYU Report.

Haemophilus influenzae (Otitis media (ear infection), pneumonia, meningitis, throat and sinus infections (including epiglottitis in children and sinusitis), and suppurative arthritis in children) inhibited and killed @ 1.25 ppm. 1/22/99 BYU Report.

Klebsiella pneumoniae (lower respiratory tract infections, nosocomial infections (infections spread in hospitals), urinary tract and wound infections, and bacteremia) inhibited and killed @ 2.5 ppm. 1/28/99 BYU Report.

Klebsiella oxytoca, (Similar to those infections caused by *K. pneumoniae*) killed in water treatment tests @ 0.1 ppm. 3/15/99 CUWCD Report.

Pseudomonas aeruginosa (severe burn and wound infections, keratitis, pneumonia, meningitis, nosocomial infections, urinary tract infections, etc.) inhibited @ 2.5 ppm and killed @ 5 ppm. 1/22/99 BYU Report.

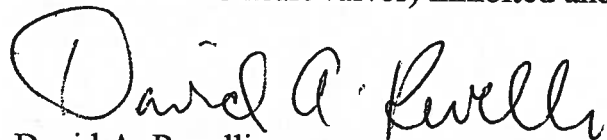
Streptococcus pneumoniae (pneumonia, meningitis, sinusitis, otitis media (ear infection) inhibited @ 2.5 ppm and killed @ 5 ppm. 4/21/99 BYU Report.

Streptococcus pyogenes (skin infections, upper respiratory infections (i.e. strep throat) impetigo, hospital-acquired infections, scarlet fever, etc.) inhibited and killed @ 1.25 ppm. 1/22/99 BYU Report.

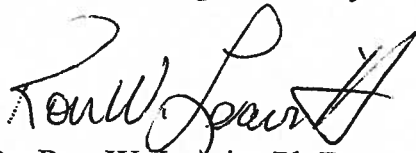
Streptococcus faecalis (Urinary tract infections, endocarditis, wound infections, etc.) inhibited @ 2.5 ppm and killed @ 5 ppm. 1/22/99 BYU Report.

Streptococcus mutans (A major cause dental plaque and tooth decay etc.) inhibited and killed @ 5 ppm. 2/3/99 BYU Report.

Streptococcus gordonii (Tooth decay, also implicated in infective endocarditis-an infection of the heart valves) inhibited and killed @ 5 ppm. BYU Report 2/12/99.



David A. Revelli
Microbiologist
Brigham Young University



Dr. Ron W. Leavitt, Ph.D.
Professor of Microbiology/Molecular Biology
Brigham Young University

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June 7, 1999

To: American Silver, LLC

From: David A. Revelli
Brigham Young University

Four potentially pathogenic bacteria were tested in MICs against American Silver's ASAP solution. These bacteria are: *Salmonella typhimurium* (food poisoning and enteric fever), *Enterobacter aerogenes* (wound infections, urinary tract infections, bacteremia, and meningitis), *Enterobacter cloacae* (causes illnesses similar the *E. aerogenes*), *Klebsiella oxytoca* (produces infections similar to *K. pneumoniae*).

The results of the MICs and MBCs are as follows: *K. oxytoca* was inhibited and killed at a concentration of 2.5ppm. *E. aerogenes* was inhibited and killed at a concentration of 2.5ppm. *E. cloacae* was inhibited and killed at a concentration of 5ppm. And *S. typhimurium* was inhibited and killed at a concentration of 2.5ppm.

Testing continues on American Silver's ASAP solution—the testing being performed tests the antimicrobial activity of American Silver's nonprescription product against the activity of five major classes of prescription antibiotics.

Sincerely,

A handwritten signature in black ink that reads "David A. Revelli".

David A. Revelli

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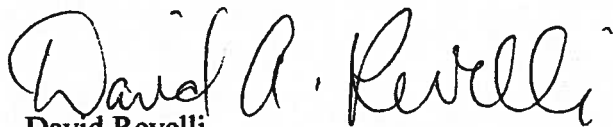


June 18, 1999

To: American Silver, LLC

From: David Revelli
Brigham Young University

We have tested and compared the activity of American Silver's ASAP solution against three commercial colloidal silver solutions using the Minimum Inhibitory Concentration test (MIC) and the Minimum Bacteriocidal Concentration (MBC) test. The data obtained suggests that American Silver's ASAP solution is at least two to three times more effective depending on the bacteria tested than the commercial colloidal silver products tested.


David Revelli

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August 9, 1999

To: American Silver, LLC

From: David A. Revelli
Brigham Young University

MICs and MBCs were performed using the solution from the new machine. *E. coli* and *S. aureus*, were chosen to test because they are two of the standard test organisms used in minimum inhibitory concentration testing in the clinical setting. The results are as follows: *S. aureus* was inhibited at a concentration of 2.5ppm and killed at a concentration of 5ppm. *E. coli* was inhibited and killed at a concentration of 1.25ppm.


David A. Revelli

Minimum Inhibitory Concentrations of Antibiotics from Five Different Classes versus the ASAP Solution
performed by D. Revelli, Brigham Young University

Bacteria Tested	tetracycline	ofloxacin	penicillin G	cefaperazone	erythromycin	ASAP
<i>E. coli</i> B	1.67±0.59 / >5 / 3	0.104±0.037 / 0.130±0.037 / 3	No inh / 3	.625 / .625 / 3	5 / >5 / 3	1.25 / 1.56±0.54 / 4
<i>E. aerogenes</i>	2.5 / >5 / 3	0.078 / 0.104±0.037 / 3	No inh / 3	2.92±1.56 / >5 / 3	No inh / 3	1.5±0.5 / 1.5±0.5 / 5
<i>E. cloacae</i>	1.67±0.59 / >5 / 3	.156 / .156 / 3	No inh / 3	No inh / 3	No inh / 3	2.0±1.5 / 3.0±1.0 / 5
<i>S. typhimurium</i>	1.25 / >5 / 3	0.078 / >5 / 3	No inh / 3	1.25 / >5 / 3	5 / >5 / 3	1±0.75 / 1.5±0.5 / 5
<i>P. aeruginosa</i>	0.078 / >5 / 3	0.156 / 0.313 / 3	0.130±0.037 / >5 / 3	2.5 / >5 / 3	2.5 / >5 / 3	1.88±0.625 / 3.75±1.25 / 2
<i>S. gordonii</i>	0.156 / >5 / 3	2.5 / 5 / 3	0.012±0.005 / 0.026±0.009 / 3	1.25 / 2.5 / 3	.005 / 0.012±0.005 / 3	3.13±1.08 / 5 / 4
<i>S. aureus</i>	0.313 / >5 / 3	0.313±0.18 / 0.625 / 3	2.5 / >5 / 3	5 / >5 / 3	0.039 / >5 / 3	4.60±3.62 / 3.29±1.44 / 12

The average was taken from all data points to obtain the average minimum inhibitory concentration for an antibiotic. Then a standard deviation was determined to give an error. All concentrations were calculated in parts per million (µg/ml). The abbreviation "No inh." stands for "No inhibition of growth." And ">" means that the measurement of the bacteriocidal concentration was beyond the limits of the test. Information is listed as "MIC (level at which bacteria was inhibited)/MBC (level at which bacteria were killed)/number of tests performed."