

## PEER-REVIEWED ARTICLE

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# Efficacy of Treatment of Reusable Grocery Bags with Antimicrobial Silver to Reduce Enteric Bacteria

## ABSTRACT

Reusable grocery bags are seldom washed in homes and can become contaminated with enteric bacteria during use in grocery shopping. The goal of this study was to evaluate the efficacy of a cloth fiber grocery bag impregnated with silver in controlling enteric bacteria and viruses in three different settings. The reusable grocery bags were evaluated for bacterial survival in the laboratory, for bacterial growth in the trunk of a car, and for bacterial contamination when used by volunteers over 4 months. In the laboratory, the treatment was found to reduce *Escherichia coli*, *Salmonella Choleraesuis*, and *Staphylococcus aureus* counts by more than 99.9% within two hours and the MS2 virus and murine norovirus by 99.5%. When placed in a car trunk during warm weather, the silver treatment was capable of reducing bacterial counts in bags containing meat juices even under conditions that ordinarily promoted growth of bacteria. After being distributed to 38 households in Southern California for use over 4 months, the bags

were found to have significantly fewer coliforms than non-treated canvas bags. The major significance of these findings is that the broad antimicrobial properties of silver make it a useful component of commercially available reusable grocery bags.

## INTRODUCTION

The Reusable Grocery bag (RGB) is now mandated as a necessity in several U.S. municipalities as an alternative to single-use disposable polyethylene plastic grocery bags. Many cities have banned the single-use plastic bags as a response to the public's perceived environmental concerns such as: degradation time in a landfill, clogging of storm drains, energy conservation issues, and damage to marine ecosystems (4, 11). The adoption of reusable grocery bags requires a new societal hygiene practice, as RGBs have a heightened potential to become contaminated with pathogens and transmit disease because of repeated use without washing (13, 15, 16). The RGB is a vehicle of transmission because it traverses the public and private food domain and can transmit microbes from private households to the public setting, such as a grocery

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store or school. One proposed solution to contamination of RGBs is to add antimicrobial silver in its ionized form. The silver is an effective antimicrobial when added to cotton or cotton-like fabrics (5, 8, 9, 10, 14). Viable *E. coli*, *S. aureus*, and other bacteria have been successfully reduced through embedding silver nanoparticles in fabrics such as surgical masks (10), cotton fibers (8), wound dressings (9, 10) and food packaging (5, 14). Silver can be easily added to materials and is usually incorporated as salt, in a mineral form or as independent silver ions in solution. The delivery method of silver ions is dependent on the antimicrobial activity desired; a slow release is usually necessary in fabrics, to extend the useful life of the added silver (14).

We have previously shown that RGBs collected from consumers were found to contain both coliforms and *E. coli*. That study also showed that bacteria will grow in RGBs when they are in the trunk of cars and when meat juices are present in the RGB. These findings justify the recommendations that meats and vegetables should be placed in separate RGBs that are washed after each use (1, 2, 3). We found that these recommendations are seldom practiced by the consumer; 97% reported never having washed their RGB (16). Low user compliance in washing RGBs justifies better public education and/or the addition of antimicrobial silver to the RGB material.

The goal of this study is to assess the antimicrobial efficacy of an RGB with silver woven into the fabric. The test hypotheses are that the antimicrobial silver RGB can prevent survival of pathogenic bacteria and viruses on the material, can prevent bacterial growth when the RGB is placed in incubating conditions, and will have no bacterial contamination after being used by volunteer households for several months without washing. This potential additive could ease the washing requirements of RGBs and protect the public health against the transmission of pathogens across the public and private food domain.

## MATERIAL AND METHODS

This study evaluates the antimicrobial efficacy of silver-containing reusable grocery bags against bacteria and virus in three different settings: for bacteria and viruses in the laboratory, for bacterial growth in the trunk of a car, and for bacterial contamination when used by volunteers. The antimicrobial silver bag was compared with a similarly constructed cotton bag that was not treated with silver and served as a control. All bags were unused and the control bag was selected based on its similar construction to the antimicrobial silver bag. The study was determined not to be human subject research by the Loma Linda University Institutional Review Board.

### Reusable grocery bags

The antimicrobial silver-containing RGBs were supplied new from PureThread Technologies Inc. (Morrisville, NC). The antimicrobial RGBs are off-white in color, are 0.75 mm

thick, measure 36 × 29 × 18 cm, and are constructed of 70% polyester and 30% silver-infused polyester. The PurThread bag is woven to appear similar to a cotton-based durable canvas. The control RGBs were chosen because of their similar appearance to that of the antimicrobial bags; they were purchased new from the Trader Joe's supermarket in Redlands, CA. The canvas control RGBs are off-white in color, are 1 mm thick, measured 36 × 34 × 18 cm and made of a durable cotton material.

### Test organisms used for survival and growth studies

*Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25293), *Salmonella enterica* Choleraesuis (ATCC 10708) and coliphage MS-2 (ATCC 15597-B1) were obtained from the American Type Culture Collection (Manassas, VA). The Murine norovirus (MNV) (strain S7-PP3) was obtained from Dr. Yukinobu Tohya from the Department of Veterinary Medicine, Nihon University (Kanagawa, Japan).

All the bacteria were grown overnight in trypticase soy broth (TSB) (Difco, Sparks, MD) at 37°C and assayed on trypticase soy agar (Difco) by the spread plate method. The cultures were then centrifuged to pellet the bacteria and re-suspended in phosphate buffered saline (PBS) (Difco, USA). This step was repeated two additional times before use in experiments. *E. coli* was assayed on MacConkey agar (Difco), *S. aureus* on mannitol salt agar (EMD Chemicals, Inc., Gibbstown, NJ) and *S. enterica* on XLD agar (EMS Chemicals). Bacterial colonies were counted after incubation for 24 hours at 37°C.

The coliphage MS2 was assayed using the double agar overlay method on its host, *E. coli* ATCC 15597. Stock virus was produced by harvesting it from agar plates containing phage by adding phosphate buffer saline (PBS), allowing it to sit for two hours, and harvesting with a pipette. The PBS was then centrifuged to remove cell debris and passed through a 0.22 µm membrane filter (Millipore Corporation, Billerica, MA). The virus was stored at 4°C until needed.

The virus was propagated on RAW 264.7 (ATCC TIB-71) cell line monolayers, using Dulbecco's modified Eagle medium (DMEM; Mediatech Inc., Manassas, VA) containing 10% fetal bovine serum (FBS; HyClone Laboratories, Logan, UT), 10 mM HEPES buffer (Mediatech Inc.), 0.113% sodium bicarbonate (Fisher Scientific, Fair Lawn, NJ), and 1.0% antibiotic-antimycotic (Mediatech Inc.). The cells were incubated at 37°C with 5% CO<sub>2</sub>. MNV was concentrated and purified as previously described, with minor modifications (7). Briefly, MNV was concentrated and purified using polyethylene glycol precipitation and Vertrel XF extraction, which promoted monodispersing of the virus and the removal of lipids. The virus stocks were stored at -80°C until needed.

Murine norovirus was assayed using the Reed-Muench method (12) to determine the tissue culture infectious dose that showed a 50% (TCID<sub>50</sub>) cytopathogenic effect (CPE). Briefly, serial 10-fold dilutions were made, using DMEM (Dulbecco Minimal Essential Media) containing 10% FBS (DMEM-FBS). Virus samples were assayed in 96-well tissue culture plates (Nunc, Roskilde, Denmark) containing monolayers of RAW 264.7 cells. Of these, 100 µl of each dilution was used to inoculate six replicate wells to ensure adequate precision of the assay, with incubation at 37°C with 5% CO<sub>2</sub> as described before. Each well was observed every day for 8 days for cytopathic effects (CPE). The highest dilution in which > 50% of the wells exhibited cytopathogenic effects (CPE) was used to determine TCID<sub>50</sub>/ml.

### Survival on RGB materials

Survival of the test organisms was determined by cutting one-inch square (6.45 cm<sup>2</sup>) swatches from the bags. The swatches were then placed in sterile plastic petri dishes and inoculated with 10<sup>5</sup> to 10<sup>6</sup> CFU (cm<sup>2</sup>)<sup>-1</sup> of the test organisms. In the case of the murine norovirus, ~10<sup>4</sup> TCID<sub>50</sub> was inoculated onto the swatch in 0.01 ml drops for a total of 0.1 ml. The swatches were then covered and placed at 37°C with a relative humidity of 36 to 47%. Swatches were collected after 2, 4, and 24 hours of incubation and placed into a tube containing one ml of DE neutralizing broth (Difco) and vortexed for 30 seconds to stop the antimicrobial action of the silver and release bacteria from the swatch. Serial dilutions were then performed in PBS. All tests were conducted in duplicate, and the chi-square test statistic was used to define significant associations among the antimicrobial RGB. Calculations were performed with 2010 Microsoft Excel.

### Assessment of bacterial growth in RGBs

To assess the potential for bacterial growth in stored reusable bags, raw chicken and beef were purchased from a grocery store and hand wiped with sterile gloves; the resulting juices were collected in a sterile beaker. The solution was then spiked with 10<sup>5</sup> to 10<sup>6</sup> CFU (ml)<sup>-1</sup> concentration of *Salmonella enterica* Choleraesuis and *E. coli*. These solutions were inoculated onto test swatches prepared as those in a foregoing section. A triplicate set of the swatches were processed immediately to determine the initial concentration of the two bacteria, a triplicate set added to resealable bags for growth assessment in a car trunk, and another parallel triplicate set of resealable bagged swatches were kept in the laboratory at 25°C for a control of the trunk experiment. The bagged and inoculated swatches were placed in the trunk of a Tucson, AZ car for two hours in mid-afternoon of March 11 and 12 of 2015. The temperature was measured at a maximum of 33.3°C, using a Fisher Scientific (Ontario, CA) traceable digital thermometer with a maximum temperature function. The laboratory- and trunk-stored swatches were assayed immediately after being

stored in the trunk for two hours and then assayed again in the lab after being stored at room temperature (25°C) for an additional two hours, at the fourth and 24th hour. *S. enterica* was assayed on XLD media (Difco) and *E. coli* assayed using the IDEXX Colilert Quanti-Tray (Irvine, CA).

### Shopping bag performance field study

Households were recruited to use the bags through a chain-referral sampling strategy (6). Laboratory technicians used their personal, work, and family networks to recruit households to host regular and anti-microbial shopping bags for 4 months. Volunteer household representatives were given verbal instructions to use the grocery bags for their routine grocery shopping, divide their goods equally among the bags and never wash the bags. Identity of volunteer participants was kept confidential; only the laboratory team member who recruited them could locate them again to collect the used RGB. Other study team members could not access any identifying information about volunteer participants. The household-use study bags were numbered from 1 to 80 with a letter designating the cloth-like antimicrobial bags supplied from PurThread and the similarly constructed canvas control bags (Trader Joe's, Redlands, CA).

After three to five months, the bags were returned to the laboratory and processed for total coliform and *E. coli*, using the IDEXX Quanti-Tray 2000 method (IDEXX, Irvine, CA). The returned bags were processed by soaking each bag with 300 ml of Buffered Peptone Water (Becton Dickinson, Franklin Lakes, NJ) and eluting by hand in a large stomacher bag (Thermo Fisher Scientific, Waltham, MA). A variable amount of the bag fluid was collected in a 50-ml Falcon polypropylene centrifuge tube (Thermo Fisher Scientific). The resulting extract was assayed using the IDEXX Quanti-Tray 2000 method to determine the concentration of *E. coli* and coliforms of each shopping bag. The reagent within each Quanti-Tray was prepared by mixing 10-ml of bag fluid with 90 ml of phosphate buffer saline and 1 package of IDEXX colilert-24 powder. This 100 ml of Colilert solution was poured into Quanti-Trays and incubated for 24 hours at 37°C. All tests were conducted in duplicate.

Scanning electron microscopy was used to image *E. coli* and *Bacillus thuringiensis* morphology when the organisms were inoculated onto RGB material. The RGB was cut into five pieces of 10 cm<sup>2</sup> material and exposed for 24 hours to a 10<sup>5</sup> to 10<sup>6</sup> solution of bacterial inoculant solution as previously described. Samples were further cut and mounted on specimen stubs for examination with the Hitachi S-3400N Type II scanning electron microscope (Hitachi High-Tech Inc., Northridge, CA, USA) at an accelerating voltage of 9kV.

## RESULTS

### Survival on RGB materials in the laboratory

Table 1 shows that the *S. enterica* and *E. coli* numbers decreased by over 3 logs (> 99.9%) within two hours at

**Table 1. Log<sub>10</sub> reductions of test bacteria by antimicrobial and control RGB swatches held at room temperature (25°C) and a relative humidity of 36% in the laboratory**

Organism	2 hours		4 hours		24 hours	
	Control	Antimicro	Control	Antimicro	Control	Antimicro
<i>E. coli</i>	0	3.8 <sup>a</sup>	0.03	4.9 <sup>a</sup>	2.63	6.3 <sup>a</sup>
<i>S. aureus</i>	0	3.4 <sup>a</sup>	0	3.8 <sup>a</sup>	0.15	4.4 <sup>a</sup>
<i>S. enterica</i>	0	4.2 <sup>a</sup>	0.44	4.6 <sup>a</sup>	1.32	> 6.8 <sup>a</sup>
Coliphage MS2	0	0.01	0	2.5 <sup>a</sup>	0.31	4.2 <sup>a</sup>
Murine norovirus	0	1.75 <sup>a</sup>	0.25	2.5 <sup>a</sup>	0.18	≥ 2.72 <sup>a</sup>

<sup>a</sup> = chi-square significant for antimicrobial RGB ( $P \geq 0.05$ ).

**Table 2. Log<sub>10</sub> reductions and growth of bacteria after storage in car trunk with meat juices**

Exposure	In laboratory at room temperature (25°C)						In car trunk for 2 hours (33.3°C) then lab (25°C)					
	Control RGB			Antimicrobial RGB			Control RGB			Antimicrobial RGB		
Time (hours)	2	4	24	2	4	24	2 (car)	4	24	2 (car)	4	24
<i>E. coli</i> with beef juice	0	0	0	0.4	1.7 <sup>a</sup>	4.2 <sup>a</sup>	0	0.75 <sup>b</sup>	1.39	0	0	5.4 <sup>a</sup>
<i>S. enterica</i> with chicken juice	0	0.15 <sup>b</sup>	0	2.6 <sup>a</sup>	2.1 <sup>a</sup>	2.8 <sup>a</sup>	0.24 <sup>b</sup>	0.7 <sup>b</sup>	0.7 <sup>b</sup>	0.3	0	3.1 <sup>a</sup>

<sup>a</sup> = chi-square significant for antimicrobial RGB ( $P \geq 0.05$ ).

<sup>b</sup> = growth of bacteria

room temperature, while no significant die-off occurred in the untreated (control) bags. Under these test conditions, the *S. enterica* appear to be the more sensitive organism, decreasing by over 6 logs in 24 hours. The viruses were more resistant, but still decreased by 2.5 logs in four hours.

#### Assessment of bacterial growth in RGBs

The antimicrobial RGBs were shown to still be effective in the presence of meat juices at room temperature against the enteric bacteria (Table 2). When the RGBs were in a car trunk at 33.3°C, both bacteria grew in the control bags, but had decreased by over 3 logs after 24 hours in the antimicrobial RGBs (Table 2).

#### Household use study results

Seventy-four bags were distributed to 38 different enrolled households: 49 antimicrobial bags and 25 control bags. Of those 74 bags, 17 (23%) were not returned, leaving 57 RGBs to be tested in the lab (38 antimicrobial RGBs and 17 control RGBs) (Fig. 1). Fifteen households had both an antimicrobial RGB and a control RGB. All comparisons of antimicrobial RGBs to control RGBs were drawn from those 15 households.

#### *Escherichia coli* concentration

*E. coli* were found in four household RGBs: three of the 38 antimicrobial RGBs and one of the 19 control RGBs. The concentration of *E. coli* was low in three of the bags, with only one Quantitray well positive for *E. coli*, giving a concentration of less than 10<sup>-3</sup> for 10 (cm<sup>2</sup>)<sup>-1</sup> of RGB material. One antimicrobial sample had an average concentration of 21 *E. coli* for the entire bag. The laboratory technician confidentially followed up with the volunteer, who admitted to having a package of raw chicken leak out into the RGB. The other three bags were heavily used by large families and left in the back seat of cars. All concentrations of *E. coli* were lower than the total coliform concentrations on the same Quantitray.

#### Total coliform concentration

Table 3 shows that the total coliform geometric mean was four orders of magnitude greater for the control cotton RGB than for the other antimicrobial bags. The geometric mean is used for this assessment because it can represent most probable number (MPN) concentration values when they are standardized to 10 cm<sup>2</sup> as a uniform area. The arithmetic mean and standard deviation in Table 4 are also standardized to show the total coliform MPN (10 cm<sup>2</sup>)<sup>-1</sup> but do not

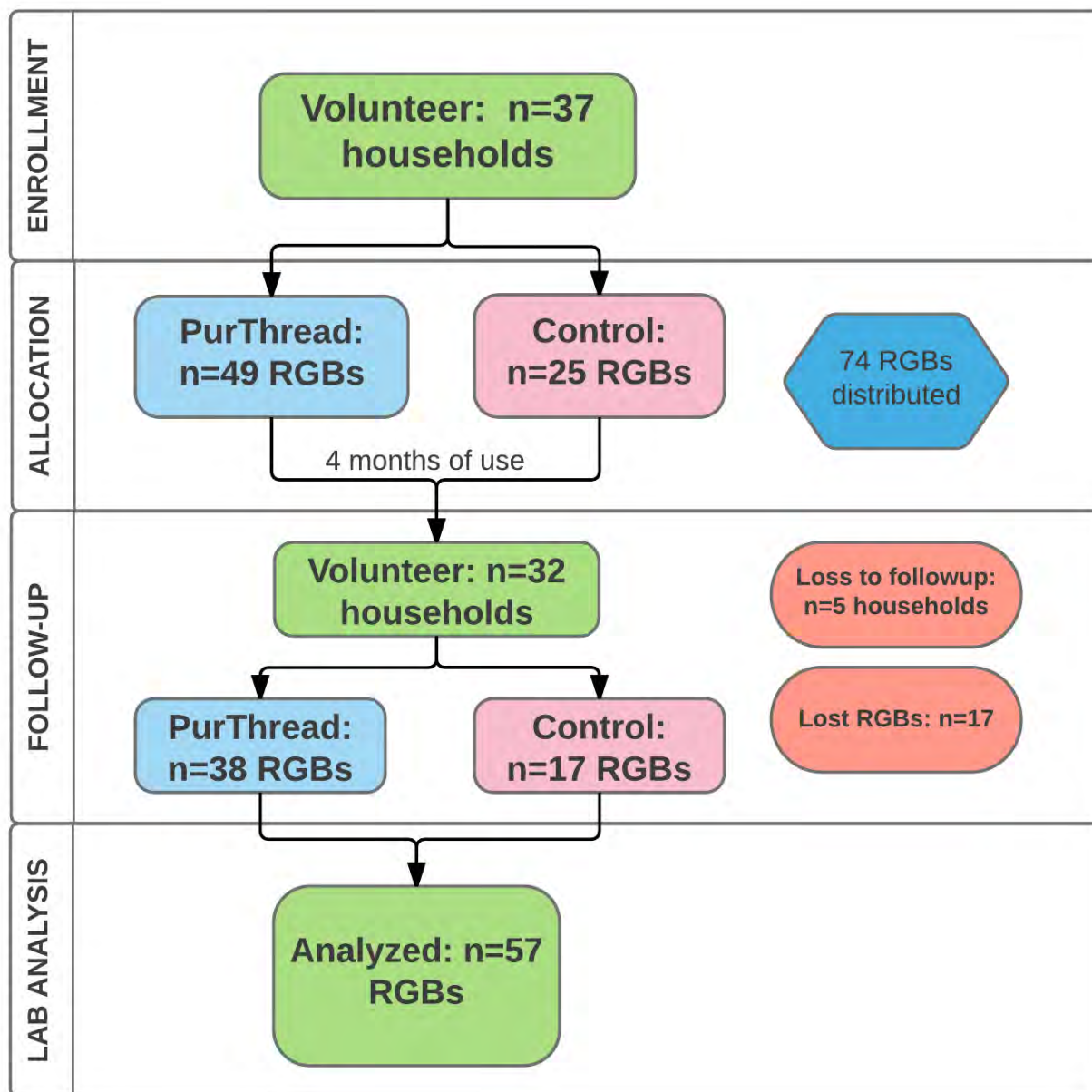


Figure 1. Flow diagram of volunteer households with RGBs

represent the differences in log concentration that are typical with bacterial growth.

#### Total coliform percent positives

Table 4 shows that all of the cotton control RGB bags were contaminated with total coliforms, meaning all volunteer users contaminated their RGBs. Of the antimicrobial RGBs, 61% were contaminated with total coliforms, demonstrating the ability to inhibit typical contamination that occurs from household use.

#### Scanning electron microscope images

The images provided in Fig. 2 show microorganisms with membranes and morphology intact on non-treated RGBs after 24 hours of exposure. Images show microbes distributed

evenly throughout exposed threads, but often clustered within pores and fabric imperfections.

#### DISCUSSION

The first two of the three hypotheses evaluated the RGBs in the lab and intermediate field scale environment, while the third study was an in-field evaluation of the RGBs as used by volunteers. The antimicrobial RGBs exhibited continuous inactivation of all five test organisms in the laboratory challenge tests and reasonable inactivation of real-world bacteria during the household use study. In the household use study, bacteria were found in nearly every RGB, but at lower concentrations in the antimicrobial RGBs. Consumers seldom or ever wash RGBs, so any reduction of contamination may be a beneficial result and will protect

**Table 3. Total coliform MPN (10 cm<sup>2</sup>)<sup>-1</sup> of reusable grocery bags (RGB) after volunteer use**

RGB	Number of bags	Mean	St. dev.	Geometric mean
Control	19	2.7	4	8.57E-01
Antimicrobial	38	21.6	124	9.53E-05
Total	57	14.9	101.4	1.98E-03

**Table 4. Number of control and antimicrobial RGBs positive for total coliform and *E. coli* after use**

RGB	n	Positive coliforms	Positive <i>E. coli</i>	% positive coliforms	% positive <i>E. coli</i>	Matched <sup>a</sup>
Antimicrobial	38	23	3	61	8	.
Control	19	19	1	100	5	15
Total	57	42	4	74	7	.

<sup>a</sup>paired within households to the antimicrobial RGB

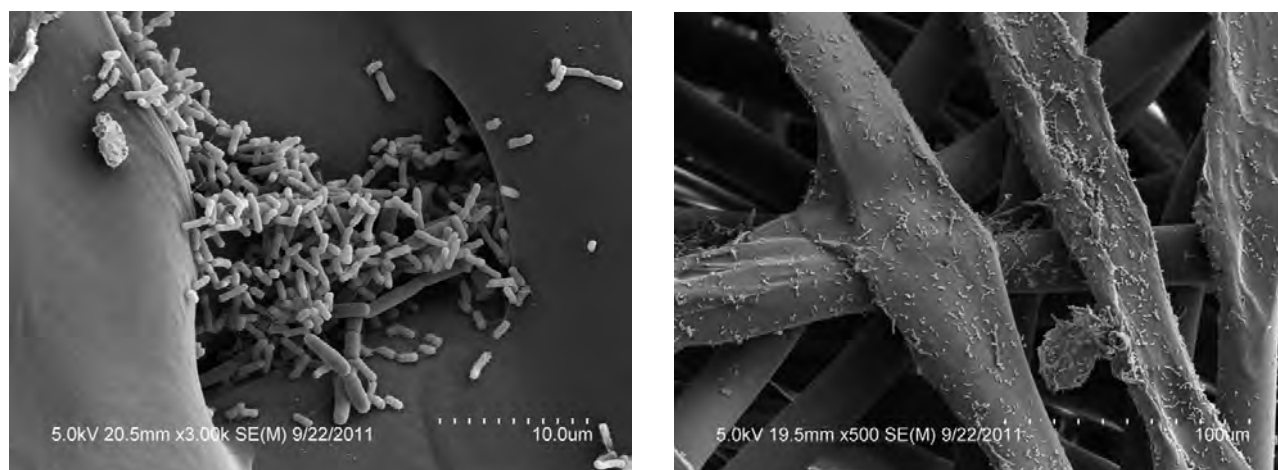


Figure 2. SEM images of *B. thuringiensis* and *E. coli* on a reusable grocery bag inoculated 24 hours prior to imaging

public health by reducing the risk of cross-contamination with pathogens.

#### LIMITATIONS

This household use study was a field investigation that had many uncontrolled factors. Some RGBs had high concentrations and were used often, while other bags may have been used only a few times over the four-month period. The study team did not disclose volunteer user identity, and therefore was not able to actively

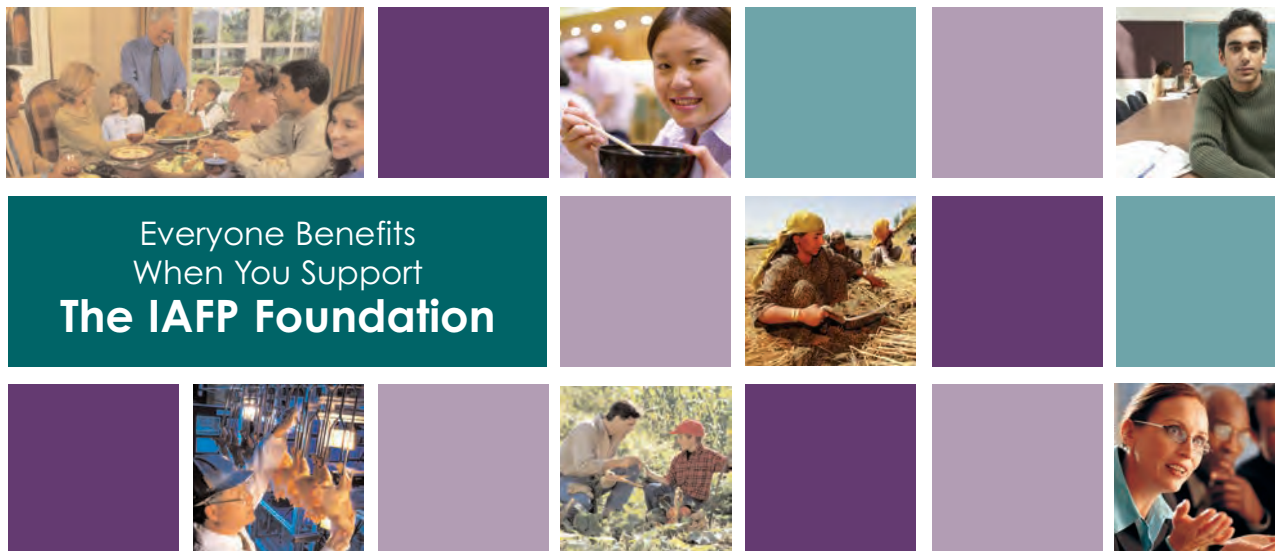
monitor volunteer use of the bags. The chain-referral sampling strategy as the recruitment method may also be a contributor to relaxed compliance and motivation on the part of some of the participants.

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