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Antibacterial efficacy of TiO₂/Ag treated fabrics

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Abstract Shared fabrics may be a source of pathogenic microorganisms that can be transmitted among individuals. Cotton towels used for hand drying become heavily contaminated with enteric bacteria, such as *Escherichia coli*, during household use. Incorporation of a titanium dioxide/silver/hydroxyapatite containing formulation (Earthplus™) was shown to be effective both in the laboratory and households in controlling the growth of gram negative bacteria in cotton towels.

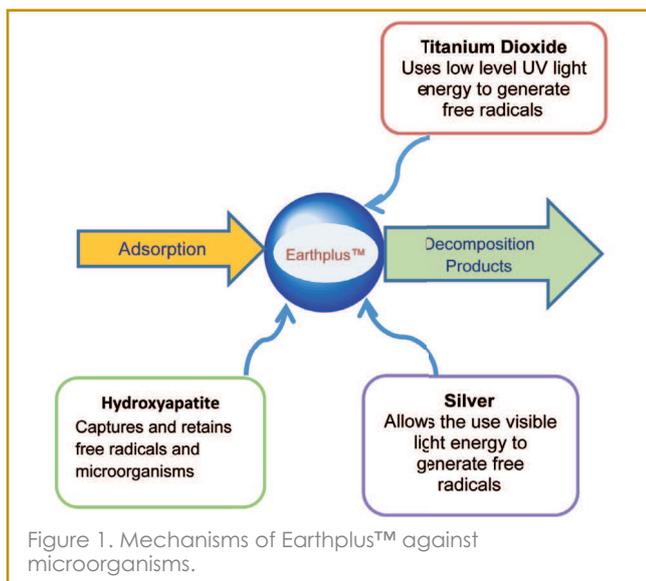
INTRODUCTION

Cotton fabric towels are used in households primarily in the kitchen and bath rooms. Bath towels are usually in two sizes in the bathroom, large bath towels used for drying the body after showering or bathing and smaller towels used for drying the hands or face after washing. Because they may be used multiple times before washing among different individuals the potential exists for the transmission of infectious diseases among users. In a study of 200 homes in the United Kingdom, Scott et al. (1) reported the occurrence of coliform bacteria and *E. coli* in both kitchen and bathroom towels, but their sampling was limited to the surface of the towel the size of a Rodac plate (an agar plate designed for surface sampling), and excluded bacteria present within the towel. Gerba (2) also reported the occurrence of coliform bacteria in large bath towels collected from households in the United States.

Bathroom hand towels have been known or suspected of being involved in the transmission of infectious diseases in households. Sharing of towels has been shown to be associated with an increased risk of blood borne hepatitis B virus (3). Sharing of towels is believed to have caused nosocomial infections of *Bacillus cereus* among hospital patients in Japan (4). Olier and Mitchell (5) found that the more absorbent a cloth towel the longer the survival of *Staphylococcus aureus*. Several studies have found that *Staphylococcus* can survive for 19 to 21 days in cotton fabrics (6). Methicillin resistant strains of *S. aureus* (MRSA) capable of causing serious life threatening infections have been isolated from towels used in a hospital (7) and sharing of towels is believed to be the cause of an outbreak of MRSA among football players (8).

The properties of titanium dioxide as a photocatalyst have been known for some time and have been shown to be

useful to degrade toxic and odour producing chemicals and inactivate microorganisms including algae, fungi, bacteria, viruses and spores (9). It is believed that it uses light energy to produce radicals which result in oxidation of chemicals and components of microorganisms resulting in a loss of infectivity. It is not consumed in the reaction and can be coated or incorporated into surfaces to act as a thin film reactor (10). The photocatalytic reaction primarily depends upon exposure to ultraviolet light (UV), but addition of certain metals such as small amounts of silver can extend the useable energy into the visible light range (11). In addition, incorporation of substances which attach or hold the contaminate near a matrix with TiO₂ results in more efficient use of photon energy because of the short half-life of oxidizing free radicals which are produced (12). Hydroxyapatite-supported Ag-TiO₂ can not only act to attach bacteria to its surface, but may also aid the photocatalytic process by production of active species (13). While laboratory manufactured materials have demonstrated the efficacy of TiO₂ devices and treated surfaces under controlled conditions in the presence of UVA light (9), little information is available on practical applications in consumer products. Recently a hydroxyapatite-binding silver/titanium dioxide (Earth-plus™) has been available for the incorporation into fabrics such as bathroom towels (Figure 1). The TiO₂/Ag act to capture light energy moving electrons to a higher energy state resulting in the production of free radicals when water and oxygen are present. The free radicals act to kill bacteria by first oxidation and denaturalization of proteins and lipids in the cell membrane and wall resulting in rapid leakage of ions from the bacterial cell causing death (9). Eventually the processes lead to the complete destruction of the organism resulting in the formation of carbon dioxide and water. The goal of this study was to assess the effectiveness of this formulation for use in towels to control the occurrence of bacteria.



MATERIALS AND METHODS

Evaluation of fabric

To demonstrate the efficacy of Earthplus™ an overnight culture of *Escherichia coli* strain 25922 was obtained from the American Type Culture Collection (ATCC; Manassas, VA) and was maintained on Tryptic Soy Agar (TSA; Difco, Sparks, MD) with incubation for 18 to 24 hours at 35°C. Prior to the start of each experiment, an Erlenmeyer flask containing 100 ml of Tryptic Soy Broth (TSB; Difco, Sparks, MD) was inoculated with the organism and incubated on an orbital shaker (Model G33; New Brunswick Scientific, Edison, NJ) at 300 rpm at 35°C overnight. After incubation, the *E. coli* were pelleted via centrifugation (9,800 \times g, 15 min, 25°C). The bacteria were then resuspended in 0.05 M phosphate buffered saline and centrifuged again and the bacteria resuspended again in fresh saline buffer. The bacteria in this suspension were then added to cotton fabric cotton towels swatches of 5 by 5 cm in 0.05 ml drops for a total of 100 μ l to yield approximately 10⁵ colony forming units (CFU). Controls consisted of the same fabric without treatment. The treated and untreated fabric was evaluated under different conditions including varying relative humidity, temperature, and light and dark conditions. The textile was placed in the centre of a translucent 100 millimetre petri dish. The samples were placed in their respective conditions and collected after various time points. After the incubation period, the sample was placed into a conical tube with 10 ml of Dey-Engley broth (EMD Chemicals, Gibbstown, NJ) to neutralize the reaction and the tube was vortexed for 10 seconds. Samples were assessed by spread plating on MacConkey media (EMD Chemicals, Gibbstown, NJ). The plates were then incubated at 35°C for 24 hours, and colony forming units (CFU) were quantified. All experiments were performed in triplicate.

Evaluation of towels in the community

To assess the ability of the treated towels to control the growth of bacteria during use in households the occurrence of bacteria in normal untreated household face towels were compared to those treated with Earthplus™. Interviews with

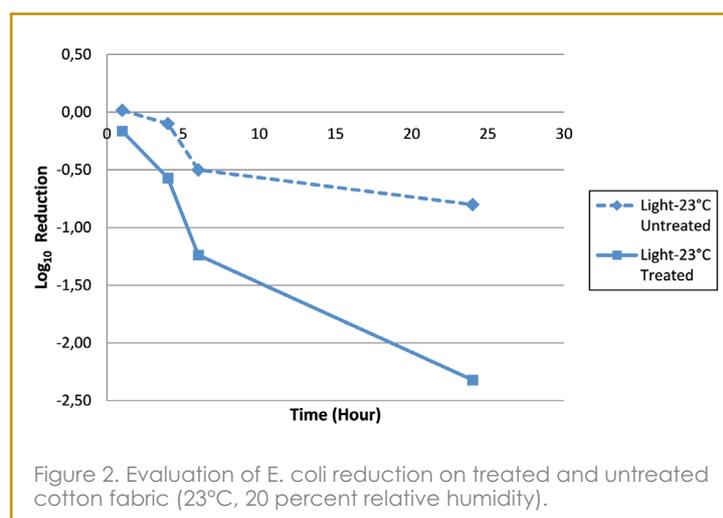
home owners indicated that face towels are used about seven days before they are washed. The average surface area of the face towels was calculated to be about 1000 cm² with a standard deviation of 150 cm². Each collected towel was submerged in peptone broth to extract bacteria. Each towel was placed in a stomacher bag with either 250 or 500 ml of peptone broth based on towel size and material's absorbency to guarantee full soaking of the towel. Each towel was then manually kneaded in the peptone broth until it was completely absorbed by the towel. The peptone broth was extracted from the towel by ringing the liquid out. The extract was assayed on selected media for the isolation of the various bacteria.

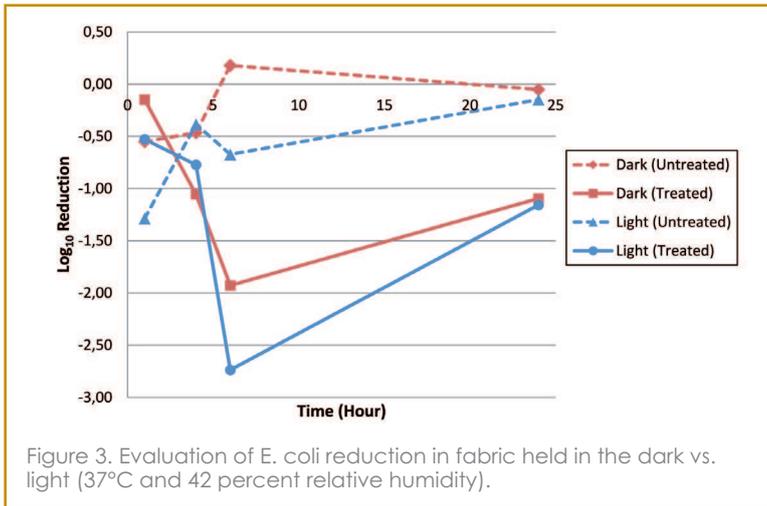
Each towel was tested for total bacteria (heterotrophic bacteria), coliform bacteria, and *Escherichia coli*. Total heterotrophic bacteria were assessed by spread plating on R2A media (Difco, Sparks, MD, USA) and incubated for five days at 25°C. Bacterial colonies were counted after incubation. Coliforms and *E. coli* were assayed using Collert method (IDEXX, Westbrook, ME, USA) and enumerated after incubation at 35°C for 24 hours. A maximum of 100 ml of the towel extract could be assayed by this method. Selected coliforms and presumptive *E. coli* isolates from randomly selected towels were identified by using API bacterial identification test kits 20E (Biomérieux, Marcy Etoile, France). Towels treated with Earthplus™ were supplied to 20 homeowners to replace existing towels and recollected one week later. The process by which Earthplus™ is incorporated into fabrics is described in detail by Kasuga et al., 2011.

RESULTS

Evaluation of fabric

The results shown in Figure 2 demonstrate that *E. coli* declined in numbers far greater in the treated swatches than the untreated swatches over a 24 hour period of time. Additional studies demonstrated that *E. coli* were also reduced when the towels were held in the dark (Figure 3) and that the towels suppress growth of the bacteria at humidity and temperatures optimal for *E. coli* growth. *E. coli* first declined and then increased in numbers in the untreated towels greater than in the treated towels.





Evaluation of towels in households

The numbers of bacteria found in treated and untreated towels collected from households is shown in Table 1. After one week of use the average number of total bacteria detected in the treated towels was 95 percent lower than untreated bath towels. There was a higher concentration of bacteria found in the untreated towels when compared to the treated. No coliform bacteria or *E. coli* was found in the Earthplus™ treated towels.

Towel Type	Total Bacteria	Coliform Bacteria	<i>Escherichia coli</i>
Cotton*	920,200,000	390,000	11,000
Earthplus™ cotton*	480,000	0	0

Table 1. The Average Concentration of bacteria detected in hand towels in treated and untreated correct from homes(CFU/ml)
*Average of 30 towels.

DISCUSSION

Large numbers of total bacteria are found in hand towels, as well as, coliform bacteria and *E. coli*. The moist, high water holding capacity of hand towels results in ideal conditions for the prolonged survival and possible growth of these bacteria. Large numbers of coliforms, *E. coli* and the presence of *Salmonella* have previously been reported in kitchen sponges and towels (14). This has been attributed to high moisture holding capability and the presence of degradable organics from use in the kitchen area. Bathroom towels appear to fall into this same category. The presence of enteric bacteria in the towels suggests they could serve as vectors for enteric bacteria and possibly enteric viruses in households if the towels are shared. The common occurrence of large numbers of bacteria, coliform and *E. coli* in household hand towels suggests long term persistence and possibly growth. Similar to kitchen dish cloths, towels are designed to hold moisture, which encourages bacterial survival and creates conditions for enough moisture for growth of bacteria. The high moisture content will also encourage the persistence of

enteric and possibly viruses. The incorporation of Earthplus™ into hand towels were shown to result in the more rapid decline of the enteric bacterium *E. coli* and prevent its growth. In addition, treated towels placed in households were found to not only harbour fewer bacteria than normal towels, but no coliform or *E. coli* bacteria were detected. Kasuga et al. (15) reported the rapid decline of *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by Earthplus woven cotton and polypropylene fabrics. They reported that less than 0.2 of silver is leached during washing, which is below the 50 µg need for effective antimicrobial activity. The primary purpose of the silver is to extend the ability to produce free radicals in the visible light range. Earthplus™ also exhibits antimicrobial activity in the dark, although the rate of killing is slower (16). The treated fabrics have also been reported to retain their antimicrobial properties after repeated washings and use (15). Incorporation of antimicrobials into fabrics can help reduce the probability of transfer of pathogenic microorganisms among individuals in households and healthcare facilities. Earthplus™ was shown to perform well both in laboratory tests and in the household under actual use conditions by consumers.

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