ANTIBACTERIAL PROPERTIES AND DRYING EFFECTS OF FLAX DENIM AND ANTIBACTERIAL PROPERTIES OF NONWOVEN FLAX FABRIC

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A modification of "AATCC Test Method 100-1999" was used for assaying for bacteriostatic/antibacterial properties of denim containing various flax concentrations. Since no direct evidence that increasing the flax content of fabric imparted the fabric with increased bacteriostatic properties was found against the control bacteria, *Staphylococcus aureus* and *Klebsiella pneumoniae*, other possible explanations for the long held presumption that flax fabric exhibited antibacterial properties was sought. Because the appearance of having antibacterial or bacteriostatic properties might be imitated if the flax content would decrease the time fabric would be moist enough for bacterial growth, the effect of drying was evaluated. When flax fabric was saturated and the moisture lost during incubation was measured, there was no improved drying associated with increased flax content. When untreated nonwoven flax was evaluated as possibly containing more 'antibacterial' or bacteriostatic components than scoured nonwoven flax material, the population densities increased. This increase suggests that the unscoured nonwoven flax contain components that support bacterial growth to the extent that bacteriostatic or antibacterial components, if any, are overwhelmed by the components that support bacterial growth. In tests involving the control bacteria, *Staphylococcus aureus* and *Klebsiella pneumoniae*, increasing the flax content of flax fabric did not demonstrate increased antibacterial properties.

Keywords: Flax; Antibacterial; Bacteriostatic; Fiber quality

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INTRODUCTION

The "AATCC Test Method 100-1999, Antibacterial Finishes on Textile Materials: Assessment of" (American Association Textile Chemists Colorists 1999) was chosen as the basic protocol for assaying bacteriostatic/antibacterial properties. The test method was modified so we could assay for bacteriostatic/antibacterial properties of denim containing various flax concentrations as well as other textile materials. Even though we could not find an in-depth report which establishes that flax possesses bacteriostatic/antibacterial properties, this claim is made widely in the textile industry (Anonymous 2005, 2006) and has even found its way into scientific publications (Cierpucha et al. 1997, 2004). In an earlier study the authors found evidence

contradicting this property of flax, that adding flax to denim failed to provide any bacteriostatic properties against the assay challenge bacteria, *Staphylococcus aureus* or *Klebsiella pneumoniae* (Chun et al. 2006). This was opposed to the long held presumption that flax was bacteriostatic or antibacterial. Lignin is found in flax fibers (Day et al. 2005) and fibers that contain lignin are more resistant to fungi and bacteria than cotton fibers that do not contain lignin (Berkley 1949). Flax can be more resistant to bacterial decomposition (Bose 1952) and more resistant to fungal growth than many other natural fibers (Basu and Bhattacharyya 1951) and bacteriostatic/antibacterial constituents have been observed in flax (Akin et al. 2003; Gamble et al. 2000).

In this paper we report more in-depth study aimed at verifying our earlier findings. Given that these findings are verified, further studies were carried out to possibly explain these discrepancies by looking at excess moisture, enhanced dryability due to addition of flax to blends, and increasing the concentration of flax to 100%. A touted feature of flax fabric is that it is very breathable and provides warm weather comfort. This type of fabric allows moisture from perspiration to readily escape so that flax fabric would not be a breeding ground for bacteria. It has been demonstrated that a significant gain in wicking rate and air permeability occurs with an increase of flax fibers in the fabric (McAlister et al. 2002; Muir and Westcott 2003). If a material were to promote rapid drying to the extent that moisture availability became limiting for bacterial growth or if a material were to promote rapid drying of an already wet fabric to the extent that moisture availability became limiting for bacterial growth, the apparent effect would be a functional antibacterial property as opposed to a purely chemical antibacterial property. Normally in the assay, the textile material and the challenge bacteria are incubated together in a sealed jar to prevent the evaporation and drying of the bacterial inoculum. A set of experiments was conducted where the canning jars were incubated sealed to prevent evaporation, or were incubated in uncovered canning jars. The objective of this study was to leave the jar unsealed, allowing normal evaporation to be simulated using the antibacterial assay for textile fabrics. Another explanation for the earlier results was that antibacterial or bacteriostatic components may have been removed in the processing and scouring process. The antibacterial assay was applied to sheets of felted nonwoven flax made from 100% retted flax and the same felted nonwoven flax following scouring which removes loose and soluble components to determine if antibacterial or bacteriostatic components may have been removed.

EXPERIMENTAL

Protocol for Assaying Antibacterial Property of Textile Materials

The method described in "AATCC Test Method 100-1999, Antibacterial Finishes on Textile Materials: Assessment of" (American Association Textile Chemists Colorists, 1999) was adapted as the protocol to measure the qualitative and quantitative antibacterial tendency of textile materials. Swatches of the textile material were 'inoculated' with the challenge bacteria used in the AATCC Test Method, *Staphylococcus aureus* or *Klebsiella pneumoniae*. The textile material and challenge bacteria were incubated together at 37°C for 24-hours. After incubation, bacteria are

eluted from swatches using known volumes of extraction solution from which the bacterial density was determined by culturing the viable bacterial present in the extraction solution. This was done for each tested textile material. In the AATCC test method, the percent reduction by the treated specimen was calculated and used for comparison. The test method was made more flexible and more efficiently carried out when only the final population densities were compared since the test materials were initially all treated with the same density of bacteria. It was also found that after incubation, the population densities instead of decreasing were often the same as the starting population density or had actually increased. The treatments were different flax fiber fabric compositions of increasing proportions of flax content. A change in population densities proportional to the flax content was expected if flax fiber contained antibacterial properties.

Flax Denim Treatments

Denim cloths were constructed to contain different flax content, and each cloth of different flax content was considered as a treatment. Yarn consisting of 100% cotton was used in the warp direction. For the weft, yarn consisting of 100%, 75%, 50%, and 25% cotton fiber with flax as the other fiber, respectively, was used The four resulting cloths contained 0%, 9.6%, 19.6%, and 27.6% flax in the fabric, respectively. Each of the fabric compositions, 0%, 9.6%, 19.6%, and 27.6% flax, was considered as a separate treatment for a total of four treatments. Before testing, fabrics were cleaned by scouring (McAlister 1994), and then cut into square swatches (18.1 cm^2, 4.25 cm on a side) and placed in small autoclave polypropylene bags before being sterilized in an autoclave (10 minutes @ 121°C, followed by a 30 minute drying cycle). Four swatches were used for each treatment replicate sample.

Bacterial Species Used for the Assay

As recommended by the AATCC Test Method 100-1999, the two bacterial species used throughout were: *Staphylococcus aureus*, American Type Culture Collection No. 6538 (a Gram-positive organism), and *Klebsiella pneumoniae*, American Type Culture Collection No. 4352 (a Gram-negative organism). Stock cultures of these bacteria were maintained on Difco Brain Heart Infusion Agar slants (Difco Brain Heart Infusion Agar, Man #237500). The stock cultures were transferred once every 3 to 4 weeks by incubating a freshly inoculated slant at 37°±2°C for 2 days before storing at 5°±1°C.

The bacteria were incubated either in a trypticase soy broth (TSB, BBL® No. 11768 Trypticase Soy Broth) or on trypticase soy agar slants (TSA, BBL® No. 11768 Trypticase Soy Broth, and 2.0% agar) at 37°±2°C for 1-3 days before being used to inoculate the broth (TSB) used for the assay. The bacteria were cultured as broth cultures in a shaker incubator (37°±2°C and 300 rpm) for 24 hr. At the end of incubation, the broth cultures were placed in an ice bath and held until chilled.

The chilled cultures were then diluted with TSB to a pre-determined turbidity to provide approximately 2 x 10^9 CFU/ml (colony forming units per milliliter) or 2 x 10^8 CFU/ml to get a known starting density of bacteria which was diluted further to get a standardized density of bacteria for inoculation. The turbidity was measured at 500 nm on a Beckman DU-7 Spectrophotometer (Beckman Instruments, Inc., Irvine CA 92713)

using chilled TSB from the same batch, as the cultures were grown in, to zero the instrument and to dilute the broth cultures to the desired turbidity. Then the diluted broth cultures were serially diluted with chilled diluent (Chun and Perkins, 1996) for a final approximate bacterial density of 1-2 x 10^5 CFU/ml. As used in-house, the diluent normally contains both Tween®-80 (T-164, Fisher Scientific company, Fair Lawn, New Jersey), a nonionic surfactant, and 0.01% gelatin. For this study, both the surfactant and the gelatin were omitted at this stage; when used for the extraction stage and for serial dilutions to determine bacterial population density, the gelatin was retained in the diluent. The bacterial suspensions were kept in an ice bath. A magnetic stirring bar and stirring plate was used to keep the bacteria suspended during inoculation.

Each fabric treatment sample replicate was inoculated with 1.0±0.1 mL of the bacterial inoculum which was dispersed over the 4 swatches using a Rainin EDP-Plus Electronic Pipette (RAININ Instrument Co., Inc., Woburn, MA), 25 10-µL droplets/swatch. The swatches were inoculated while in pre-sterilized 237 ml (half pint) canning jars (Mason or Kerr Brand, locally obtained), one swatch at a time. The band and lid of the canning jar were screwed on the jar to prevent evaporation or left off depending on the experimental protocol. After all the treatment sample replicates had been inoculated, the jars were either incubated at 37°±2°C for 24 hours before being assayed for bacterial population density or immediately assayed for bacterial population density as the zero-time population density.

The bacterial population density was determined by extracting the bacteria from the test swatches and then by culturing the viable bacterial present in the extraction solution. The bacteria were extracted from the swatches by adding 100 mL of diluent to each jar and shaking the jars on a tabletop shaker for one minute. Then aliquots were removed and plated directly into Petri dishes or further diluted before being plated. The pour plate method (Chun and Perkins, 1996) was used to determine the bacterial density. No antibiotics were used and incubation was at 37°±2°C for at least 24 hours before the plates were counted.

Testing the Drying Effect of Flax on Antibacterial Property

If a material were to promote rapid drying to the extent that moisture availability became limiting for bacterial growth, this would suggest that the material exhibited functional antibacterial property as opposed to a purely chemical antibacterial property. To see if this may be the case with adding flax fibers to fabric, a set of experiments was conducted where the canning jars were incubated sealed to prevent evaporation, or were incubated in uncovered canning jars. This was done because normally the antibacterial assay attempts to keep conditions optimal to support bacterial growth, which included keeping moisture optimal for growth. The four fabric treatments were challenged first with the *Klebsiella pneumoniae* bacteria and then by the *Staphylococcus aureus* bacteria -- 4 sample replicates per fabric treatment for a total of 64 samples for each test; two tests for each of the two challenge bacteria, for a total of 256 samples. The fabric treatment applications were completely randomized.

Testing the Drying Effect of Flax on Wet Fabric

In the previous test, the fabric was dry except for the added moisture used as the carrier for the bacteria. In that case, the amount of moisture was low and the fabric could have returned to a dry state quickly and perhaps may have obscured the effect of flax to enhance drying. So another test was set up using swatches saturated with moisture to prolong moisture availability during the drying period. Likewise, if a material were to promote rapid drying of an already wet fabric to the extent that moisture availability became limiting for bacterial growth, this would suggest that the material exhibited functional antibacterial property as opposed to a purely chemical antibacterial property. To see if this may be the case with adding flax fibers to fabric, a set of experiments was conducted where the canning jars were incubated sealed to prevent evaporation, or were incubated in uncovered canning jars. The four fabric treatments were tested using first the *Klebsiella pneumoniae* bacteria and then the *Staphylococcus aureus* bacteria. In both tests, 4 sample replicates per fabric treatment was used for a total of 64 samples per test. The fabric treatment applications were completely randomized. One test was done for each of the two bacteria. In this experiment, after all the samples had been inoculated but before the jars were either incubated at 37°±2°C for 24 hours or immediately assayed for bacterial population density, 1.0 ml of diluent (without surfactant or gelatin) was added to the swatches in each jar to simulate wet saturated fabric.

Testing the Drying Effect of Flax

The influence of flax as a component of the fabric on the rate of drying was observed by weighing wet fabric over time. The four flax fabric treatments were conditioned for several days (20°C and 65%RH) at the start. Then 4 swatches of each of the treatment fabric were placed in canning jars. Then 1.0 ml of deionized water was added to the swatches in each jar. The jars were then incubated uncovered, but were covered during the time the jars were removed from incubation to be weighed on an hourly basis. One set of jars was incubated at 37°±2°C and a second set at room temperature (20°±2°C) on the laboratory counter. Each set consisting of the four treatment fabrics. This test was done twice for each incubation temperature, and the results from each incubation temperature combined.

Testing for Antibacterial Properties in Nonwoven Flax

Sheets of felted nonwoven flax made from retted flax which had not been scoured, bleached, or washed were tested for antibacterial properties to see if antibacterial properties could be detected. This nonwoven flax material was approximately 0.6 cm thick, but not uniform in thickness. Some sheets were scoured (McAlister 1994) and then dried. These sheets were cut into square swatches (18.1 cm^2, 4.25 cm on a side). Because these scoured sheets were wrinkled and had to be smoothed over by ironing before cutting, both unscoured nonwoven flax sheets and the scoured nonwoven flax sheets were ironed before being cut into square swatches (18.1 cm^2, 4.25 cm on a side). A household cloth iron was used at a cotton setting, where the temperature fluctuated from 85-97.8°C; and contact with the swatches was kept to a minimum. The scoured and unscoured flax swatches were sterilized before testing (10 minutes @ 121°C, followed by a drying cycle).

The nonwoven flax treatments were assayed as described, except that instead of four swatches per treatment replicate sample, only a single nonwoven flax swatch was used because of their thickness. The inoculum was dispersed over each swatch as 25 40-μL droplets. The treatments consisted of scoured nonwoven flax swatches, untreated nonwoven flax swatches (the unscoured nonwoven flax swatches) and a control. The control was the inoculum placed in jars with no swatches. For each treatment, 4 replicate samples were used. The population density was determined as before after the 24-hr incubation at 37°±2°C.

RESULTS AND DISCUSSION

In the drying effect test of flax, the starting bacterial levels of both challenge bacteria were about the same and did not differ significantly (Table 1 and Fig. 1). In the covered jars which were not permitted to dry, the bacterial densities of both challenge bacteria were higher than their starting population densities after 24-hours of incubation, and these higher densities were usually significantly higher (Fig. 1). For the most part, the differences between population densities were not significantly different for the different flax fabric treatments; except for the *Klebsiella pneumoniae* challenge, but no trend in regard to flax fabric content was observed (Table 1). In the jars incubated without lids, the swatches dried during 24-hr incubation. The population densities of both challenge bacteria in the uncovered jars were generally statistically significantly lower than in the covered jars (Fig. 1); however, the population densities were not significantly different for the *Staphylococcus aureus*, and the significant differences in regard to the *Klebsiella pneumoniae* challenge did not follow any trend related to the flax fabric treatments (Table 1). With the *Klebsiella pneumoniae* challenge, while the 24-hr uncovered population density showed a decreasing population density trend with fabric containing increasing flax, the overlapping error bars of the two high flax fabric content treatments indicates that the population densities are not significantly different from the controls (Fig. 1). The *Staphylococcus aureus* challenge appear to be less sensitive than the *Klebsiella pneumoniae* challenge to drying and did not show any statistically significant trend with increasing flax content and what trend was shown for the bacterial density to be lower with increasing flax content was weak in the uncovered jars (Table 1 and Fig. 1). As expected, the population densities showed more variability after 24-hr incubation for both challenge bacteria whether covered or not (Fig. 1).

In the test where the fabric was already saturated with moisture and incubated with the challenge bacteria, at the zero time incubation, the approximate population densities of both bacterial challenges were about the same as in the tests where the swatches were not saturated with liquid (Tables 1 and 2). But after the 24-hours of incubation, the average population densities of the saturated swatches were higher (Table 2) than swatches that were not saturated with liquid (Table 1).

Table 1. Average Population Density [Log_{10}(CFU+1)/ml] at Start and End of 24 Hour Incubation for Flax Fabric Treatments, Uncovered and Covered to Reduce Drying

Staphylococcus aureus	Zero Time[1,2]		24-hr[1,2]	
Flax Fabric Blend, %	No Lids	Lids	No Lids	Lids
27.6	4.373 A	4.234 A	3.810 A	4.748 A
19.6	4.379 A	4.415 A	3.928 A	5.762 A
9.6	4.423 A	4.405 A	4.159 A	5.604 A
0.0	4.553 A	4.412 A	4.098 A	5.751 A
Klebsiella pneumoniae	Zero Time[1,2]		24-hr[1,2]	
Flax Fabric Blend, %	No Lids	Lids	No Lids	Lids
27.6	4.537 A	4.542 A	0.598 B	5.052 B
19.6	4.485 A	4.462 A	2.084 A	6.684 AB
9.6	3.945 A	4.551 A	2.551 A	7.231 A
0.0	4.572 A	4.509 A	0.598 B	5.396 B

[1]Stating inoculums made from broth cultures diluted to provide ~1-2 x 10^9 or ~1-2 x 10^8 CFU/ml, based on turbidity at OD_{500}, which were then serially diluted to a final ~1-2 x 10^5 CFU/ml densities.
[2]Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

Table 2. Average Population Density [Log_{10}(CFU+1)/ml] at Start and End of 24 Hour Incubation for Saturated Flax Fabric Treatments, Uncovered and Covered to Reduce Drying

Staphylococcus aureus	Zero Time[1,2]		24-hr[1,2]	
Flax Fabric Blend, %	No Lids	Lids	No Lids	Lids
27.6	4.269 B	4.357 A	4.666 A	5.947 B
19.6	4.276 B	4.445 A	3.861 A	6.999 A
9.6	4.312 B	4.517 A	3.981 A	6.977 A
0.0	4.685 A	4.807 A	5.089 A	7.545 A
Klebsiella pneumoniae	Zero Time[1,2]		24-hr[1,2]	
Flax Fabric Blend, %	No Lids	Lids	No Lids	Lids
27.6	4.767 A	4.987 A	2.242 A	7.972 A
19.6	4.648 A	4.633 B	1.195 A	8.032 A
9.6	4.736 A	4.817 AB	2.176 A	8.317 A
0.0	4.826 A	4.858 AB	1.227 A	8.120 A

[1]Stating inoculums made from broth cultures diluted to provide ~1-2 x 10^9 or ~1-2 x 10^8 CFU/ml, based on turbidity at OD_{500}, which were then serially diluted to a final ~1-2 x 10^5 CFU/ml densities.
[2]Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

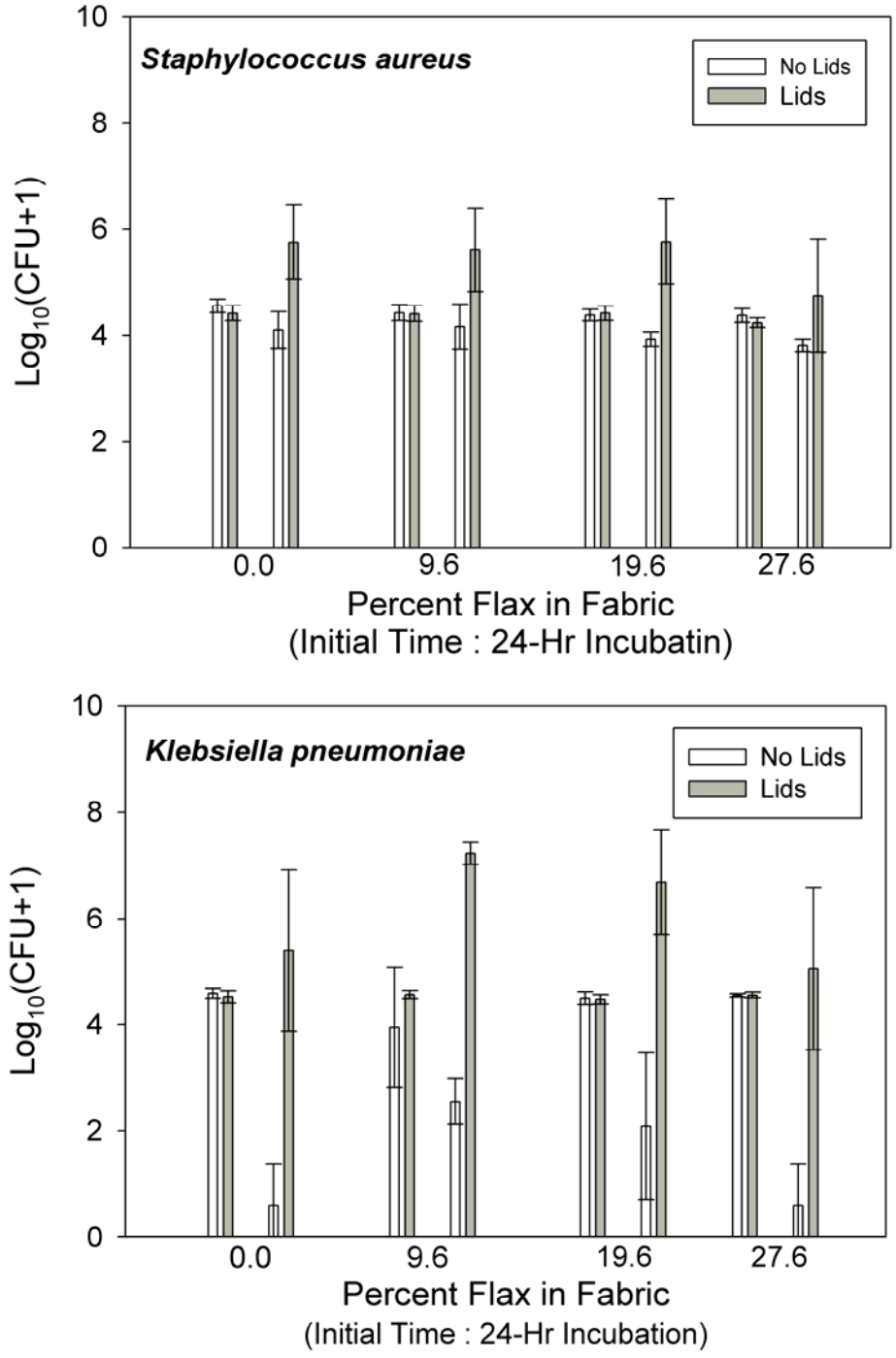

Fig. 1. Average Population Densities at start (bars on left) and the end of 24 hours of incubation (bars on right) of the uncovered (no lids) and the covered jars (Lids). Each half-error bar represents 2 s.e.

This is understandable in that the time for the saturated swatches to become dry enough to affect the survivability of the challenge bacteria in the uncovered jars was probably longer, and the additional moisture in the covered jars was probably more conducive for bacterial growth and survivability. With both challenge bacteria and after 24-hr incubation, the population densities in the covered jars were all statistically significantly higher than their corresponding starting population densities (Fig. 2); but the 24-hr population densities were not significantly different in the covered or the uncovered jars for the different flax fiber treatments, except for the 27.6% flax fabric treatment and *Staphylococcus aureus* challenge (Table 2). Even so, no strong decreasing trend with flax content was shown (Table 2 and Fig. 2). This time there were more differences in the initial population densities for both challenge bacteria (Table 2). This is probably because even though the zero time samples were assayed before incubation began, a time lag existed between the time of inoculation and the actual assay for the bacterial population, caused by the additional time needed to add the additional saturation liquid, which may have been enough to exaggerate differences between the populations. Still, the differences were less than half an order of magnitude for the zero time samples, whether covered or uncovered, for both bacterial challenges (Table 2). As before, after the 24-hours of incubation, most of the populations decreased in the uncovered jars, with the *Klebsiella pneumoniae* challenge being more sensitive to drying than the *Staphylococcus aureus* challenge (Table 2). Even though the population densities were generally lower in the uncovered jars, the population densities of *Staphylococcus aureus* after the 24-hr incubation were not significantly lower than their corresponding initial population density (Fig. 2). This was not the case with *Klebsiella pneumoniae*, where the uncovered population densities after the 24-hr incubation were significantly lower than the corresponding starting population density (Fig. 2). But the population densities of both challenge bacteria for the flax treatments in the uncovered jars were not significantly different, nor did they show a decreasing trend associated with increasing flax content (Table 2 and Fig. 2).

During the test for antibacterial properties, occasional small non-significant trends associated with increased flax with lower bacterial densities were observed. This suggested that during the processing/scouring of flax that some surface component or chemical property had been altered/changed/removed which might affect bacterial growth. However, it is possible that in the brief time the nonwoven swatches were ironed to remove wrinkles, the swatches were exposed to a short duration to heat which may have denatured or inactivated potential bacteriostatic components; this possibility has not been addressed in this study. The maximum flax content in the test swatches was only 27.6%, which may not have contained a high enough content of antibacterial compound(s) to effectively exhibit bacteriostatic properties. To avoid low flax fiber levels, a fabric containing 100% flax fiber was tested.

A nonwoven material was tested which potentially contains greater levels of bacteriostatic components. Because fabrics were scoured and antibacterial compounds potentially removed, a scoured and non-scoured comparison was performed. Results were consistent in that the scoured and unscoured flax nonwoven fabric had the same bacterial density or that the unscoured nonwoven flax fabric supported higher population densities (Table 3 and Fig. 3).

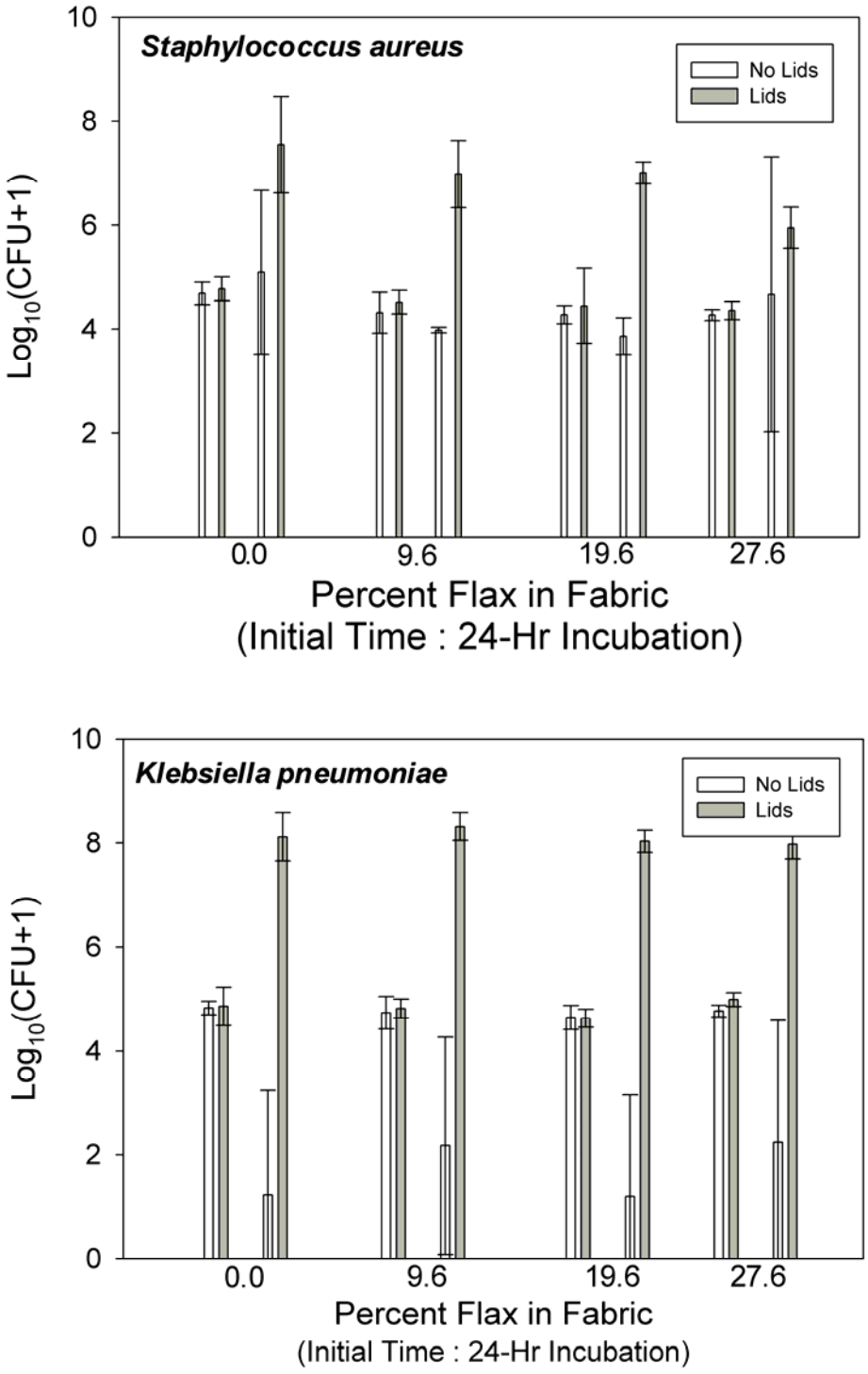

Fig. 2. Average population densities at start (bars on left) and the end of 24 hours of incubation (bars on right) of the uncovered (no lids) and the covered jars (Lids) using wet fabric at the start of the test. Each half-error bar represents 2 s.e.

The nonwoven flax fabrics, both scoured and unscoured, supported statistically significantly higher bacterial population densities than the control jars, and the three treatments all showed significant differences in bacterial population densities. The lower population densities found with the scoured nonwoven flax compared to the unscoured flax can be explained. Instead of assuming that the unscoured nonwoven flax would have more antibacterial material, one could change the assumption to that the scoured nonwoven flax would have had surface components removed that would otherwise support bacterial growth or that the scouring process altered the surface features to become less suitable for bacterial growth. Regardless, the fact that both nonwoven flax fabric treatments exhibited greater population densities than the control suggested that the flax had components in it that supported bacterial growth that overwhelmed the effects, if any, of components that may be bacteriostatic. Thus while components may have been isolated that exhibit bacteriostatic properties from flax (Akin et al. 2003; Gamble et al. 2000), flax as a whole does not appear to suppress the bacterial growth of *Staphylococcus aureus* or *Klebsiella pneumoniae*.

Another possible explanation for flax antibacterial properties is that hollow flax fibers make linen fabrics very absorbent by increased wicking (Muir and Westcott 2003), and increasing flax content can improve the fabric wicking properties (McAlister et al. 2002). Body odor becomes more apparent when perspiration remains on clothing longer to become a breeding ground for bacteria, so the faster perspiration is permitted to escape, the less body odor is expected. This led to measuring the moisture lost at room temperature and at 37°C. At both room temperature and at 37°C incubation, evaporation was observed as decreasing moisture weight of the 1.0 ml deionized water added to the swatches (Fig. 4). The drying rates of the different flax content fabrics did not appear to be visually very different from one another at either temperature, which is in keeping with the observations on flax content and population densities so far. At room temperature, the slopes were -0.09, -0.10, -0.10, and -0.10, for the 0.0%, 9.6%, 19.6%, and 27.6% flax fabrics, respectively. For the 37°C incubation, the slopes were -0.23, -0.13, -0.21, and -0.21, for the 0.0%, 9.6%, 19.6%, and 27.6% flax fabrics, respectively. At room temperature, the swatches did not completely dry out after 8 hours, while for the jars incubated at 37°C, the swatches dried out completely between 2 and 4 hours. Many of the swatches demonstrated a negative weight, indicating that the applied moisture had been lost, as well as the moisture associated with the fabric wet-weight at this elevated temperature. This helped explain the low population densities associated with the uncovered jars during the 24-hour incubation at 37°C (Tables 1 and 2 and Figs. 1 and 2). The approximate 3 hours that it takes for the added moisture to completely evaporate is very likely too short a time for bacterial growth to be substantial, and this rapid moisture removal shortens the time before low moisture availability affects the survival of the bacterial. Future tests should be conducted at room temperature instead of 37°C to study the effect of drying with the challenge bacteria which would have been more realistic of fabric usage, although the current observations did not indicate that this would have changed the results.

Table 3. Average Population Density [Log_{10}(CFU+1)/ml] at 24 Hour Incubation on the Control, Nonwoven Flax and Scoured Nonwoven Flax

Treatment Swatch	Both Challenge Bacteria[1,2]	*Staphylococcus aureus*[1,2]	*Klebsiella pneumoniae*[1,2]
Control, no swatch	6.188[C]	5.162[B]	7.214[B]
Scoured Nonwoven Flax	7.233[B]	6.906[A]	7.561[B]
Nonwoven Flax	7.712[A]	7.330[A]	8.094[A]

[1]Stating inoculums made from broth cultures diluted to provide ~1-2 x 10^9 or ~1-2 x 10^8 CFU/ml, based on turbidity at OD_{500}, which were then serially diluted to a final ~1-2 x 10^5 CFU/ml densities.
[2]Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

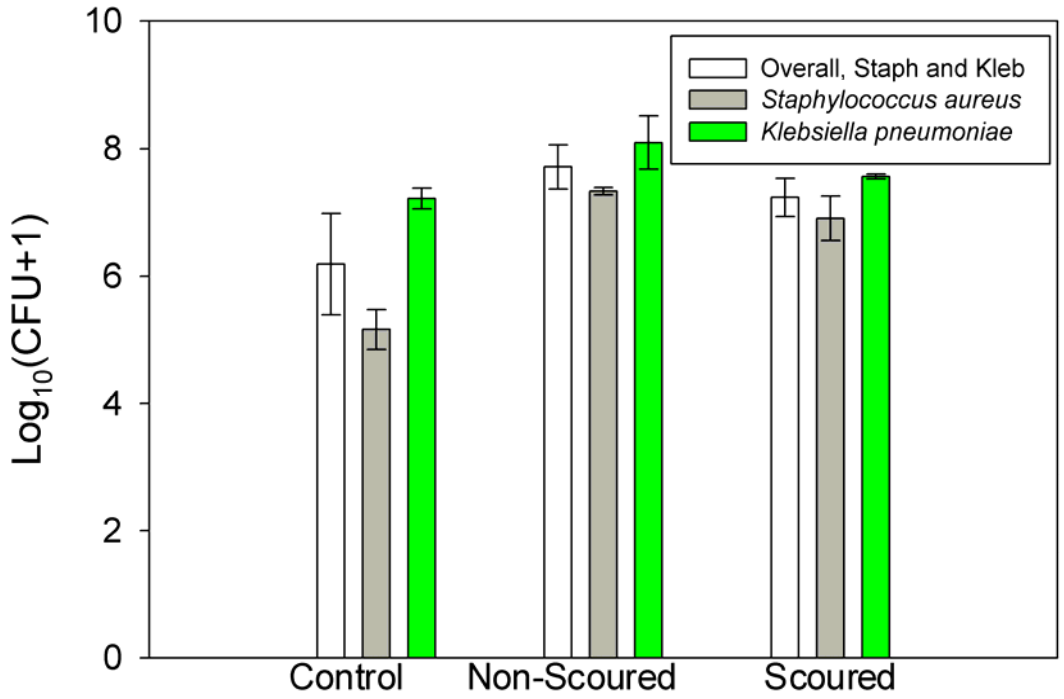

Fig. 3. Average population densities after 24-Hour incubation on the control (no fabric), nonwoven flax fabric and the nonwoven flax fabric that had been scoured. Each half-error bar represents 2 s.e.

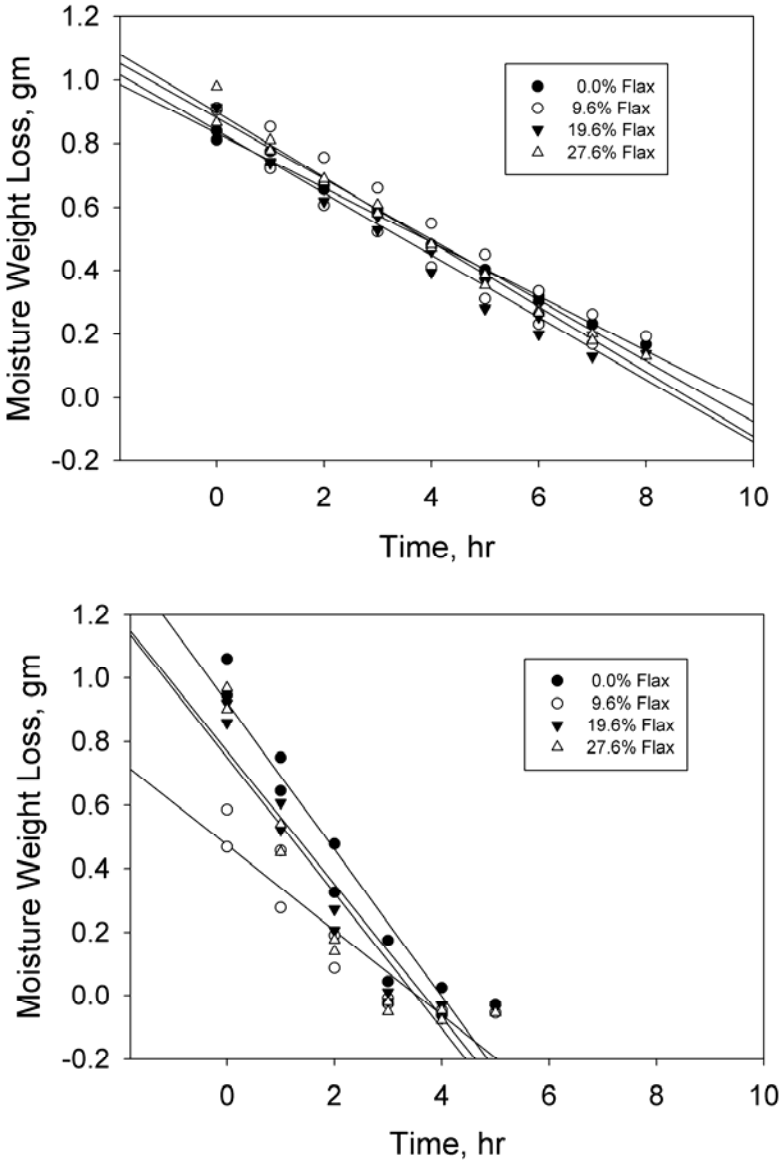

Fig. 4. Drying of the 0%, 9.6%, 19.6% and 27.6% Flax swatches at room temperature (RT, 20°C; top) and at 37°C (bottom).

CONCLUSIONS

In conclusion, earlier studies did not support the long-held belief that flax fabric possessed antibacterial/bacteriostatic properties. So additional studies using the modified AATCC Test Method 100-1999 were conducted to examine other explanations of why flax fabric may appear to have bacteriostatic properties. Drying of flax fabric and drying of already wet flax fabric was tested, since faster drying would make moisture availability limiting for bacterial growth and would give the apparent appearance as a functional antibacterial property. In addition, nonwoven flax was looked at as possibly

containing more 'antibacterial' components than scoured nonwoven flax material. In both studies, the results supported our earlier findings that addition of flax did not impart additional bacteriostatic/antibacterial properties to fabric. No improvement in drying rate was demonstrated by increasing flax content. Nevertheless, drying was found to decrease the population density below that of the original inoculum level, but this was not influenced by the proportion of flax in the fabric. When the flax fabric was saturated and then dried during incubation, there was again no improved drying associated with increased flax content. When nonwoven flax was looked at as possibly containing more 'antibacterial' components than scoured nonwoven flax material, the population density was found to be actually higher, which suggested that unscoured nonwoven flax may contain substrate components that may support bacterial activity more than inhibit it. These studies did not take into account that a step in the processing may have inactivated one or more labile bacteriostatic component in nonwoven flax. The results from this study from a practical stand point did not support the long-held presumption that flax fabric is bacteriostatic or antibacterial against the challenge bacteria, *Staphylococcus aureus* or *Klebsiella pneumoniae*, used in the antibacterial assay.

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