Applied and Environmental Microbiology

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Appl. Environ. Microbiol. **1983, 46(4):817.**

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1983, p. 817–820
0099-2240/83/100817-04$02.00/0
Vol. 46, No. 4

Endotoxins in Baled Cottons and Airborne Dusts in Textile Mills in the People's Republic of China

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Received 17 June 1983/Accepted 2 August 1983

Bulk cotton samples and airborne vertical elutriated cotton dusts were obtained from textile mills in Shanghai, People's Republic of China. Analyses of endotoxin contents revealed that baled cottons which were grown in different countries varied in endotoxin contamination. The two textile mills, which operated at similar overall airborne dust levels, differed markedly in the levels of airborne endotoxins. The data suggest that the biological activity or "toxicity" of airborne cotton dusts may not be correlated directly with gravimetric dust levels.

Gram-negative bacteria and their endotoxins have been shown experimentally to exert profound effects on the functional characteristics and histology of the lungs. Rabbits developed circulating leukopenia and fever within hours after an aerosol challenge with endotoxin, and marked edema and cellular infiltration could be seen in their lungs at 24 h (17). Dyspnea was observed as a major effect in another study of rabbit exposure to aerosolized endotoxins (13), and granulocyte recruitment into the airways of guinea pigs followed aerosol challenge with *Salmonella typhosa* endotoxin (6) and endotoxins from *Escherichia coli* and *Klebsiella oxytoca* (18). In addition, laboratory rabbits developed functional respiratory impairment as demonstrated by marked changes in arterial oxygen tensions after inhalation of endotoxin-containing bacteria (2). The results from animal exposure studies correlate well with clinical signs and symptoms reported to occur in workers who are exposed to airborne endotoxins at work (3, 8).

Cotton dusts, to which a large workforce is exposed worldwide, contain gram-negative bacteria and their endotoxins (11, 13, 14, 16). Correlations between acute pulmonary function decrements in cardroom workers and numbers of gram-negative bacteria in baled cotton provided an improved statistical relationship over the comparison with vertical elutriated gravimetric dust levels (15). A recent study of human subjects who were experimentally exposed to airborne cotton dust in a model cardroom reported that pulmonary function decrements were more closely correlated with endotoxin levels in the airborne dusts than with gravimetric dust concentrations (10). In addition, it was suggested that quantification of airborne endotoxin levels represents a good biological indicator of the "cleanliness" or "potency" of cotton dust (10).

Cottons of various grades (quality) or even cottons of the same grade but grown in different geographic regions within the United States can vary with respect to the endotoxin contamination of their airborne dusts, even when gravimetric dust levels are held constant (10, 12). It is the purpose of this paper, therefore, to report differing levels of gram-negative bacterial endotoxins in cottons which were grown in different countries throughout the world. In addition, we present environmental sampling data for airborne endotoxins in cotton textile mills in which dust control technologies differ significantly from those found in textile mills within the United States.

MATERIALS AND METHODS

Cottons and dusts. Random grab samples of bulk cottons were taken from bales in the opening rooms of two textile mills in Shanghai, People's Republic of China. The samples were taken from bales which originated in different countries or areas in the world and were being processed at the two mills. Vertical elutriators were operated in various work areas of the same textile mills. Environmental sampling was performed in accordance with National Institute for Occupational Safety and Health recommended guidelines (9), with the exception that the height of the vertical elutriator was lowered from the recommended 1.50 m to between 1.35 and 1.40 m to compensate for the slightly shorter workforce. Polyvinyl chloride filters (37 mm; 5-μm pore size; Millipore Corp., Bedford, Mass.) were used to collect the elutriated dust.

Endotoxin analyses. Samples of baled cottons were received in the laboratory in the United States in sealed plastic bags. Samples of the cottons (206 to 281 mg) were extracted in 10 ml of sterile nonpyrogenic water (Travenol Laboratories, Inc., Deerfield, Ill.) by rocking at room temperature for 60 min. The insides of unused plastic bags were washed with 10 ml of water for 60 min at room temperature and served as controls. Filters from the air sampling studies were wrapped individually dust-face down in Parafilm M laboratory film (American Can Co., Greenwich, Conn.). Filters and Parafilm M wrappers were extracted separately with 10 ml of sterile, nonpyrogenic water in a manner similar to that of the baled cottons. Blank (unused) filters and folded unused Parafilm M were used as controls and treated similarly. Sterile, nonpyrogenic plasticware was used throughout the assays. All extract fluids were centrifuged at 1,000 × *g* for 10 min, and the gram-negative bacterial endotoxin levels in the supernatant fluids were quantified in duplicate by a spectrophotometric modification of the *Limulus* amoebocyte lysate gel test (Pyrostat; Millipore Corp.). Results were analyzed by linear regression, compared to a standard curve, and reported in nanograms of United States reference endotoxin. The results of each filter and its respective Parafilm M wrapper were added together and reported as a single value for that sample.

RESULTS

Analyses of the contents of baled cottons randomly grabbed in two different mills and grown in different countries and areas showed marked differences in endotoxin contamination (Table 1). The cotton with the least amount of endotoxins was obtained from Morocco (8.1 ng/mg), whereas the sample with the highest content came from cotton grown in rural counties adjacent to Shanghai (6,159.8 ng/mg). In both mills, the cottons which were obtained from areas around Shanghai contained the highest levels of endotoxins.

Airborne dust samples were assayed for the presence of endotoxins. This sampling represents the actual working environments of various functional areas within the two cotton textile mills. Table 2 presents the gravimetric dust concentration as measured by the vertical elutriator, as well as the endotoxin concentration in the dust and air at various work areas. The airborne elutriated dust levels ranged from 0.53 to 1.56 mg/m^3 in mill 1 and 0.55 to 1.54 mg/m^3 in mill 2. Mean dust concentrations in the various work areas within each textile mill reflected overall mill mean (± standard error of the mean [SEM]) of 1.07 ± 0.23 mg/m^3 in mill 1 and 1.01 ± 0.24 mg/m^3 in mill 2, showing that the elutriated dust concentrations at both mills were similar when different work areas were studied. The endotoxin contamination of the dusts differed greatly, however. Dusts from mill 1 contained 165.7 to 459.2 ng of U.S. reference endotoxin per mg of dust, whereas the range in mill 2 was 4.3 to 163.2 ng/mg. Overall mean (± SEM) levels of endotoxins in the airborne dusts from each mill were 322.3 ± 62.2 and 90.3 ± 33.1 ng/mg in mills 1 and 2, respectively. Consequently, a greater than threefold difference in airborne concentration of endotoxins existed. Mill 1 airborne levels of endotoxins ranged from 103.2 to 535.8 ng/m^3 (mean ± SEM, 331.5 ± 83.0 ng/m^3), and airborne levels in mill 2 ranged from 2.1 to 220.8 ng/m^3 (mean ± SEM, 100.5 ± 45.7 ng/m^3). The carding and cleaning areas were the most contaminated with endotoxins in both mills.

Analysis of unused plastic bag wash, unused filters, and Parafilm M resulted in levels of endotoxin in the supernatant fluids which were below the detectable range of the test.

TABLE 1. Endotoxin concentrations in baled cottons from various sources

Mill	Source	U.S. reference endotoxin (ng/mg)[a]
1	United States	28.1
1	Sudan	120.1
1	Pakistan	2,719.9
1	China, outside Shanghai area	4,222.5
1	China, Shanghai suburb	6,159.8
2	Morocco	8.1
2	Egypt	113.5
2	China, outside Shanghai area (Xinjiang region)	413.5
2	China, Shanghai suburb	1,704.0

[a] Mean of duplicate value.

DISCUSSION

Studies of cottons which were grown in distinctly different geographic areas within the United States demonstrated previously that the cottons differed with respect to the endotoxin contents of their airborne dusts (10, 12). Likewise, as reported here, cottons which were obtained from different countries showed wide differences in their respective concentrations of endotoxins. Different strains or types of cotton are probably grown in the various growing areas in the different countries, and one might speculate that the growth temperatures and conditions such as irrigation, rain, moisture, and soils might predispose the resulting cotton to different quantities and different types of bacterial infestation. It is suggested by Table 1 that the differences in endotoxins do not result from the apparently longer shipment of the baled cotton. The most highly contaminated cottons were those which were obtained from the local Shanghai areas, and not those which traveled greater distances. It must be noted that the randomly grabbed samples which we collected and analyzed may not be reflective of the entire bale of cotton. However, our sampling was entirely without prejudice or knowledge of the contamination.

TABLE 2. Airborne dust and endotoxin concentrations in various work areas within two textile mills

Mill and area (no.)	Dust level (mg/m^3)[a]	U.S. reference endotoxin	
		Dust (ng/mg)[a]	Air (ng/m^3)[a]
1, opening (8)	1.56 ± 0.19	198.0 ± 65.4	232.4 ± 38.4
1, cleaning (3)	1.35 ± 0.27	340.5 ± 80.5	498.1 ± 216.6
1, carding (4)	1.37 ± 0.39	448.0 ± 82.7	535.8 ± 82.3
1, drawing (4)	0.54 ± 0.17	165.7 ± 40.0	103.2 ± 48.9
1, roving (4)	0.53 ± 0.04	459.2 ± 197.5	288.2 ± 87.3
2, opening-cleaning (4)	1.30 ± 0.20	84.2 ± 19.8	108.1 ± 29.2
2, carding (9)	1.54 ± 0.20	163.2 ± 37.5	220.8 ± 55.4
2, combing (6)	0.66 ± 0.07	109.4 ± 8.6	71.1 ± 8.4
2, fine spinning (7)	0.55 ± 0.07	4.3 ± 0.8	2.1 ± 0.3

[a] Mean ± standard error for elutriated dust.

Therefore, our samples should be considered as "representative" of the cottons we studied.

The randomly grabbed samples of baled cottons were taken approximately midway through the environmental study of the two mills. Both mills were similar in their general operations, although the fine spinning operation was studied in mill 2 only, and roving was studied in mill 1 only. The overall airborne concentrations of vertical elutriated dusts were remarkably similar in both mills (1.07 and 1.01 mg/m^3). The 8-h time-weighted average of vertical elutriated cotton dust in yarn preparation areas is limited by law in the United States to 200 μg/m^3 (5). Although our data do not present the time-weighted averages for the vertical elutriated cotton dusts in the two textile mills, it can be observed readily from Table 2 that the dust levels which we report are approximately fivefold greater than the legal standard in the United States. The concentrations of airborne dusts in both mills, although similar in magnitude, differ greatly in their levels of gram-negative bacterial endotoxins. In agreement, therefore, with the results reported in a previous study of airborne endotoxins in a model cotton cardroom (12), the biological activity of cotton dusts as measured by *Limulus* amoebocyte lysate gelation can vary even when gravimetric dust levels are held constant. Airborne levels of biologically active endotoxins varied over threefold between two different textile mills in Shanghai. Mill 1, which had the most contaminated baled cottons, had the higher overall airborne concentrations of endotoxins. Within each mill, job areas/functions likewise were associated with different levels of airborne endotoxins. There is a 5-fold difference in airborne endotoxin concentration between drawing and carding in mill 1, but only a 2.5-fold difference in mean dust concentration exists. Similarly, a 105-fold difference exists between airborne endotoxins in fine spinning and carding operations in mill 2, with only a 2.8-fold difference in airborne dust concentration, as measured by the vertical elutriator. It should be noted that substances such as gram-positive cell walls (7), certain proteins and polynucleotides (4), and pyrogenic exotoxins (1) may react nonspecifically with the *Limulus* amoebocyte gel test. However, the levels we observed are of such magnitude and range as to lessen the contribution of potential nonspecific reactivity in our tests.

In conclusion, the data from this study confirm on a worldwide scale the observations obtained from regional studies in the United States (10, 12) that cottons obtained from different growing areas can differ in their endotoxin contamination. Additionally, at relatively similar overall airborne dust concentrations, the airborne endotoxin levels varied between two textile mills, and the mill with the more contaminated baled cotton had the greater airborne concentration of endotoxins. Finally, intramill variations in airborne endotoxin concentrations were not associated with vertical elutriated dust concentrations. The data suggest, therefore, that potential biological activity or "toxicity" of cotton dusts may not be correlated directly with airborne gravimetric dust levels but should be examined separately.

ACKNOWLEDGMENTS

We thank Beverly J. Wilhelm for helping us prepare the manuscript. We also thank the workers and staff of the Shanghai numbers 1 and 2 cotton textile mills for their cooperation.

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