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RESPONSE OF FURNITURE FACTORY WORKERS TO WORK-RELATED AIRBORNE ALLERGENS

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Abstract: The aim of this work was to determine the reactivity of furniture factory workers to microbial allergens associated with wood dust. Allergological examinations by skin and precipitin tests were performed in 48 workers employed in a factory producing furniture from fibreboards and chipboards, and in 32 healthy urban dwellers not exposed to organic dusts (referents). The skin test was performed by the intradermal method with the saline extracts of the cultures of 3 microbial species (*Rahnella* sp., *Arthrobacter globiformis*, *Aspergillus fumigatus*) associated with wood dust. Skin reactions were recorded after 20 minutes, 8 hours and 24 hours and graded 1–4, depending on the diameter of the reaction. The agar-gel test for the presence of precipitins in serum was performed with the extracts of 15 microbial isolates. The furniture factory workers showed a high skin response to the extracts of environmental microbes. The frequency of early grade 2 reactions (diameter 10 mm) to the extract of *Rahnella* sp. was 64.6% among furniture workers, being significantly higher ($p < 0.001$) compared to reference group (18.7%). High frequencies of grade 2 reactions in furniture workers were also found with the extracts of *A. globiformis* and *A. fumigatus* (52.1% and 62.5%, respectively). The frequencies of grade 2 delayed (after 8 h) and late (after 24 h) reactions to *Rahnella* sp. in furniture workers were non-specifically high (97.9%/93.7%) while the response rates to *A. globiformis* and *A. fumigatus* were much lower (10.4%/25.0%, and 4.2%/37.5%, respectively). In agar-gel test for detection of precipitins, in most cases very low percentages of positive reactions (0–2.1%) were noted in furniture factory workers. The only exception was a high percentage of positive reactions (27.1%) to the antigen of *Pseudomonas maltophilia*, which was significantly greater in furniture workers compared to the reference group ($p < 0.01$). The obtained results suggest that early allergic reactions to microorganisms associated with wood dust are common among workers of furniture industry, which may increase a potential risk of work-related disease in this occupational group.

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INTRODUCTION

Workers of furniture industry could be exposed to various allergenic, immunotoxic and cancerogenic substances originating from wood itself (e.g. resin acids, monoterpenes), from bacteria and fungi associated with wood, and from chemicals used in the production processes (glues, dyes, varnishes, solvents, hardeners) [1, 2, 3, 8, 13, 18, 19, 20,

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28, 29, 30, 37, 42. 43, 45]. The exposure may cause impairment of lung function, bronchial hyperresponsiveness and various diseases, such as: asthma, bronchitis, chronic obstructive pulmonary disease (COPD), allergic alveolitis, pharyngitis, rhinitis, and nasal cancer [3, 5, 7, 8, 20, 21, 37, 38, 39, 42, 43, 45]. Occupational disorders of the respiratory tract may be common among workers of the furniture industry [21, 30, 37, 42] but their etiology is not fully known.

The concentration of microorganisms in the air of furniture factories is much lower than in sawmills [1, 2, 3, 9, 14, 16, 24, 28, 29, 45] and their role in causing work-related disease in this occupational group has not been documented as clearly as in sawyers or workers handling wood chips [4, 16, 17, 29, 35, 44, 46]. It is presumed that most of adverse effects caused by airborne microorganisms in furniture industry workers has an immunological background. Minárik *et al.* [33] described a case of allergic alveolitis in a female furniture industry worker. The disease was caused by fungi *Aspergillus fumigatus* and *Aspergillus clavatus* which abundantly developed in a powder used for filling gaps in manufactured doors. Wilhelmsson *et al.* [45] found that 3% of Swedish wood furniture workers had nasal allergy to moulds.

The aim of this work was to determine the immunological reactivity of Polish furniture factory workers to a wide spectrum of biological allergens associated with wood dust as potential disease agents. Allergens for this study were selected on the basis of the earlier microbiological analysis of the air in the examined factory [23, 24].

MATERIALS AND METHODS

Examined population. A group of 48 furniture industry workers (17 males + 31 females) aged 29.6 ± 5.1 yrs (mean ± S.D.) were examined. They worked in a big furniture factory located in the Lublin region (eastern Poland), in which furniture were made from fibre- and chipboards. Previously in this factory (marked in the study design as factory "A") microbiological studies of the air were performed for selecting the antigens for allergological examinations [23, 24].

Out of 48 examined workers, 17 were employed in the department of initial processing at sawing of large boards into small ones, 10 in the department of board processing at trimming, veneering, and sanding of small boards and 21 in the varnishing department at painting of boards with nitric and polyester varnishes.

Thirty two healthy office workers living in the city of Lublin and not exposed to organic dusts were examined as a reference group. This group comprised 11 males and 21 females, aged 36.4 ± 8.6 yrs (mean ± S.D.).

All furniture factory workers and members of the reference group were examined by skin and precipitin tests with the saline extracts of cultures of microorganisms isolated from the air polluted with the dust. Human subjects protocols were approved by the Ethics Commission of the Institute of Agricultural Medicine and all subjects gave informed consent.

Preparation of allergens. The antigens of the following 15 microorganisms associated with organic dusts were used in the study:

- Gram negative bacteria: *Acinetobacter calcoaceticus, Pantoea agglomerans* (syn. *Erwinia herbicola, Enterobacter agglomerans*), *Pseudomonas maltophilia*, *Rahnella* sp.;
- Gram-positive bacteria: *Arthrobacter globiformis, Bacillus subtilis, Corynebacterium xerosis*;
- Actinomycetes: *Saccharopolyspora rectivirgula* (syn. *Micropolyspora faeni, Faenia rectivirgula*), *Streptomyces albus, Thermoactinomyces vulgaris*;
- Fungi: *Alternaria alternata, Aspergillus candidus, Aspergillus fumigatus, Cephalosporium glutineum, Penicillium citrinum.*

The antigen of *Pseudomonas maltophilia* was prepared from the strain isolated from the air of examined furniture factory, while the other antigens were prepared from the reference strains used for research and diagnostic purposes in the Institute of Agricultural Medicine in Lublin [10, 15].

All 15 antigens were used in the agar-gel precipitation test, while for the skin test, on the basis of the common occurrence in the air of the furniture factory and potential pathogenic properties, the following 3 antigens were selected: *Rahnella* sp., *Arthrobacter globiformis* and *Aspergillus fumigatus.* For technical reasons, skin test with the antigens of *A. globiformis* and *A. fumigatus* were not performed in the reference group.

Both in skin and agar-gel precipitation tests, lyophilised saline extracts of bacterial or fungal mass, produced in the Institute of Agricultural Medicine in Lublin, were used as antigens. In the case of Gram-negative and Gram-positive bacteria the mass was harvested from nutrient agar cultures, while in the case of actinomycetes and fungi the mass was harvested from sugar broth cultures. The mass was then homogenised and extracted in saline (0.85% NaCl) in the proportion 1:2 for 48 hrs at 4°C, with intermittent disruption of cells by 10-fold freezing and thawing. Afterwards, the supernatant was separated by centrifugation, dialysed against distilled water for 24 hrs, concentrated by evaporation to 0.1-0.15 of initial volume and lyophilised. In skin test, the antigens were used at the concentration of 1 mg/ml and in agar-gel precipitation test at the concentration of 30 mg/ml [15, 31, 41].

Skin test. The test was performed by intradermal method. The antigens were dissolved in 0.85% NaCl, sterilised by filtering and checked for sterility and lack of toxicity. The test was performed by intracutaneous injecting 0.1 ml of the antigenic extracts and of saline (as a control) into the forearm of the subject. The test sites were observed at 20 min for immediate reactions, at 8 hrs for delayed reactions and at 24 hrs for late reactions. The

Table 1. Skin response of furniture factory workers to the extract of *Rahnella* sp.

Furniture factory workers	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Workers employed at initial processing (N = 17)	16*** (94.1%)	9* (52.9%)	0	0	16 (94.1%)	16 (94.1%)	0	0	17 (100%)	16 (94.1%)	7 (41.2%)	0
Workers employed at board processing (N = 10)	10*** (100%)	6* (60.0%)	0	0	10 (100%)	10 (100%)	1 (10.0%)	0	10 (100%)	10 (100%)	4 (40.0%)	0
Workers employed at varnishing (N = 21)	20*** (95.2%)	16*** (76.2%)	3 (14.3%)	0	21 (100%)	21* (100%)	6^{+} (28.6%)	0	21 (100%)	19 (90.5%)	13 (61.9%)	0
Total furniture factory workers (N = 48)	46*** (95.8%)	31*** (64.6%)	3 (6.3%)	0	47 (97.9%)	47* (97.9%)	7 (14.6%)	0	48 (100%)	45 (93.7%)	24 (50.0%)	0
Reference group (N = 32)	8 (25.0%)	6 (18.7%)	4 (12.5%)	0	30 (93.7%)	26 (81.2%)	11 (34.4%)	1 (3.1%)	30 (93.7%)	26 (81.2%)	21 (65.6%)	5 (15.6%)

Grade 1 = reactions weakly positive, diameter ≥ 5 mm. Grade 2 = reactions positive, diameter ≥ 10 mm. Grade 3 = reactions strongly positive, diameter ≥ 20 mm. Grade 4 = reactions very strongly positive, diameter ≥ 40 mm. *–***: significantly greater compared to reference group; * $p < 0.05$, *** $p < 0.001$. $^{+}$–$^{+++}$: significantly greater compared to subgroup of workers employed at initial processing; $^{+}$ $p < 0.05$.

wheal and/or erythema reactions of 5 mm or more in diameter (at negative control) were regarded as positive. The intensity of positive reactions was graded on the basis of diameter as follows: ≥ 5 mm - grade 1, ≥ 10 mm - grade 2, ≥ 20 mm - grade 3, ≥ 40 mm - grade 4 [15].

Agar-gel precipitation test. The test was performed by Ouchterlony double diffusion method in purified 1.5% *Difco* agar. The subject's serum was placed in the central well and antigens, dissolved in 0.85% NaCl, in the peripheral wells. Each serum was tested twice: not concentrated, and three-fold concentrated, for the detection of low levels of precipitins. The agar plates were incubated for 6 days at room temperature, then washed in saline and in 5% sodium citrate solution (to prevent false positive reactions), and stained with azocarmine B [15, 31, 36, 41].

Statistical analysis. The obtained results were analysed by Student's t-test, assuming $p < 0.05$ as a significance level. The incidence of allergic reactions in furniture factory workers were compared with the occurrence of work-related symptoms, respiratory abnormalities, and spirometric values in these workers (including values of the overshift drop expressed in milliliters) which have been presented by our group in a separate paper [32]. The comparison was carried out by Pearson's correlation test.

The study was performed mostly in the years 1987–1989 and continued during 1998–2001. Preliminary results of this work have been reported elsewhere [23].

RESULTS

Skin reactions. The skin responses of furniture factory workers to the extracts of *Rahnella* sp., *Arthrobacter globiformis* and *Aspergillus fumigatus* are presented in the Tables 1, 2, and 3, respectively. The workers responded to the extract of Gram-negative bacterium *Rahnella* sp. with a very high frequency at all time intervals (20 min, 8 hrs, 24 hrs) (Tab. 1). The incidence of grade 1 and grade 2 positive early reactions was distinctly and significantly greater in furniture workers compared to the reference group ($p < 0.001$). At longer time intervals, a slightly significant difference ($p < 0.05$) between these groups could be observed only in the case of grade 2 delayed reactions (Tab. 1).

The frequencies of early skin reactions of furniture workers to the extracts of *Arthrobacter globiformis* and *Aspergillus fumigatus* were similar to those to *Rahnella*, while the frequencies of delayed and late reactions were much lower except for grade 1 late reactions (Tables 2-3). Unfortunately, the results of skin tests with *A. globiformis* and *A. fumigatus* in furniture workers could not be compared with the reference group.

Comparing the frequencies of skin response rate to the extracts of environmental microbes in the particular subgroups of furniture factory workers, it was found that on the average the greatest incidence of positive reactions was found in the workers employed at varnishing. The frequency of positive skin response in this subgroup of furniture factory workers was significantly greater ($p < 0.05$) compared to the workers employed at initial

Table 2. Skin response of furniture factory workers to the extract of *Arthrobacter globiformis*.

Furniture factory workers	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Workers employed at initial processing (N = 17)	15 (88.2%)	6 (35.3%)	0	0	11 (64.7%)	1 (14.3%)	0	0	16 (94.1%)	3 (17.6%)	0	0
Workers employed at board processing (N = 10)	10 (100%)	3 (30.0%)	0	0	6 (60.0%)	1 (10.0%)	0	0	10 (100%)	2 (20.0%)	0	0
Workers employed at varnishing (N = 21)	21 (100%)	$16^{+\#}$ (76.2%)	2 (9.5%)	0	15 (71.4%)	3 (14.3%)	1 (4.8%)	0	21 (100%)	7 (33.3%)	0	0
Total furniture factory workers (N = 48)	46 (95.8%)	25 (52.1%)	2 (4.8%)	0	32 (66.7%)	5 (10.4%)	1 (2.1%)	0	47 (97.9%)	12 (25.0%)	0	0

Grade 1 = reactions weakly positive, diameter ≥ 5 mm. Grade 2 = reactions positive, diameter ≥ 10 mm. Grade 3 = reactions strongly positive, diameter ≥ 20 mm. Grade 4 = reactions very strongly positive, diameter ≥ 40 mm. $^{+}$–$^{+++}$: significantly greater compared to subgroup of workers employed at initial processing; $^{+}$ $p<0.05$. $^{\#}$–$^{\#\#\#}$: significantly greater compared to subgroup of workers employed at board processing; $^{\#}$ $p<0.05$.

Table 3. Skin response of furniture factory workers to the extract of *Aspergillus fumigatus*.

Furniture factory workers	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Workers employed at initial processing (N = 17)	16 (94.1%)	9 (52.9%)	0	0	5 (29.4%)	0	0	0	14 (82.4%)	5 (29.4%)	0	0
Workers employed at board processing (N = 10)	10 (100%)	5 (50.0%)	0	0	6 (60.0%)	1 (10.0%)	0	0	9 (90.0%)	4 (40.0%)	0	0
Workers employed at varnishing (N = 21)	18 (85.7%)	16 (76.2%)	2 (9.5%)	0	10 (47.6%)	1 (4.8%)	1 (4.8%)	0	21^{+} (100%)	9 (42.9%)	0	0
Total furniture factory workers (N = 48)	44 (91.7%)	30 (62.5%)	2 (4.2%)	0	21 (43.7%)	2 (4.2%)	1 (2.1%)	0	44 (91.7%)	18 (37.5%)	0	0

Grade 1 = reactions weakly positive, diameter ≥ 5 mm. Grade 2 = reactions positive, diameter ≥ 10 mm. Grade 3 = reactions strongly positive, diameter ≥ 20 mm. Grade 4 = reactions very strongly positive, diameter ≥ 40 mm. $^{+}$ - $^{+++}$: significantly greater compared to subgroup of workers employed at initial processing; $^{+}$ $p<0.05$.

processing in the cases of grade 3 delayed reactions to *Rahnella* (Tab. 1), grade 2 early reactions to *A. globiformis* (Tab. 2) and grade 1 late reactions to *A. fumigatus* (Tab. 3). Moreover, the frequency of grade 2 early skin reactions to *A. globiformis* in the workers employed at varnishing was significantly greater ($p < 0.05$) compared to the workers employed at board processing (Tab. 2).

Precipitin reactions. With the majority of microbial antigens tested, a very low percentages of positive reactions were noted, which were similar to or even lower compared to the results obtained in reference group (Tab. 4). The only exception was a high percentage of positive reactions to the antigen derived from the strain of Gram-negative bacterium *Pseudomonas maltophilia* isolated from the air of the furniture factory, which was significantly greater in factory workers compared to the reference group ($p < 0.01$). No differences in antibody response between the particular departments were noted (data not shown).

Relationship between the occurrence of work-related symptoms, spirometric values and allergic reactions. No significant correlation could be found between the incidence of allergic reactions and the occurrence of work-related symptoms, respiratory abnormalities and spirometric values in the examined furniture factory

Table 4. Precipitin reactions of furniture factory workers to antigens occurring in the air of work environment.

Antigen	Furniture factory workers (N = 48)		Reference group (N = 32)	
	Sera not concentrated	Sera 3-fold concentrated	Sera not concentrated	Sera 3-fold concentrated
Gram-negative bacteria				
Acinetobacter calcoaceticus	1 (2.1%)	1 (2.1%)	5 (15.6%)	15 (46.9%)
Pantoea agglomerans	0	1 (2.1%)	1 (3.1%)	1 (3.1%)
Pseudomonas maltophilia	13 (27.1%)**	16 (33.3%)***	0	0
Rahnella sp.	0	2 (4.2%)	7 (21.9%)	14 (43.7%)
Gram-positive bacteria				
Arthrobacter globiformis	0	1 (2.1%)	0	2 (6.2%)
Bacillus subtilis	0	0	0	0
Corynebacterium xerosis	0	1 (2.1%)	0	0
Actinomycetes				
Saccharopolyspora rectivirgula	0	0	0	2 (6.2%)
Streptomyces albus	0	0	0	0
Thermoactinomyces vulgaris	1 (2.1%)	1 (2.1%)	0	0
Fungi				
Alternaria alternata	0	0	0	0
Aspergillus candidus	0	1 (2.1%)	0	0
Aspergillus fumigatus	0	2 (4.2%)	0	0
Cephalosporium glutineum	0	0	0	0
Penicillium citrinum	0	0	0	5 (15.6%)

*–***: significantly greater compared to reference group; ** $p < 0.01$, *** $p < 0.001$.

workers ($p > 0.05$). A significant correlation was found only between the values of overshift drop in forced expiratory volume in one second (FEV_1) and the incidence of early skin reactions to *Arthrobacter globiformis* ($p < 0.05$), and between the values of overshift drop in vital capacity (VC) and the incidence of delayed and late skin reactions to *Rahnella* sp. and *Aspergillus fumigatus* ($p < 0.05$).

DISCUSSION

The skin response of furniture factory workers to the extract of Gram-negative bacterium *Rahnella* sp. was very high at all time intervals. The relationship between the degree of exposure to *Rahnella* and skin response was best expressed by early reactions, which occurred significantly more frequently in furniture factory workers than in members of the reference group. The delayed and late reactions to *Rahnella* sp. were non-specific, occurring with equally high frequency in factory workers and referents. It cannot be excluded that small quantities of endotoxin, which is produced by *Rahnella* sp. [12], might contribute to non-specific irritation and false-positive reactions.

The frequencies of the positive early reactions to the extracts of *Arthrobacter globiformis* and *Aspergillus fumigatus* among furniture factory workers were similar to those to *Rahnella*, while the frequencies of delayed and late reactions were lower except for weak late reactions. Unfortunately, the latter 2 antigens could not be tested in the reference group, which was a limitation preventing a full assessment of the results.

It is noteworthy that when the frequency of positive skin reactions to environmental microbes among the workers of 3 departments of the examined furniture factory was compared, it proved to be highest in most cases in the workers of the varnishing department, when the degree of exposure to bioaerosols was expected to be low. This may be explained by a synergistic effect of biological and chemical air pollutants in the work environment which may lead to sensitisation and disease even at low bioaerosol levels. Irritant and/or toxic chemicals may damage ciliated epithelium of the respiratory tract, increasing penetration of biological allergens. Some toxic substances may suppress the natural defence system and potentiate effects of biological agents. Minárik *et al.* [34] has described occupational cases of allergic alveolitis caused by biological aeroallergens which were severely exacerbated by chemical factors (ammonia, acetone, gasoline) present in the work environment. As many as 88.6% of workers of the facility

producing mushroom spawn reported occurrence of work-related symptoms, which were associated with a parallel exposure to biological allergens and formaldehyde [11]. Furniture factory workers employed at varnishing are exposed to potentially hazardous dyes, varnishes, solvents, glues and hardeners which may aggravate the effects of biopollutants. Our findings corroborate with those of Bokov *et al.* [6] who demonstrated that sensitization to fungi and mycotic infections occur significantly more often among furniture factory workers exposed to polymers.

Generally, the skin response of furniture factory workers to the tested microbial allergens was greater compared to that of sawmill workers who were tested by the same method with the extracts of *Rahnella* sp., *Brevibacterium linens* and *Penicillium citrinum* [15]. This is rather an unexpected result as the degree of exposure to airborne microorganisms is much greater in sawmills than in furniture factories [1, 2, 3, 14, 16, 24, 28, 29, 45]. A high degree of skin sensitivity of the furniture factory workers to the allergens of environmental microbes increases a risk of respiratory disorders in this occupational group, all the more so as all the tested species may be potential disease agents. A Gram-negative bacterium *Rahnella* sp. belongs to family *Enterobacteriaceae* and is characterized by strong allergenic and endotoxic properties [12, 15, 40]. A coryneform bacterium *Arthrobacter globiformis* may cause allergic alveolitis [31] and peptidoglycan constituting cell wall of these bacteria may reveal immunotoxic properties [27]. *Aspergillus fumigatus* is a known fungal pathogen causing allergic alveolitis, asthma, pulmonary aspergillosis and possibly mycotoxicoses [22, 25, 26, 35].

The antibody response of furniture factory workers to airborne antigens, as assessed by agar-gel precipitation test, was very low and not related to the exposure. The only exception was the frequency of positive precipitin reactions to the antigen of *Pseudomonas maltophilia* which was significantly greater compared to reference group. In contrast to other antigens used in the test, this antigen has been prepared from the strain isolated from the air of the furniture factory. So far, little is known about the potential allergenic properties of bacteria belonging to the genus *Pseudomonas*.

The antibody response found in the present work was much lower compared to other serological surveys of woodworkers in Scandinavia [4, 16, 17, 45, 46] and in Poland [15] which may be explained, at least in part, by the lower degree of exposure to airborne microbes [4, 15, 16, 17, 46] and the differences in methods used [16, 17, 45, 46].

The lack of the correlation between the allergic reactions and occurrence of symptoms suggests that among the workers of furniture industry there is no direct relationship between positive allergic response to environmental microbes and appearance of symptoms. Nevertheless, sensitization to environmental allergens may predispose to respiratory disorders, as evidenced by significant correlation between skin reactivity and overshift drop of spirometric values.

CONCLUSIONS

- The workers of furniture factory show a high frequency of positive skin reactions to microbial allergens present in the air of work environment while the frequency of positive precipitin-mediated reactions to these allergens is low.
- No significant relationship could be found in furniture factory workers between the incidence of positive allergic reactions to environmental microbes and occurrence of work-related symptoms and spirometric values, except for correlation between skin response and overshift drop of VC and FEV_1 values.

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