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# Exposure of water consumers to mesophilic actinomycetes

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## SUMMARY

In autumn 1978 an epidemic of respiratory disease resembling allergic alveolitis occurred in a small Finnish community. The disease was caused by repeated exposures to tap water aerosol. The raw water of the community and the sand filters of the purification system were heavily contaminated with mesophilic actinomycetes. Fourteen different strains of actinomycetes were isolated. Exposed persons with and without symptoms as well as unexposed control persons were tested for antibodies against five of these actinomycetes and against *Enterobacter agglomerans*. Both the exposed and the control persons had antibodies to actinomycetes but the exposed persons had antibodies against more actinomycete strains than the control persons. Precipitating antibodies against *E. agglomerans* were also found in control persons as well as in patients. There was a significant difference between the patients and the exposed healthy persons in bacterial agglutination tests with flagellar antigen of one *E. agglomerans* strain. However, the role of mesophilic actinomycetes and *E. agglomerans* in the aetiology of the disease could not be firmly established.

## INTRODUCTION

Mesophilic actinomycetes are part of the normal microbial flora of natural waters. Fresh water is not, however, an ideal environment, and the number of actinomycetes is usually low; less than 10 colonies/ml have been detected in lake and river water samples in Finland (Seppänen & Jokinen, 1969; Persson, 1979). The number is dependent on seasonal variation associated with the presence of blue green algae which serve as nutrients for the actinomycetes (Silvey & Roach, 1959; Silvey, 1963). High numbers of actinomycetes are often recovered from the mud of lakes and rivers (Johnston & Cross, 1976; Willoughby, 1969). The most common

species of mesophilic actinomycetes found in fresh waters are *Streptomyces*, *Nocardia* and *Micromonospora* (Lechevalier, 1964). *Actinoplanes* as well as *Streptomyces* and *Micromonospora* have also been isolated from an eutrophic lake in Finland (Jokinen, 1970).

Mesophilic actinomycetes are not known to cause any diseases. On the other hand, a repeated exposure to thermophilic actinomycetes *Thermoactinomyces vulgaris* and *Micropolyspora faeni* present in mouldy hay dust may lead to an allergic respiratory illness known as farmer's lung (Pepys & Jenkins, 1965). The association between the microbes and the illness is indicated by the presence of circulating antibodies in the sera of the patients. Mesophilic actinomycetes have also been found in mouldy hay, but their significance for the development of farmer's lung is not known (Gregory & Lacey, 1963; Terho & Lacey, 1979).

In autumn 1978 an epidemic of respiratory disease resembling farmer's lung occurred in a small Finnish community. The epidemic was caused by repeated exposure to tap water aerosol (Muittari *et al.* 1980). Antibody tests with the traditional farmer's lung antigens gave negative results. Microbiological analysis revealed mesophilic actinomycetes in high numbers both in the lake which was the water source of the community and in the sand filters of the communal water works. This led us to investigate by antibody analysis the possible association between the actinomycetes and the epidemic. Antigens were prepared from actinomycete strains isolated, and the sera of the patients were tested for antibodies with the gel precipitation technique. The sera were also tested for antibodies against *Enterobacter agglomerans*, a bacterium predominating in the airborn flora of cotton mills, and suspected to cause respiratory symptoms (Rylander & Lundholm, 1978).

## MATERIALS AND METHODS

### *Water source*

The water source of the community was a small shallow lake. The surface area of the lake is 0·33 km$^2$ and the maximum depth is 5 m the total water volume being $0{\cdot}5 \times 10^6$ m$^3$. The theoretical retention time in the lake is relatively long, approximately 290 days, due to the small drainage area of 2–3 km$^2$. Farming and numerous summer cottages on the shores of the lake have an adverse effect on the quality of the water. The lake is eutrophic having typical blooming of blue green algae during summer. The blooming was exceptionally heavy during the summer 1978, before the epidemic, because of the drainage from recently fertilized forests surrounding the lake.

The lake has served as a raw water source for approximately 1000 consumers of the community until the year 1979. The purification process comprised only sand filtration and chlorination. During the summer 1978 the sand filters became covered by a mat of blue green algae and also by an exceptionally dense growth of mesophilic actinomycetes.

### *Test persons*

Numerous cases of respiratory diseases appeared between August and December of 1978 in the community using tap water purified from the contaminated lake water. Respiratory symptoms included fever, cough and dyspnoea. The episodes

appeared after an exposure to tap water aerosol in a bath or in sauna. The causative role of tap water was confirmed by provocation tests (Muittari *et al.* 1980). Nineteen persons with these symptoms were tested for antibodies with 14 actinomycete antigens. Antigens showing positive reactions were used to test three further groups of persons: ten persons with the respiratory symptoms described above (group I), ten persons without respiratory symptoms (group II), both groups consuming tap water from the contaminated lake and ten healthy control persons (group III) consuming water from a different water source. None of the persons had any chronic lung diseases, asthma or allergic rhinitis. The age of the persons varied between 24 and 61 years in all groups. These persons were also tested for *E. agglomerans* antibodies.

### *Antigen preparation*

Mesophilic actinomycetes were isolated from lake water and from sand filters. The isolation from water samples was made by the membrane filter technique using 100–500 ml aliquots of water. Spread plate technique was used for samples taken from sand filters. Samples were grown on Actinomycetes Isolation Agar (Difco) at 20 °C for 2 weeks.

Antigens were prepared from strains of actinomycetes isolated in the study. Microbes were grown in the synthetic medium VII (Tendler & Burkholder, 1961) at room temperature for 1 week. Cells were harvested by centrifugation at 800 × g for 15 min and washed twice with PBS (0·05 M phosphate buffered saline pH 7·0) and suspended into PBS. Cells were disrupted by an ultrasonic treatment (MSE 1174 MK 2. Crawley, U.K.) and the cell debris was separated by centrifugation at 800 × g for 15 min. The supernatants were used as antigens.

Antigens were also prepared from four strains of *E. agglomerans* isolated from sedimentation plates exposed to air flow from an air conditioning system. For immuno-diffusion, three strains of bacteria were grown in nutrient broth (0·3% beef extract and 0·5% peptone, Difco) at 37 °C for 20 h. Antigen preparations were made in the same way as for the actinomycetes. The technique used for immunodiffusion was a micromodification of gel precipitation (Wadsworth, 1962). For bacterial agglutination, H- and O-antigens of four *E. agglomerans* strains were prepared separately as described for salmonella antigens (Hallman & Burkhardt, 1974). Bacteria were grown in tryptone-soya broth (Oxoid) at 37 °C for 24 h. Formalin was added to 0·3% of the total volume and left at 4 °C for 48 h. These preparations were used as H-antigens. For O-antigen preparations bacteria were grown on tryptone-soya agar at 37 °C for 24 h. Bacterial growth was harvested in saline, and ethanol was added to 80% of the final volume. Bacteria were shaken for 30 min and left for 48 h at room temperature. After washing, the bacteria were used for agglutination tests. Agglutination tests were performed on microtitre plates and the results were expressed as titres indicating the last serum dilution agglutinating the bacteria. Controls without serum excluded bacterial auto-agglutination.

## RESULTS

Fourteen different mesophilic aerobic actinomycetes were isolated. They belonged to the genus *Streptomyces* but were not identified further. Six of the strains marked

Table 1. *Precipitation reactions between actinomycete antigens and sera of patients with respiratory symptoms* ($N = 19$)

| | Actinomycete strains | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $W_1$ | $W_2$ | $W_3$ | $W_4$ | $W_5$ | $W_6$ | $W_7$ | $W_8$ |
| No. of positive reactions | 0 | 0 | 0 | 0 | 2 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 3 |

Table 2. *Number of sera reacting with one or more of the actinomycetal antigens*

| | Positive against | | | |
|---|---|---|---|---|
| Test group | One microbe | Two microbes | Three microbes | Four microbes |
| I | 1 | 2 | 2 | 0 |
| II | 2 | 0 | 0 | 3 |
| III | 3 | 1 | 0 | 0 |

Group I: exposed persons with symptoms ($N = 10$), Group II: exposed persons without symptoms ($N = 10$), Group III: unexposed persons ($N = 10$).

Table 3. *Precipitating antibodies against actinomycetes and* E. agglomerans

| | Number of positive reactions against | | | | | |
|---|---|---|---|---|---|---|
| | Actinomycete strain | | | | | |
| Test group | $F_5$ | $F_6$ | $W_1$ | $W_3$ | $W_8$ | *E. agglomerans* |
| I | 4 | 2 | 0 | 3 | 1 | 2 |
| II | 3 | 2 | 3 | 2 | 0 | 0 |
| III | 3 | 0 | 0 | 2 | 0 | 2 |

Group I: exposed persons with symptoms ($N = 10$). Group II: exposed persons without symptoms ($N = 10$). Group III: unexposed control persons ($N = 10$).

as $F_1$ to $F_6$ were isolated from the sand filters, and eight strains ($W_1$ to $W_8$) from the lake water. Nineteen persons who had consumed water purified through the sand filters and had respiratory symptoms were tested for antibodies with the actinomycete antigens. The results are presented in Table 1.

Seven of the nineteen persons were shown to have antibodies to one or more of the microbes. All except two of them had antibodies to only one microbe; one person had antibodies to three and one person to two microbes. Based on these results five actinomycete antigens $F_5$, $F_6$, $W_1$, $W_3$, and $W_8$ were selected for further tests.

These five actinomycete antigens as well as *E. agglomerans* antigens were used to test sera of additional persons of the groups I, II and III. The results are shown in Tables 2 and 3. Five persons with symptoms had antibodies to one or more of the actinomycetes; five exposed persons without symptoms also reacted with some of the antigens. Antibodies to one or two of the actinomycetes were found in four control persons. People consuming water purified from the contaminated lake water (groups I and II) showed more positive reactions against the actinomycete panel than the control persons (group III). Of the sensitized people who had

Table 4. *Antibodies to four* E. agglomerans *strains tested by bacterial agglutination*

| | Mean titre ($\log_2$) ± S.D. against | | | | | |
|---|---|---|---|---|---|---|
| | O antigen group | | | H antigen group | | |
| Strain | I | II | III | I | II | III |
| $E_1$ | 5·7 ± 1·8 | 4·8 ± 2·3 | 4·3 ± 1·6 | 11·3 ± 1·3 | 10·6 ± 1·3 | 8·8 ± 3·2 |
| $E_2$ | 7·6 ± 2·2 | 7·7 ± 1·9 | 6·6 ± 1·2 | 4·8 ± 1·4 | 5·1 ± 1·1 | 4·3 ± 1·4 |
| $E_3$ | 6·8 ± 2·3 | 6·8 ± 1·9 | 5·8 ± 1·9 | 5·0 ± 1·6 | 5·1 ± 1·1 | 4·7 ± 1·2 |
| $E_4$ | 5·1 ± 1·9 | 4·5 ± 1·9 | 3·8 ± 1·5 | 10·4 ± 1·0* | 8·6 ± 1·3* | 8·4 ± 1·7 |

Group I: exposed persons with symptoms ($N = 10$). Group II: exposed persons without symptoms ($N = 10$). Group III: unexposed control persons ($N = 10$).

* A significant difference ($0{\cdot}001 < P < 0{\cdot}01$) between the groups.

antibodies (a total of 10) three had antibodies to four actinomycetes, two to three actinomycetes, two to two actinomycetes and three to one actinomycete only. In the control group one person had antibodies to two antiomycetes and the others only to one actinomycete.

Antibodies to *E. agglomerans* were also found in patients (2/10) as well as in control persons (2/10) by the immunodiffusion method (Table 3). The three *E. agglomerans* strains used gave identical results. For a more detailed analysis of *E. agglomerans* antibodies bacterial agglutination was used. The mean serum titres of *E. agglomerans* agglutination tests are presented in Table 4. The mean titres were generally higher in the two groups consuming the contaminated water than in the control group. Antibodies to one H-antigen (strain E 4) were associated with the symptoms. By the *t*-test the differences between group I and group II and also between group I and group III were statistically significant ($t = 3{\cdot}42$ and $t = 3{\cdot}26$, respectively; $0{\cdot}001 < P < 0{\cdot}01$ in both cases).

## DISCUSSION

According to normal standards, drinking water is considered microbiologically safe if the number of faecal indicator bacteria remains below a certain level. Such water can still be an infection hazard, but it can also be a health risk through other mechanisms: respiratory exposure to water mist containing biological material, either living microbes or microbial debris, may lead to pulmonary symptoms by immunological or toxic reactions. Allergic alveolitis is one example. In most cases the respiratory disorders of this type have been associated with air humidification systems (MRC Symposium, 1977), but also with hot baths and sauna (Atterholm *et al.* 1977; Metzger *et al.* 1976). In most cases the causative agents have not been definitely identified. Amoeba (Edwards, Griffiths & Mullins, 1976; Edwards, 1980) and fungi *Cephalosporium* (Patterson *et al.* 1978, 1981) and *Pullularia* (Metzger *et al.* 1976) have been suspected. Enterobacteria or their endotoxins have also been suggested to cause respiratory symptoms (Pickering *et al.* 1976; Rylander *et al.* 1978).

A group of persons was exposed to contaminated water during an episode of water-associated respiratory disorders in Finland in 1978 (Muittari *et al.* 1980). The

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exact nature of the material causing the symptoms still remains unknown. Our results indicate, however, that people using the water apparently had been exposed to microbial material as revealed by the presence of circulating antibodies. One of the microbes incriminated was *E. agglomerans* (Dutkiewicz, 1976; Rylander & Lundholm, 1978). Unfortunately no *E. agglomerans* strains isolated from the water source were available; the use of other strains may be complicated by the variety of *E. agglomerans* serotypes. In spite of this, we were able to segregate exposed symptomatic and asymptomatic persons by the titres of agglutinating antibodies against the H antigen of one *E. agglomerans* strain. On the other hand, antibodies to O-antigens correlated poorly with the symptoms, unlike the results of Rylander *et al.* (1978) obtained with some other enterobacteria. Our results might have been more conclusive if the actual contaminating strain had been available.

Our results also show that mesophilic aerobic actinomycetes can act as sensitizing agents. These bacteria have not been mentioned previously in this connection. Usually their numbers in lake water in Finland is low, but summer 1978 was exceptional in this respect for the lake described in this study. The abundance of actinomycetes in raw water led to a heavy growth in the sand filters of the water works. This apparently resulted in contamination of the tap water (Niemi, Knuth & Lundström, 1982). Antibodies to the actinomycetes were found in persons exposed to the contaminated water but also in control persons. Here again the variety of the species isolated from the raw water and from the water purification system as well as the small number of cases prevented more definite conclusions about the association between the episode and actinomycetes.

Antibody tests are a useful method for revealing sensitization against the test antigens, but the causal relationship between antibodies and symptoms is more difficult to establish. For example, in farmer's lung, elevated antibody titres are associated with the disease, but only a fraction of antibody positive persons has symptoms (Katila & Mäntyjärvi, 1978). In the present episode *E. agglomerans* or other bacterial endotoxins may well have been responsible for the symptoms (Muittari, Rylander & Salkinoja-Salonen, 1980), and the specific antibodies, although indicating an exposure and sensitization, may have had no role in the pathogenesis of the disease. On the other hand, immune mechanisms have a significant role in the pathogenesis of allergic alveolitis (Schatz, Patterson & Fink, 1977) and their participation in this episode can not be excluded.

## REFERENCES

ATTERHOLM, I., HALLBERG, T., GANROT-NORLIN, K. & RINGERTZ, O. (1977). Unexplained acute fever after a hot bath. *Lancet* ii, 684–686.

DUTKIEWICZ, J. (1976). Studies on endotoxins of *Erwinia herbicola* and their biological activity. *Zentralblatt fur Bakteriologie und Hygiene. I. Abteilung Originale A* **236**, 487–508.

EDWARDS, J. H., GRIFFITHS, A. J. & MULLINS, J. (1976). Protozoa as sources of antigen in 'humidifier fever'. *Nature* **264**, 438–439.

EDWARDS, J. H. (1980). Microbial and immunological investigations and remedial action after an outbreak of humidifier fever. *British Journal of Industrial Medicine* **37**, 55–62.

GREGORY, P. H. & LACEY, M. E. (1963). Mycological examination of dust from mouldy hay associated with farmer's lung disease. *Journal of General Microbiology* **30**, 75–88.

HALLMAN, L. & BURKHARDT, FR. (1974). *Klinische Mikrobiologie*, 4th ed., pp. 606–607. Georg Thieme Verlag Stuttgart.

JOHNSTON, D. W. & CROSS, T. (1976). The occurrence and distribution of actinomycetes in lakes of the English Lake District. *Freshwater Biology* **6**, 457–463.

JOKINEN, S. (1970). Eräiden järvien pohjalietteen sädesienistä. Laudaturtyö. Helsingin Yliopiston Mikrobiologian laitos, 82 pp. (in Finnish).

KATILA, M.-L. & MÄNTYJÄRVI, R. A. (1978). The diagnostic value of antibodies to the traditional antigens of farmer's lung in Finland. *Clinical Allergy* **8**, 581–587.

LECHEVALIER, H. (1964). *The Actinomycetes. – Principles and applications in aquatic microbiology*, pp. 230–253. Proceeding of Rudolfs Research Conference, Rutgers, The State University, New Brunswick, New Jersey. New York: John Wiley.

METZGER, W. J., PATTERSON, R., FINK, J., SEMERDJIAN, R. & ROBERTS, M. (1976). Sauna-takers disease. *Journal of American Medical Association* **236**, 2209–2211.

MRC SYMPOSIUM (1977). Humidifier fever. *Thorax* **32**, 653–663.

MUITTARI, A., KUUSISTO, P., VIRTANEN, P., SOVIJÄRVI, A., GRÖNROOS, P., HARMOINEN, A., ANTILA, P. & KELLOMÄKI, L. (1980). An epidemic of extrinsic allergic alveolitis caused by tap water. *Clinical Allergy* **10**, 77–90.

MUITTARI, A., RYLANDER, R. & SALKINOJA-SALONEN, M. (1980). Endotoxin and bath-water fever. *Lancet* ii, 89.

NIEMI, R. M., KNUTH, S. & LUNDSTRÖM, K. (1982). Actinomycetes and fungi in surface waters and in potable water. *Applied and Environmental Microbiology* **43**, 378–388.

PATTERSON, R., FINK, J. N., ROBERTS, M., KELLY, J. F. & SOMMERS, H. M. (1978). Antibody activity in sera of patients with humidifier disease. *Journal of Allergy and Clinical Immunology* **62**, 103–108.

PATTERSON, R., FINK, J. N., MILES, W. B., BASICH, J. E., SCHLEUTER, D. B., TINKELMAN, D. G. & ROBERTS, M. (1981). Hypersensitivity lung disease presumptively due to *Cephalosporium* in homes contaminated by sewage flooding or by humidifier water. *Journal of Allergy and Clinical Immunology* **68**, 128–132.

PEPYS, J. & JENKINS, P. A. (1965). Precipitin (F.L.H.) test in farmer's lung. *Thorax* **20**, 21–35.

PERSSON, P. E. (1979). The source of muddy odor in bream (Abramis bramo) from the Porvoo Sea area (Gulf of Finland). *Journal of the Fisheries Research Board of Canada* **36**, 883–890.

PICKERING, C. A. C., MOORE, W. K. S., LACEY, J., HOLFORD-STREVENS, V. C. & PEPYS, J. (1976). Investigation of a respiratory disease associated with an air-conditioning system. *Clinical Allergy* **6**, 109–118.

RYLANDER, R. & LUNDHOLM, M. (1978). Bacterial contamination of cotton and cotton dust and effects on the lung. *British Journal of Industrial Medicine* **35**, 204–207.

RYLANDER, R., HAGLIND, P., LUNDHOLM, M., MATTSBY, I. & STENQVIST, K. (1978). Humidifier fever and endotoxin exposure. *Clinical Allergy* **8**, 511–516.

SCHATZ, M., PATTERSON, R. & FINK, J. (1977). Immunopathogenesis of hypersensitivity pneumonitis. *Journal of Allergy and Clinical Immunology* **60**, 27–37.

SEPPÄNEN, P. & JOKINEN, S. (1969). Sädesienten merkityksestä sinilevien aiheuttamissa hajuilmiöissä. Limnologisymposion, Suomen Limnologinen Yhdistys, 69–87 (in Finnish).

SILVEY, J. K. G. (1963). The relationship between aquatic organisms and tastes and odors *Public Works* **94**, 106–194.

SILVEY, J. K. G. & ROACH, A. (1959). Laboratory culture of taste- and odorproducing aquatic actinomycetes. *Journal of American Water Works Association* **51**, 20–32.

TENDLER, M. D. & BURKHOLDER, P. R. (1961). Studies on the thermophilic actinomycetes. *Applied Microbiology* **9**, 394–399.

TERHO, E. O. & LACEY, J. (1979). Microbiological and serological studies of farmer's lung in Finland. *Clinical Allergy* **9**, 43–52.

WADSWORTH, C. (1962). A microplate technique employing a gel chamber compared with other micro- and macroplate techniqes for immune diffusion. *International Archives of Allergy* **21**, 131–137.

WILLOUGHBY, L. G. (1969). A study of the aquatic actinomycetes of Blelham Tarn. *Hydrobiologia* **34**, 465–483.