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DETERMINATION OF BACTERIAL AIR POLLUTION IN VARIOUS PREMISES

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INTRODUCTION

Various methods have been used to determine the quantity and quality of the bacterial pollution of the air. Impingement of the air on the solid media and counts of 'total bacteria' on agar and 'streptococcal count' (*Streptococcus salivarius* in particular) on selective media were most commonly used. Despite the extensive investigations undertaken by numerous authors, there is still need for more data on the actual bacterial pollution of the air and on the methods of detection, in order to provide a basis for a more accurate and useful interpretation of the findings.

In view of this, air samples were taken in various premises in order to determine the bacterial air pollution in an effort to find a more suitable method for routine use.

METHODS

The main purpose was to find a simple, but reasonably accurate, method of measuring bacterial air pollution which could be applied in routine work.

Bacterial air pollution was measured by:

(*a*) Total bacterial colony counts on serum agar plates, as an indicator of the total number of air-borne bacteria present in the air;

(*b*) *Streptococcus salivarius* on S. 1 media (the selective medium of Williams & Hirch (1950)) as the indicator of buccal pollution of the air (Wells, 1955); and

(*c*) *Bacterium coli* on Eosin-methylene blue (E.M.B.) agar, as an indicator of faecal and dust-borne bacteria in the air.

The bacterial count was performed by a simple enumeration of the typical colonies on plates, though for the control a small proportion of colonies were examined microscopically by the nigrosin technique; this was found not to be absolutely necessary for routine purposes.

The sampling of the air was performed by slit-sampler (Bourdillon, Lidwell & Lovelock, 1948) and by the modified sieve devices (Cvjetanović, 1955). These instruments were matched one against the other and their relative effectiveness established (Cvjetanović, 1956). It was observed earlier by several authors that sieve devices are about ten times less effective for the detection of fine particles than the slit-sampler, but only a little less effective for the detection of the coarse particles (Crumb & Wells, 1947). We also found that modified sieve devices, when compared with the slit-sampler, are much less effective (about ten times) as far as the total bacterial count is concerned, but very close to the slit-sampler in the detection of *Bact. coli* and *Str. salivarius*. This suggests that the 'total bacteria' represent mostly small particles, while the particles carrying *Bact. coli* and *Str.*

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salivarius are much larger. This was proved by the measurement of the sedimentation rates of bacteria-carrying particles. Therefore, the samples for 'total bacteria' were taken by slit-sampler, while those for *Str. salivarius* and *Bact. coli* were taken by modified sieve devices. Modified sieve devices were constructed so as to take relatively much greater quantities of air (in the same period of time) than the slit-sampler (sieve device: 1000 l./m.; slit-sampler 30 l./m.). This is necessary if *Str. salivarius* and *Bact. coli*, which are relatively rare in the air, are to be detected.

The samples were taken simultaneously for 'total bacteria', *Str. salivarius* and *Bact. coli* by instruments situated close together in order to obtain comparable samples of air. Three instruments were used: one slit-sampler and two modified sieve devices. (The use of this technique and, in particular, the use of sieve devices, was partly determined by the fact that one slit-sampler was available.)

In order to simplify the air sampling, the detection of *Str. salivarius* and *Bact. coli* was performed with a single sampler and a single Petri dish, using a plate with media so placed that one-half was covered by E.M.B. agar, while the other was covered by S. 1 agar. The use of such method of sampling, however, was justified only in cases of heavy air pollution; in most instances the number of sampled bacteria was too small.

In order to obtain more detailed knowledge of the variations of bacterial air pollution, the same premises were examined consecutively at short intervals. One measurement comprised six to thirty separate air samples. The actual number of samples was determined by the expected changes in the air pollution. In quiet premises with no, or limited, activity fewer samples were taken than in those where numerous and varied activities took place. For each such examination an *air bacteriogram* was constructed showing the quantitative and qualitative consecutive changes of the bacterial air pollution in particular premises. In some instances, in order to determine the source of air pollution by pathogens, epidemiological inquiries were also carried out and, in addition, samples for streptococci and staphylococci were taken. When it was deemed necessary, samples were taken in the whole period of 24 hr. at 15–30 min. intervals.

Numerous factors which might influence the results were taken into account and, *therefore, additional measurements were made, including temperature of the air,* humidity and movement as well as an estimation of the size of air-borne bacteria-carrying particles by Petri dish ratio technique (Bourdillon *et al.* 1948). Careful consideration was given to the manner of air sampling and also to apparatus and media, incubation, counting and determination of the colonies and other factors as has been described elsewhere (Cvjetanović, 1956).

Bacterial counts were expressed as colonies developing per 30 l. of air sampled in order to facilitate their comparison with the findings of others which were mostly expressed as colonies per cu.ft. (28 l.).

RESULTS

Using the method described, over 1000 measurements were taken in various premises in the City of Zagreb (Table 1). They are expressed as arithmetical averages, but when several sets of findings differed from one another substantially

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the averages of the two extreme sets of observations are indicated. In all cases, however, the single findings differed a great deal from the average, as will be described later.

Table 1. *Bacterial pollution of the air in various premises*

Premises	No. of simultaneous samples	Average number of bacteria-carrying particles in 30 l. of air: Total aerobic bacteria on serum agar (sampled by slit-sampler)	*Str. salivarius* on S. 1 agar (sampled by sieve device)	*Bact. coli* on E.M.B. agar (sampled by sieve device)
Operating theatres				
General surgery	128	15–53	0–0·4	0–0·5
Neuro-surgery	28	78	0·5	0·1
Traumatology and burns	217	107	0·3	0·7
Hospital wards:				
Children's hospital				
Corridor	38	182	0	0
Waiting room	12	168	0	5·8
Cubicles	88	92	0·3	4·0
Fever hospital				
Children's ward	123	259	0·7	0·5
General hospital				
Waiting room	30	56	0·3	0
X-ray unit	30	172	1·6	0·05
Department for burns				
Shock room	26	105–376	0·1	0·4
Patients' room	65	224	0·1	0·2
Cinemas	18	40–400	0·3	0·2
Lecture theatres	33	36–123	3·2	0
Gymnastic halls	108	25–414	0	0–12
Kindergartens	30	65	0·2	0·5
Day nurseries	30	178	0	0·2
Laboratories	47	16–75	0·07	0·1
Animal houses	108	223–2000	0	0·4

The data presented as averages in Table 1 give only a general idea of the degree of air pollution in the premises examined. A closer study was, therefore, made of the air pollution from the records of the measurements taken under various conditions and at different times.

Operating theatres. The study of air bacteriograms of the operating theatres showed that the total number of bacteria was influenced by the type of activity and number of people present in the theatre; increase in the number of people increased the *Str. salivarius* count, while preparation for operations, dusting and cleaning, etc., increased the total count and sometimes also the *Bact. coli* count. The poor construction of the operators' masks favoured a high number of *Str. salivarius*, while *Bact. coli* was present only in those theatres which were not properly cleaned and had unoiled floors.

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Children's hospitals. An extremely high *Bact. coli* count was observed in waiting rooms and in cubicles for sick babies. *Bact. coli* were spread in the air from babies' napkins when mothers handled them in waiting rooms, or when nurses did likewise in the cubicles. While in crowded waiting rooms the total bacterial count was high, in cubicles it was found to be low but with a high *Bact. coli* count. This was due to poor nursing technique. The nurses were often in a hurry, washing napkins in the cubicle and drying them on the radiators, thus spreading *Bact. coli*-carrying particles in the atmosphere. Also it is possible that the upward stream of hot air from the, radiators projected the particles carrying *Bact. coli* from the napkins into the air and spread them throughout the room. It is worth mentioning that in this same ward an outbreak of diarrhoea due to pathogenic strains of *Bact. coli* was observed. The infection spread for a long time in spite of the precautionary measures taken. *Bact. coli* was isolated from the air and also from the blankets and other objects in the ward. It was not proved that the *Bact. coli* strains detected in the air were identical with those pathogenic ones causing the diarrhoea in the infants, but the air-borne spread of the infection cannot be ruled out.

A high *Bact. coli* count was a constant characteristic of the air of some premises in the infants' wards. It was not possible to find any considerable difference in the bacterial air pollution of any one of the various premises in the children's hospital in the total bacterial count, but they differed considerably in *Bact. coli* count.

Gymnasia. The only other places where the *Bact. coli* count was sometimes very high were *gymnastic halls* where dust from outside was brought in and caused the high *Bact. coli* pollution of the air during full attendance and activities as shown in Table 2.

Table 2. *Bacterial pollution of the air in gymnastic halls*

No. of people in the hall	No. of samples	Average number of bacteria in 30 l. of air: Total bacteria on serum sugar	Average number of bacteria in 30 l. of air: *Bact. coli* on E.M.B. agar
0	35	24·6	0
5–25	35	191	9·6
26–50	38	414	12

In a more detailed study of a number of gymnasia (Cvjetanović, 1956) it was shown that the linear increase of people in the halls gave a geometrical rise of bacteria in the air.

A high count of *Str. salivarius* was observed in lecture rooms (as might be expected), presumably due to the fact that droplets were expelled by lecturers and students. The high number of *Str. salivarius* observed in the X-ray unit was probably due to the frequent coughing and vomiting of the patients who took Contrast Media by mouth.

Burns Unit. In the hospital department for the treatment of burns the conditions did not appear, at first sight, as poor as was disclosed by the air-sampling records. When unfavourable results were obtained from the air sampling, the question arose as to whether these necessarily meant that infection of burns, which

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were not rare at that time, were spread by the air. Close epidemiological inquiries and examination of the burns of patients, swabbing of the throats of the patients and staff, as well as the testing of clothes and other objects for pathogenic streptococci and staphylococci were carried out. The same organisms were found in the air, floor dust, bedclothes, and even in throat swabs from some personnel, as were found in cultures from the burns. However, it was not possible to discover the exact way the infection was spread as there were so many obvious possibilities.

Other observations in various premises more or less confirmed the facts already stated fully by various authors on the number and behaviour of air-borne bacteria. A full account of these observations may be found elsewhere (Cvjetanović, 1956).

DISCUSSION

Though the degree of air pollution in the examined premises is stated in exact figures and types of bacteria, there is the crucial problem of how to interpret these data. If comparison is made with similar examinations elsewhere, one may say that, generally speaking, the results fall more or less within the range of those obtained in England (Bourdillon *et al.* 1948; Bourdillon & Colebrook, 1946; Begg, Smellie & Wright, 1947; Williams & Hirch, 1950; Air Hygiene Committee M.R.C. Report, 1954; Blowers, Mason, Wallace & Walton, 1955) or in the U.S.A. (Wells, 1955). If the results obtained are matched with the arbitrary standards established by some authors (Bourdillon & Colebrook, 1946; Bedford, 1950) a somewhat more definite but still uncertain conclusion might be drawn. These authors presented as minimum requirements for air cleanliness in various premises the following limits for the total number of bacteria-carrying particles per cu.ft.:

(1) For operating theatres in neurosurgical wards and in dressing rooms for burns, 0·1–2.

(2) For operating theatres in general surgery, 10.

(3) For small surgery and dressing rooms, 20.

(4) For dwelling and working premises, 50.

It would appear, in view of these standards, that, except in very few premises, the degree of bacterial air polution recorded in Table 1 is heavy and particularly so in the operating theatres.

The *total bacterial* count gives little information about the source of bacterial air pollution and, therefore, additional information offered by both *Bact. coli* and *Str. salivarius* counts are useful in this respect. In the case of respiratory diseases the *Str. salivarius* count or, in the case of the possible air-borne spread of *Bact. coli* infection, the *Bact. coli* count, might give a much better lead than the total bacterial count. It seems, therefore, that the simultaneous total bacterial sampling, *Str. salivarius* and *Bact. coli* counts by the technique described, may be used with advantage in the routine determination of bacterial air pollution.

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SUMMARY

A simple method, applicable in routine work, for simultaneous air sampling of total bacteria, on serum agar, and *Str. salivarius* and *Bact. coli* on specific media, is described.

Simultaneous air sampling including, in addition to detection of total bacteria, *Str. salivarius* and *Bact. coli* as indicators of buccal and faecal air-borne bacteria, respectively, reveals the kind and source of air-borne pollution much better than the simple total bacterial count.

The air in various medical and public institutions was examined; the results are presented and discussed. The air pollution of the premises examined was relatively high, especially in the operating theatres. The air of infants' wards was found to be heavily polluted with *Bact. coli* as a result of poor nursing technique and this might have had bearing on the epidemic of infant diarrhoea.

REFERENCES

AIR HYGIENE COMMITTEE (1954). Air disinfection with ultra-violet radiation. *Spec. Rep. Ser. med. Res. Counc., Lond.*, No. 283.

BEDFORD, T. (1950). Heating and ventilation. *Yugoslav Academy of Arts and Science, Zagreb.*

BEGG, N. D., SMELLIE, E. W. & WRIGHT, I. (1947). Dust control in a measles ward with note of sulphadiazine prophylaxis. *Brit. med. J.* i, 209.

BLOWERS, R., MASON, J. A., WALLACE, K. R. & WALTON, M. (1955). Control of wound infection in a thoracic surgery unit. *Lancet*, ii, 786.

BOURDILLON, R. B. & COLEBROOK, L. (1946). Air hygiene in dressing rooms for burns or major wounds. *Lancet*, i, 561, 601.

BOURDILLON, R. B., LIDWELL, O. M. & LOVELOCK, J. E. (1948). Studies in air hygiene. *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 262.

CRUMB, C. & WELLS, W. F. (1947). Air sampling performances. *J. Bact.* **54**, 277.

CVJETANOVIĆ, B. (1955). Aerogene infekcije, II. *Arhiv. za Higijenu rada*, **6**, 97.

CVJETANOVIĆ, B. (1956). Higijenska Ocjena Bakterijskog. *Zagadenja Zraka* (*thesis*), University of Zagreb.

WELLS, W. F. (1955). *Air-borne contagion and air hygiene.* Harvard University Press.

WILLIAMS, R. E. O. & HIRCH, ANN (1950). The detection of streptococci in air. *J. Hyg., Camb.*, **48**, 504.

WILLIAMS, R. E. O., HIRCH, ANN & LIDWELL, O. M. (1950). Bacterial contamination of air in underground trains. *Lancet*, i, 128.

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