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Potential Health Hazards from Microbial Aerosols in Densely Populated Urban Regions

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Aerosolized bacteria were recovered up to 930 m downwind of three sewage treatment plants in Jefferson County, Ky. This distance includes homes in the proximity of several hundred such plants in that county. Bacterial counts were elevated on foliage near activated sludge tanks; although these counts decreased rapidly, at 48 h after exposure they were significantly higher than the counts on unexposed leaves. The 50% lethal dose of aerosolized *Klebsiella pneumoniae* was comparable to the 50% lethal dose of a virulent clinical isolate, and enteric bacteria were recovered from the respiratory organs of mice after forced inhalation adjacent to an aerated sludge tank. The coliform density in the effluents of the plants tested was inversely related to the airborne bacterial load at those plants. This relationship was attributed to the correlation between effluent quality and extent of aeration of activated sludge. Wind direction and distance influenced the airborne counts, but the extreme variation in counts indicates that it is not possible to predict emission rates accurately in an open ecosystem. Airborne enteric bacteria also were isolated near a decorative fountain used by humans for wading. The discovery of these sources of aerosolized microorganisms from polluted waters in densely populated areas suggests that a potential health hazard may be created by the increased probability of inhaling and ingesting microorganisms of fecal origin.

There have been sufficient field studies demonstrating the dispersal of bacteria-laden aerosols from aerobic sewage treatment plants to assume that such facilities might be hazardous to plant workers or others exposed to the aerosols (6). However, there is an absence of consistent epidemiological data that verify or negate this assumption (4, 6, 7), and there are reasons to question a link between exposure to these aerosols and disease. For example, most of the pathogens from domestic sewage are assumed to be enteric bacteria, whose effective portal of entry is ingestion. Furthermore, there is evidence that aerosolized cells are damaged, which might affect the virulence of the bacteria distributed in these droplets (6). Even if one assumes that exposure to these aerosols creates a risk, it is difficult to predict the magnitude of that risk, since there are no models for predicting emission rates of the aerosols under the variety of environmental conditions prevailing at plant sites.

Jefferson County, Ky., which includes the large metropolitan area of Louisville, is an example of the regions concerned about this potential source of environmental pollution. Outside the area served by the Louisville metropolitan centralized treatment facility, all sewage treatment depends upon small units. There are over 400 such units in this county, many of which rely on activated sludge processes. Virtually every subdivision in this large county contains one treatment plant, and the aerated basins are commonly close to several residences. Figure 1 is a photograph of one such plant.

This study examined whether the risk of exposure to microbial aerosols in the vicinity of these facilities could be determined with sufficient accuracy to assist in public policy decisions regarding the design and location of such plants. To this end, aerosols were collected in close proximity upwind and downwind of three of these plants under a variety of environmental conditions, and the leaf surfaces of foliage plants in these same areas were tested for bacterial contents. In addition, the virulence of aerosolized bacteria was tested by direct exposure of experimental animals. In a related study, I tested the emission of aerosols from a decorative fountain that is an example of the additional sources of aerosols found in densely populated regions.

MATERIALS AND METHODS

Aerosol sampling. Samples of air upwind and downwind of the treatment plants were collected with disposable two-stage Andersen samplers (Andersen 2,000, Inc., Atlanta, Ga.). Each sample contained a total volume of 226 liters of air and was collected over a 10-min period. Total bacterial counts and total en-

FIG. 1. *Small activated sludge plant in a subdivision in Jefferson County, Ky., showing the proximity of aerated sludge to residences.*

teric bacterial counts were determined by running samplers in series at each collection site. The total bacterial count was the number of colony-forming units developing on plate count agar (Difco Laboratories) within 48 h at 37°C; the total enteric count was the number of colony-forming units which developed on mFC medium (Difco) under the same incubation conditions and identified as one of the species under group I *Enterobacteriaceae* in *Bergey's Manual of Determinative Bacteriology* (3). Identification was made by the API-20 method (Analytab Products, Inc., Carle Place, N.Y.) unless the assignment by these tests was equivocal; in such cases additional biochemical tests were used (5), or the colony was not included in the enteric count. The same techniques were used to collect samples downwind of the decorative fountain. Grab samples of the effluent of the plants and of the water in the pools that received the fountain spray were tested for total and fecal coliforms by the fecal coliform most-probable-number method (1).

Three treatment plants were selected for air sampling. Two of these facilities (referred to in this study as plants 1 and 2) are typical of the small activated sludge plants which are located in subdivisions and treat domestic sewage. The third (plant 3) is the largest facility in the area which is separate from the Louisville, Ky., centralized treatment facility, and it treats a combination of domestic and industrial wastes. From the viewpoint of this study, the major differences between the plants were the larger volume of the aerated tanks at plant 3 and the vigorous aeration provided by a mechanical aerator at this plant, compared with the diffused aeration at the other two plants.

The decorative fountain is part of a large popular recreational facility. The fountains splash into standing pools of recirculating water that are fed by city water only when the volume requires replenishing. There are stepping stones traversing the pools, and the pools were commonly used by visitors for wading during the period of this study.

Deposition and retention of aerosolized bacteria on leaf surfaces. Geranium plants and pepper plants in clay pots were placed upwind and downwind of an aerated basin at one of the treatment facilities, and the plants remained at these sites from May through September. Control geranium and pepper plants were maintained in an environmental chamber in the laboratory. At intervals, pairs of leaves from each plant were removed, and the total and enteric bacterial counts on the leaves were determined by the viable plate count method on plate count agar or mFC medium after the sample was macerated in sterile buffer. These counts included the number of bacteria per square centimeter for leaves immediately after they had been collected and for paired leaf samples which had been maintained after collection for 48 h at room temperature in a sterile petri dish. Except for the sterile container, this environment was considered typical of the conditions under which home garden products are maintained after collection.

Conditions affecting dispersal of aerosols with viable bacteria. Aerosol samplers were placed at distances up to 150 m upwind and 930 m downwind of the aerated sludge tanks. A total of 29 of the samples were collected at the same distances upwind and downwind of the tanks to provide comparative data with wind direction as the only variable. Samples at the sewage treatment plants were collected over a 2-year period, which permitted sampling over the variety of environmental conditions found in the central southeastern United States, except for the extremely cold months of the 1977–1978 winter, during which the collection system malfunctioned. Samples could be collected at the fountain only during the summer, when the fountains were turned on. Samples were placed 2 to 23 m from the fountain.

For each sampling period, the wind speed, light

intensity, relative humidity, and water and air temperatures were recorded while the samples were collected. Ozone levels were obtained from the records of the Jefferson County Air Pollution Board.

For the study of bacterial counts on exposed leaves, plants were placed from 3.5 to 12 m upwind and downwind of an aerated tank. The same environmental parameters noted above were recorded when the leaves were removed from the plants for processing.

Virulence of aerosolized bacteria. *Klebsiella pneumoniae*, a common isolate in aerosols from treatment plants, was selected for the assay of virulence. Confirmed isolates of *K. pneumoniae* recovered from an air sampler exposed at a treatment plant were prepared in physiological saline as an inoculum. The numbers of cells injected were calculated by turbidimetric methods. All injections were intraperitoneal in a volume of 0.25 ml. The maximum elapsed time between recovery at the plant site and inoculation was 72 h, the minimum time possible for isolation in pure culture, verification of identification, and preparation of inoculum.

Strain CFW mice (mixed sexes; 8 to 16 weeks old) were used to construct a 50% lethal dose curve. Each animal was weighed immediately before injection, and the dose was calculated as cells per gram of animal. A total of 121 mice were assayed. After inoculation, the animals were housed separately and maintained on laboratory chow and water ad libitum. Dead animals were autopsied and examined for gross pathology. The lungs and diaphragms of dead animals were also macerated and cultured; the API profiles of isolated colonies were compared with the profile of the original inoculum.

After the 50% lethal dose of the aerosolized *K. pneumoniae* was calculated, a comparable dose of a virulent strain of *K. pneumoniae* (recovered from the sputum of a hospitalized patient) was injected into 12 CFW mice in the dose determined as the 50% lethal dose for the aerosolized bacteria. Deaths were recorded for 7 days, and cultures from the tissues of dead animals were tested as described above.

An additional in vivo assay of virulence was performed by forcing the inhalation of air by 12 CFW mice in respirators at a sewage treatment plant. The respirators, constructed of chambers attached to a vacuum pump, were placed on catwalks directly above an activated sludge tank. Air was forced through the chamber by a vacuum source maintained at 8-inch (20.3-cm) mercury pressure for 30 min. Six animals, tested on six separate runs, were returned to the laboratory and observed for clinical symptoms for 14 days. An additional six animals were autopsied in the field immediately after exposure, and for each animal the respiratory tree from the pharyngeal-tracheal junction through the lungs was removed and inoculated in Trypticase soy broth (BBL Microbiology Systems). After incubation at 37°C for 24 h, samples of the broth were streaked onto mFC medium. Presumed enteric colonies were isolated on Trypticase soy agar (BBL) and identified by the API-20 method.

RESULTS

Aerosol samples. The total bacterial and enteric bacterial counts confirmed the assumption that activated sludge tanks are a source of aerosolized bacteria; however, my counts were generally lower than those reported by others who utilized reusable six-stage Andersen samplers. The highest enteric count in my study was 1,455 bacteria per m^3 at 3 m downwind of an aerated tank, whereas the highest enteric count upwind was 173 bacteria per m^3 at 3 m.

There was extreme variation in the bacterial counts at the three plants over the 2-year period, but there was a consistency in the differences among the plants and in the enteric bacteria recovered at all three plants. Table 1 shows that plant 3 had the highest-quality effluent and the highest enteric counts in airborne samples within 183 m of an activated sludge tank; plant 1 had the poorest-quality effluent and 24% less frequency of airborne enterics at 183 m downwind compared with plant 3 (183 m was used as the standard for this comparison, since that was the greatest distance samples could be taken at plant 3 without interference from a road). There were minor differences in the relative frequencies of the enteric species isolated, and five species predominated. These were *Enterobacter agglomerans*, *Enterobacter cloacae*, *K. pneumoniae*, *Citrobacter freundii*, and *Escherichia coli*. *Shigella flexnerii* was identified from two isolates at plant 2.

A total of 11 air samples were collected at the decorative fountain. Of these, 10 yielded enteric bacteria, with values ranging from 3 bacteria per

TABLE 1. *Frequency of isolates of enteric bacteria during a 2-year sampling period*

Plant	No. of samples	% of samples positive for airborne enteric bacteria[a]	Effluent quality[b]		Airborne enteric bacteria recovered
			Total coliform (%)	Fecal coliform (%)	
1	35	43	76	76	*E. cloacae* > *K. pneumoniae* > *E. agglomerans* > *E. coli* > *C. freundii* > *E. aerogenes*
2	38	37	80	57	*E. agglomerans* > *E. cloacae* > *C. freundii* > *K. pneumoniae* > *E. coli*
3	54	67	31	13	*E. agglomerans* > *K. pneumoniae* > *C. freundii* > *E. cloacae* > *E. coli*

[a] Percentage of samples yielding airborne enteric bacteria, using the arbitrary standard for positive of 5 enteric bacteria per m^3 at 183 m from an aerated tank.

[b] Percentage of grab samples with more than 100 total or fecal coliform bacteria per 10 ml.

m^3 for one sample at 9 m to too numerous to count for one sample at 3 m. Grab samples from the pool water showed that airborne enteric bacteria were recovered on any day that the pool contained coliforms; however, on 2 days airborne enteric bacteria were detected when the standing water was negative for coliforms. The standing pool water was assayed by the fecal coliform most-probable-number procedure (1), and 70% of the samples that were positive for total coliforms were also positive for fecal coliforms. Bacteria recovered from the aerosols dispersed by this fountain included *E. agglomerans*, *E. coli*, *K. pneumoniae*, and *S. flexnerii*.

Environmental effects on emission rates. The statistical relationships among the total and enteric counts and the environmental factors were tested by multiple regression (10). The predictor variables were direction (upwind or downwind, used as a discrete variable with a value of 0 for upwind and 1 for downwind), relative humidity (percent), air and water temperatures (degrees centigrade), wind speed (kilometers per hour), light intensity (lux), distance from source (meters), and ozone levels (microliters per liter). The counts on the two stages of the sampler were distributed approximately evenly, and the combined counts were used for the analysis. The regression analysis for the enteric counts is shown in Table 2. R^2, which indicates the proportion of variance explained by a linear relationship between the predictor and predicted variables, was significant for two plants. Distance was the only variable that influenced both of the significant regressions, but relative humidity and wind speed also contributed significantly at one plant. The comparable analysis for the total counts yielded a significant regression only at plant 3 ($P < 0.01$), and distance was the only factor that appeared to contribute to this significance. When the standardized residuals were plotted against the predicted variables, there appeared to be a slight deviation from a linear model. Since the shape of the scatter plots indicated greater variability at higher counts, a regression analysis was done on transformed data by using the formula $Y = 1/(X + 1)$, where Y equals predicted variables and X equals observed predictor variables; the resultant plots were compatible with a linear model, and there was no dramatic change in the regression. For example, the enteric counts at plant 3, untransformed, yielded $R^2 = 0.336$ and $F_s = 3.162$ ($P < 0.005$) (F_s = sample values of Fisher's statistic for analysis of variance); transformation yielded $R^2 = 0.410$ and $F_s = 4.344$ ($P < 0.001$). Therefore, the untransformed data were used for the analysis, and a linear regression model was assumed to be valid.

Since wind direction did not have a significant

TABLE 2. *Environmental influences on the dispersal of aerosolized enteric bacteria*

Plant	R^2	F_s (df)	P (F_s)	Variable	t	P (t)
1	0.178	0.758 (8, 28)	$0.5 < P < 0.75$	Direction[a]	1.04	NS[b]
				Relative humidity	0.37	NS
				Air temp	1.17	NS
				Wind speed	0.17	NS
				Light intensity	0.55	NS
				Distance	1.19	NS
				Water temp	1.32	NS
				Ozone level	0.50	NS
						NS
2	0.419	3.959 (8, 44)	<0.002	Direction	1.56	NS
				Relative humidity	3.27	<0.01
				Air temp	1.97	NS
				Wind speed	2.45	<0.02
				Light intensity	1.68	NS
				Distance	2.90	<0.01
				Water temp	1.91	NS
				Ozone level	2.04	NS
3	0.336	3.1621 (8, 50)	<0.005	Direction	1.52	NS
				Relative humidity	0.49	NS
				Air temp	1.40	NS
				Wind speed	0.19	NS
				Light intensity	1.43	NS
				Distance	3.14	$0.001 < P < 0.01$
				Water temp	0.75	NS
				Ozone level	1.46	NS

[a] Upwind or downwind.
[b] NS, Not significant.

influence when all variables were combined, separate analyses were done for the 29 paired samples at 6, 9, 15, 23, 46, 92, and 138 m upwind and downwind of the three plants. Of the downwind samples, 62% were positive for enteric bacteria, compared with 41% of the upwind samples; the maximum upwind and downwind recoveries were 44 and 1,323 bacteria per m^3, respectively, and the mean upwind and downwind recoveries were 3 and 70 bacteria per m^3, respectively. Despite these indications of a downwind influence, there was extreme variation in the downwind data, and both the parametric t test and the nonparametric Wilcoxon signed rank test for paired data (10) failed to show significant differences between upwind and downwind samples at the 95% level of confidence.

Deposition and retention of bacteria on foliage. Table 3 summarizes the total and enteric counts on foliage maintained upwind and downwind of the aerated tanks, and Table 4 shows the statistical analysis of the differences between counts on exposed and control leaves. The differences between total and enteric counts for downwind samples were highly significant when tested by a parametric statistic allowing for unequal variance and by the nonparametric Mann-Whitney test. The total count, but not the enteric count, of the upwind samples was significantly different from that of the control, but the average count for the upwind enteric samples was considerably lower than the average count for the downwind samples.

TABLE 3. *Total and enteric bacterial counts on foliage upwind and downwind of aerated tanks*

No. of levels tested	Location[a]	Assay[b]	% of positive samples[c]	Range (cells/cm^3)[d]
16	Upwind	Enteric	81	33–20,000
19	Downwind	Enteric	100	100–83,000
27	Control	Enteric	30	33–47
18	Upwind	Total	95	130–28,000
19	Downwind	Total	100	1,000–62,000
31	Control	Total	81	33–630

[a] Each location was upwind or downwind of an aerated basin.

[b] The samples were tested for total enteric counts or total bacterial counts.

[c] Percentage of leaves yielding either enteric or total counts over the sampling period.

[d] Range of counts of all samples tested.

A multiple-regression analysis for the influence of environmental factors (relative humidity, atmospheric temperature, wind speed, and distance) was performed on the total and enteric counts of upwind and downwind leaf samples. The regression coefficients were not significant, but that for downwind samples was close to significance ($0.5 < P < 0.10$). Wind speed was the only predictor variable with a consistent influence on the counts for total and enteric counts. Distance did not contribute significantly to the counts at the downwind sites (3.5 to 12.0 m from the aerated tank).

There was a rapid decrease in both enteric and total bacterial counts at 48 h after the leaves were removed from the field. Table 5 summarizes the analysis by the Wilcoxon signed rank test for samples tested after 48 h paired with leaves assayed immediately after exposure. Although the numbers declined sharply by 48 h, the residue was still considerably higher than the counts on unexposed leaves (Table 4).

Virulence of aerosolized bacteria. Of 121 mice injected with aerosolized *K. pneumoniae*, 28 died, and organs of all dead animals yielded

TABLE 4. *Bacterial contamination of foliage exposed to bacterial aerosols*

Sample	No. of cells/cm^2 of the leaf surface			t test		Mann-Whitney test	
	Mean	Standard deviation	Median	t	P	W	P
Downwind							
Total bacterial count, sewage treatment plant	20,500	19,186	13,000	4.617	<0.0001	437	<0.0001
Total bacterial count, control	176	203	100				
Enteric bacterial count, sewage treatment plant	18,463	25,084	4,700	3.201	<0.005	247	<0.007
Enteric bacterial count, control	42	4	40				
Upwind							
Total bacterial count, sewage treatment plant	4,088	7,035	1,300	2.359	<0.03	364	<0.0003
Total bacterial count, control	174	182	100				
Enteric bacterial count, sewage treatment plant	2,406	5,295	365	1.794	<0.09	208	<0.07
Enteric bacterial count, control	31	16	33				

TABLE 5. *Retention of bacteria on foliage at 48 h after exposure to bacterial aerosols*

Type of count	Time after exposure (h)	Count		P^a
		Mean	Standard deviation	
Total bacteria	0	13,270	14,985	$0.01 < P < 0.025$
Total bacteria	48	5,620	8,639	
Enteric bacteria	0	17,981	30,485	<0.0039
Enteric bacteria	48	1,066	1,292	

[a] The difference between samples assayed at 0 and 48 h was tested by the Wilcoxon signed rank test.

K. pneumoniae with an API profile matching that of the inoculum. The 50% lethal dose of the aerosolized *K. pneumoniae*, as calculated by a modified Reed-Muench analysis, was 14.7×10^6 cells per g of mouse. This dosage of the virulent *K. pneumoniae* obtained as a fresh clinical isolate was lethal to 45% of the mice tested, and tissues of each of these animals also yielded *K. pneumoniae* matching the API profile of the inoculum. The API biotypes of the aerosolized and hospital-derived strain were similar, but the antibiotic sensitivity profiles showed that the environmental strain was sensitive and the hospital strain was resistant to most of the antibiotics tested.

Six mice forced to inhale air at a sewage treatment plant and observed for 2 weeks did not exhibit any clinical symptoms, and cultures of their respiratory organs after 2 weeks were negative for enteric bacteria. Cultures of the respiratory organs of three of the six mice sacrificed immediately after exposure contained enteric bacteria, including *E. coli*, *E. agglomerans*, and *K. pneumoniae*.

DISCUSSION

This study confirms, in the case of the small treatment plants, the presence of airborne bacteria from aerated sewage. I believe that the comparatively low counts in this study were due to the use of disposable two-stage Andersen samplers. The only apparent difference between my techniques and those of comparable studies in which Andersen samplers were used was the use of the six-stage reusable sampler in those studies. I compared total counts on a two-stage sampler with total counts on a six-stage reusable sampler and consistently obtained higher counts on the six-stage device. Since the differences in counts between the samplers was constant in this comparison, the use of data from the two-stage sampler was considered valid for the statistical analyses performed.

It is particularly noteworthy that enteric bacteria were isolated frequently from upwind samples taken close to aerated sludge tanks. Since this study was not designed to prove that aerated sewage treatment creates aerosols, the upwind sites were not selected as background controls, but to determine the extent that local turbulence might distribute the aerosols upwind within the perimeter of a treatment plant. Aerosol samples collected at least 10 km from any known source of aerated sewage seldom yielded enteric bacteria; therefore, the enterics collected upwind and downwind at a plant site were considered to be from the sludge tanks.

These results indicate that the variation in bacterial counts over a long period of time is so great that one cannot construct a statistically reliable model of emission rates within the perimeter of a plant, although the probability of contact with sewage-derived bacteria increases with proximity downwind of the source. The inability to attribute statistical significance to such factors as relative humidity and temperature does not mean that these factors have no influence on emission patterns. For example, in a recent study of coliforms emitted from effluent sprays, Teltsch and Katznelson (11) studied the effects of relative humidity and solar irradiation by using a design that essentially removed the effects of variation from other factors. Under these conditions, there was a positive correlation between bacterial counts and relative humidity and a negative correlation with solar irradiation. However, under nonstandardized conditions there are extreme variations in the counts and essentially nonlinear fluctuations in the predictor variables.

The hazards associated with exposure to airborne enteric microorganisms are not known, but the risk cannot be disregarded, based on available evidence (6, 7). This study indicates that persons residing or working close to such sewage plants may have an increased probability of contact with enteric organisms, but the distance beyond which there is no risk of exposure from such a source cannot be stated precisely, and the risk cannot be specified as a function of environmental conditions in an open ecosystem. This study also emphasizes one of the dilemmas in controlling environmental pollution from aerated treatment processes. The quality of the effluent, which is critical in considering contamination of receiving streams, is partially dependent upon the extent of aeration (2), but the large mechanical agitators commonly used to increase aeration also create greater numbers of aerosolized bacteria (Table 1).

The deposition and retention of bacteria on foliage near these facilities is reason to extend the concern over such contamination beyond the relatively restricted environments that have been reported (7). These results are compatible with the implications of the studies of Knittel et al. (8) on *Klebsiella* of vegetables near pulp treatment plants. The rapid decline in bacterial counts in this study compared with the increasing numbers Knittel et al. obtained may be explained by the differences in techniques of exposure and incubation. The potential exposure of humans to enteric pathogens by ingestion of edible products grown near treatment plants, added to the increased probability of inhalation of aerosolized organisms by persons residing in the same vicinity, is an additional reason to question the policy of locating such plants in densely populated areas. A number of the residences observed in this study had vegetable gardens well within the distance of aerosol dispersion from the activated sludge tanks.

The results of the 50% lethal dose assays of aerosolized *Klebsiella* indicate that the virulence of such cells (tested by intraperitoneal inoculation) is not diminished by this method of dispersion. The related study on direct exposure of mice at a plant is evidence that aerosolized microorganisms are deposited in the respiratory tract. Although clinical disease did not follow such exposure in these animals, the combined finding of virulence and deposition of *Klebsiella* is presumptive evidence of a potential risk to susceptible hosts, as might be found in humans with compromised immunological defenses. These studies should be considered with the results of Pereira and Benjamison (9), who obtained lesions in guinea pigs with aerosolized *Mycobacterium*, and with the findings of Knittel et al. (8), who found that *Klebsiella* retained its virulence after prolonged exposure in a botanical environment.

The recovery of fecal bacteria from the aerosols generated by a decorative fountain does point to an unnecessary potential health hazard in many communities. Although the solution to the problem of aerosols from treatment plants is complicated by the need for sewage treatment, there is no compelling reason for permitting contamination of such decorative fountains by humans. In Louisville, Ky., this study was presented in evidence for an ordinance which now prohibits wading in such pools. Although such measures do not prevent contamination by other sources, they do reduce the particular risk associated with microorganisms of human origin.

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