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PAPER

PATHOLOGY/BIOLOGY

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Air, Water, and Surface Bacterial Contamination in a University-Hospital Autopsy Room*

ABSTRACT: Today, little is known about the bacteriological environment of the autopsy room and its potential interest for medico-legal practices. Seven hundred fifty microbiological samples were taken from surface (n = 660), air (n = 48), and water (n = 42) to evaluate it in a French University Forensic Department. Median bacterial counts were compared before and during autopsy for air samples, and before and after autopsy for surface samples, using Wilcoxon matched pairs signed ranks test. Bacterial identification relied on traditional phenotypic methods. Bacterial counts in the air were low before autopsy, increased significantly during procedure, and seemed more linked to the number of people in the room than to an important production of aerosol-containing bacteria. Despite cleaning, human fecal flora was omnipresent on surfaces, which revealed insufficient disinfection. Bacteriological sampling is an easy way to monitor cleaning practices in postmortem rooms, but chiefly a way to improve the reliability of medico-legal proofs of infectious deaths.

KEYWORDS: forensic science, autopsy, postmortem bacteriology, environmental microbiology, hygiene, disinfection, practice management

The European and French legislations relative to the practice of medico-legal autopsy have evolved during the last decade showing the determination of health-policy decision makers to develop strategies to improve forensic practices and control its risks (1–4). The forensic scientists and personnel involved in postmortem examinations are currently exposed to various infectious agents from the dead body (5). Occupational exposures to infectious diseases such as the human immunodeficiency virus, hepatitis C virus, hepatitis B virus, or tuberculosis are now well documented (6–8). The environment of an autopsy room can also be contaminated by other infectious agents from the cadaver. Babb et al. (9) have suggested that bacteriological sampling of air and surfaces might be interesting to point out the role of the environment in the spread of infection. Although they have finally concluded that such an environmental contamination poses little risk of infection for the staff provided basic hygienic precautions, they underlined the fact that some microorganisms present in the autopsy room environment and coming from the cadaver, including Gram-negative bacilli, may remain present despite cleaning/disinfection (9,10). These infectious agents might be linked to the contamination of samples taken from the cadaver for diagnosis purposes, including those taken to look for an infectious cause of death.

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Today, there seems to be no information about the prevalence and the nature of such contamination of the environment in the autopsy room. Here, we report a study on the bacterial contamination of the environment of an autopsy room in a French university forensic department and assess the risk of contamination of forensic samples.

Materials and Methods

This prospective study was conducted between March 3 and May 10, 2008 in a university forensic department (Lyon, France) that had two autopsy rooms. In the main room that was 62 m^2 large, there were three necropsy tables used for autopsy of fresh cadavers. In the adjacent room of 16.5 m^2, a necropsy table was dedicated to the examination of putrefied bodies. Both rooms were equipped with an air conditioning system. The ventilation rates were, respectively, 12 air changes per hour (ACH) in the main autopsy room and 16 ACH in the adjacent room. Incoming and outcoming air was, respectively, filtered through air filters G4 and fine air filters F9, as defined in the New European Standard for Coarse and Fine Filters (EN779).

Air Sampling

Air samples were collected using a Biomérieux Air Ideal Biocollector (Lyon, France) to analyze 250 L of sampled air (2.5-min samples at 100 L/min). The sampler was placed 1 m above the median part of each necropsy table and the samples taken before autopsy and during autopsy at bowel removal.

All air samples were collected twice: once on culture dishes containing a nonselective bacterial growth medium (Trypticase soy

agar [TSA]), and once on dishes with tryptose sulfite cycloserine agar (TSC) to detect sulfite-reducing anaerobes.

Surface Sampling

Surface samples were collected from necropsy tables (n = 14), draining boards (n = 14), dissection boards (n = 6), instrument trays (n = 8), scales (n = 10), blocks (n = 10), and reusable aprons (n = 10). Each sample was taken three times: once using a 25-cm^2 nutrient agar contact plate (25-cm^2 CP) to count the bacterial colonies and twice using swabs to be cultured separately on TSA and on TSC. For each necropsy table, the sampling was made in three different sites, respectively, in upper, median, and lower parts, to collect nine samples per necropsy table at every time point. Reusable gloves (n = 8) and boots (n = 7) were only sampled twice, using only swabs. All surfaces were sampled both before autopsy after cleaning and disinfection and after autopsy before cleaning and disinfection.

Water Sampling

Five hundred milliliter water samples were collected from water sources after 1-min purging periods using sterile recipients containing 0.5% sodium thiosulfate. Two plate count agars were inoculated with 1 mL of each sample. A membrane filtration procedure for enumerating *Pseudomonas aeruginosa* was also followed for each sample. One hundred milliliters of each sample was first filtered through membrane. The membrane filter was then transferred to the surface of a cetrimide agar plate.

Bacteriology Analyses

Analyses of the Air Samples—TSA were incubated aerobically at 37°C for 24 h. TSC were incubated anaerobically at 46°C for 24 h. Whenever possible, bacterial colonies were counted as colony-forming units (CFUs). Bacteria grown on TSA were subcultured aerobically on bromocresol purple agar (BCP) at 37°C for 24 h. Those grown on TSC were also subcultured, both aerobically and anaerobically, on blood nutrient agar at 37°C for 24 h. Bacterial identification relied on traditional phenotypic methods, using Gram staining, culture, and biochemical methods. Presumptive *Staphylococcus aureus*-positive cultures were confirmed by coagulase tests. The identification of Gram-negative bacilli and sulfite-reducing anaerobes was made using Analytical Profile Index API® 20E and API® 20A systems, respectively (Biomérieux).

Analyses of the Surface Samples—Nutrient agars were incubated aerobically at 37°C for 24 h. Concerning TSA and TSC, they were incubated using the same procedure described earlier for the air samples. Bacteriological analyses for the identification of species were also the same than those described for the air samples.

Analyses of the Water Samples—The two plate count agars were incubated separately, once at 22°C during 72 h and once at 37°C during 48 h. The cetrimide agar plate was incubated at 41°C during 48 h. Bacterial colonies were counted as CFUs from plate count agars, and *P. aeruginosa* colonies were counted as CFUs from cetrimide agar plate.

Statistical Analysis

Median bacterial counts from air samples taken before and during autopsy were compared using Wilcoxon matched pairs signed-rank tests. The same statistical nonparametric tests were used to compare the median bacterial counts between surface samples before and after autopsy.

The analyses used SPSS software for Windows, version 12.0 (SPSS Inc., an IBM Company, Chicago, IL).

Results

Overall, 750 samples were collected among which 660 (88%) were taken from surfaces, 48 (6.4%) from air, and 42 (5.6%) from water, as shown in Table 1.

Air Contaminants

Table 2 shows the median CFUs found in the air samples collected on TSA from the autopsy rooms before or during postmortem procedures. The median CFUs before autopsy were rather low whatever the autopsy room considered. Most isolated organisms were environmental saprophytes, and their distribution did not differ between the two autopsy rooms: *Bacillus* sp. (33.3% of all preprocedural bacterial flora), *Staphylococcus nonaureus*

TABLE 1—*Distribution of the surface, air, and water samples, according to the type of sampling.*

	Before Autopsy N Samples		During Autopsy N Samples		After Autopsy N Samples		Total N Samples
Surface sampling	CP	Swab			CP	Swab	660
Necropsy table (n = 14)							
Main autopsy room (n = 9)	27	54			27	54	162
Adjacent autopsy room (n = 5)	15	30			15	30	90
Draining board (n = 14)	14	28			14	28	84
Dissection board (n = 6)	6	12			6	12	36
Instrument tray (n = 8)	8	16			8	16	48
Scale (n = 10)	10	20			10	20	60
Block (n = 10)	10	20			10	20	60
Reusable apron (n = 10)	10	20			10	20	60
Reusable glove (n = 8)	0	16			0	16	32
Boots (n = 7)	0	14			0	14	28
Air sampling	TSA	TSC	TSA	TSC			48
Main autopsy room (n = 9)	9	9	9	9			36
Adjacent autopsy room (n = 3)	3	3	3	3			12
Water sampling							42
Main autopsy room (n = 11)	33						33
Adjacent room (n = 3)	9						9

TABLE 2—*Median number of colony-forming units (CFUs) on TSA from air samples made before and during autopsy.*

	Before Autopsy			During Autopsy			
Place	Samples	Median CFUs	Range of CFUs	Samples	Median CFUs	Range of CFUs	*p*
Main autopsy room (fresh cadavers)	9	5	2–37	9	35	16–19	0.01
Adjacent autopsy room (putrefied cadavers)	3	7	2–25	3	30	12–46	0.2

TABLE 3—*Median [range] colony-forming units (CFUs) per sample from various surfaces before and after autopsy.*

Sampled Surface	Before Autopsy	After Autopsy
Necropsy table—fresh cadaver (n = 9)	15 [1–1000]	>500 [30–1000]
Necropsy table—putrefied body (n = 5)	2 [0–26]	>500
Draining board—clean sector (n = 6)	5 [5–38]	50 [1–1000]
Draining board—dirty sector (n = 8)	4 [0–120]	>500
Dissection board (n = 6)	3 [0–100]	>500
Instrument plate (n = 8)	11 [4–1000]	>500
Scale (n = 10)	3 [1–35]	>500
Block (n = 10)	67.5 [1–1000]	>500 [200–1000]
Apron (n = 10)	1 [0–30]	>500 [150–1000]

(57.1%), and *Pseudomonas* sp. (4.8%). *Enterococcus fecalis* was isolated once, but these did not exceed 1 CFU per 250 L of sampled air. During autopsy, the median CFUs were significantly higher in the main autopsy than in the before autopsy room (p = 0.01). Besides *Enterobacter cloacae*, the Gram-negative bacilli found belonged to genera *Serratia*, *Kluyvera*, and *Pantoea*. No sulfite-reducing anaerobe was isolated neither before nor during postmortem procedures.

Surface Contaminants

The results of surface sample analyses are shown in Tables 3 and 4.

Quantitatively, few surfaces were heavily contaminated before autopsy and after cleaning/disinfection. The median CFU counts from most sampled surface points before autopsy and after cleaning/disinfection varied from 1 to 15 CFUs per sample, except head-support for which the median was 67.5 CFUs per sample. The isolated organisms were largely environmental saprophytes, including *S. aureus*, coagulase-negative staphylococci, nonpathogenic streptococci, *Bacillus* sp. *Pseudomonas* sp., and *Enterococcus* sp. Gram-negative bacilli and sulfito-reducing aerobes were also isolated, but in lower number.

On necropsy tables, only three tables of nine showed the same degree of contamination at their upper, median, and lower parts before autopsy and after cleaning/disinfection. For the six other necropsy tables, no correlation was found between the degree of contamination of their three parts. Less than 9% of the samples had more than 10 CFUs of Gram-negative bacilli per CP. Eleven percent of the samples were contaminated with sulfito-reducing aerobes, but none of them exceeded 10 CFUs per sample. However, surface contamination with human fecal flora including *Enterococcus* sp., Gram-negative bacilli, and sulfito-reducing aerobes was qualitatively found in most samples before autopsy, but scale, if considering only the presence of at least one of these bacteria, whatever its quantity. Autopsy tables, instrument plates, and dissection boards were the more often contaminated surfaces with fecal flora before autopsy and after cleaning/disinfection (89%, 75%, and 67% of samples, respectively), whereas only half other surfaces were contaminated (50%).

Postprocedural median CFUs were significantly higher than preprocedural median CFUs, whatever the surface considered (Wilcoxon matched pairs signed-rank test, $p < 0.01$), exceeding 500 CFUs per sample in most surfaces. Almost 75% of the samples had more than 10 CFUs of Gram-negative bacilli per CP. Postprocedural bacterial flora did not differ qualitatively from the preprocedural one but appeared more variate. The number of bacterial saprophytes was smaller. Most isolated bacteria were Gram-negative bacilli, and some species were found only after autopsy such as *Strenotrophomonas maltophilia*, *Clostridium botulinum*, *Acinetobacter* sp., and *Prevotella* sp.

Water Contaminants

The results of water sample analyses are shown in Table 5. The median bacterial count from water samples at 22°C was 800 CFUs/mL. The total bacterial count at 22°C varied from 90 to 9000 CFUs/mL; it was more than 100 CFUs/mL in 39 samples (92.9% of all samples) and exceeded 1000 CFUs/mL in 12 samples (28.6%). At 37°C, the median bacterial count was 340 CFUs/mL. The total bacterial count ranged from 35 to 2000 CFUs/mL, exceeding the standard threshold of 10 CFUs/mL. It was higher than 100 CFUs/mL in 36 samples (85.7%). *P. aeruginosa* was isolated in 6 (14.3%) of the water samples, in excess to 500 CFUs/per mL. No fecal contamination was found in water samples whatever the incubation temperature.

Discussion

The present study describes the bacterial flora present in an autopsy room in a rather original way, using environmental microbiological sampling from air, water, and surfaces contaminants.

Regarding the air contamination, the bacteria present before autopsy and after cleaning/disinfection were mostly environmental saprophytes, but *E. fecalis* that was isolated only once in a small number. Before autopsy, air samples were collected several hours after cleaning. If dispersion of microorganisms into the air is known to be unlikely during wet cleaning methods, Gram-negative bacilli usually die on dry air. The results showed a significant increase in the median bacterial counts before and during autopsies of fresh bodies. It is well established that people activity and moving equipment within close spaces are the main factors of spread of airborne bacterial contamination (9–12), another important emission source of bacterial pollutants being the air conditioning systems (13). Consequently, aerosol-containing bacteria in the air samples collected during autopsy were more likely associated with the number of people present in the room than with a direct aerosol from the cadaver during the procedure because fecal bacteria were isolated only in 25% of the samples and in small numbers (<6 CFUs per 250 L of sampled air). This increase was not found in the adjacent room where less staff usually works. These results confirmed previous studies by Babb et al. (9) and Newsom et al. (10).

TABLE 4—*Distribution of Gram-negative bacilli and sulfito-reducing aerobes on surfaces before and after autopsy. The values are numbers (percentages).*

		Gram-Negative Bacilli		Sulfito-Reducing Aerobes	
Sampled Surface	CFU per 25 cm^2 CP	Before	After	Before	After
Necropsy table (fresh cadaver)					
Upper part (n = 9)	0	5 (55.6)	2 (22.2)	9 (100)	6 (66.7)
	1–10	3 (33.3)	2 (22.2)	0	1 (11.1)
	>10	1 (11.1)	5 (55.6)	0	2 (22.2)
Median part (n = 9)	0	4 (44.4)	0	8	5 (55.6)
	1–10	5 (55.6)	1 (11.1)	1 (11.1)	2 (22.2)
	>10		8 (88.9)	0	2 (22.2)
Lower part (n = 9)	0	7 (77.8)	0	8 (88.9)	6 (66.7)
	1–10	2 (22.2)	2 (22.2)	1 (11.1)	1 (11.1)
	>10		7 (77.8)	0	2 (22.2)
Necropsy table (putrefied cadaver)					
Upper part (n = 5)	0	4 (80.0)	3 (60.0)	5 (100)	5 (100)
	1–10	1 (20.0)		0	
	>10		2 (40.0)	0	
Median part (n = 5)	0	5 (100)	2 (40.0)	5 (100)	4 (80.0)
	1–10	0	1 (20.0)	0	1 (20.0)
	>10	0	2 (40.0)	0	
Lower part (n = 5)	0	5	2 (40.0)	5 (100.0)	4 (80.0)
	1–10	0	1	0	1 (20.0)
	>10	0	2 (40.0)	0	
Dissection boards (n = 6)	0	5 (83.3)	0	5 (83.3)	5 (83.3)
	1–10	0	0	1 (16.7)	1 (16.7)
	>10	1 (16.7)	6 (100)	0	0
Draining boards—dirty sector (n = 8)	0	6 (75.0)	1 (12.5)	7 (87.5)	6 (75.0)
	1–10	1 (12.5)	0	1 (12.5)	2 (25.0)
	>10	1 (12.5)	7 (87.5)	0	0
Draining boards—clean sector (n = 6)	0	5 (83.3)	5 (83.3)	6 (100)	6 (100)
	1–10	0	0	0	0
	>10	1 (16.7)	1 (16.7)	0	0
Instrument plate (n = 8)	0	3 (37.5)	2 (25.0)	6 (75.0)	7 (87.5)
	1–10	2 (25.0)	0	2 (25.0)	0
	>10	3 (37.5)	6 (75.0)	0	1 (12.5)
Scale (n = 10)	0	10 (100)	1 (10.0)	10 (100)	9 (90.0)
	1–10	0	0	0	1 (10.0)
	>10	0	9 (90.0)	0	0
Blocks (n = 10)	0	7 (70.0)	0	8 (80.0)	7 (70.0)
	1–10	1 (10.0)	0	2 (20.0)	3 (30.0)
	>10	2 (20.0)	10 (100)	0	0
Apron (n = 10)	0	10 (100)	0	7 (70.0)	5 (50.0)
	1–10	0	0	3 (30.0)	4 (40.0)
	>10	0	10 (100)	0	1 (10.0)

TABLE 5—*Distribution of the total bacterial count from water samples, in colony-forming unit per mL (CFUs/mL), according to the incubation temperature.*

Total Bacterial Count (CFUs/mL)	22°C Incubation	37°C Incubation
≤10	0	0
11–100	3	6
101–1000	27	36
>1000	12	0

Regarding the surfaces, before autopsy and after cleaning/disinfection, all the median bacterial counts were lower than the 25 CFUs per sample threshold defined by the Guide du Bionettoyage (14) as a medium infectious risk in hospital rooms.

Comparisons are difficult to make with other studies because only one study (9) has already focused on environmental bacterial contamination of autopsy rooms. This study was carried out 20 years ago in 30 British hospitals and coroners' postmortem rooms. It reported only on surface contamination by Gram-negative bacilli. Its authors concluded that very few surfaces were heavily contaminated before autopsy and that more than 90% of samples lacked Gram-negative bacilli.

Here, the proportion of surfaces contaminated with Gram-negative bacilli before autopsy was slightly higher than that reported by Babb et al. (9), as we reported 16% of surface samples showing at least 1 CFU of Gram-negative bacilli. We also showed that human fecal flora were omnipresent before autopsy and after cleaning/disinfection whatever the surfaces, which may be due to insufficient cleaning/disinfection.

Although the considered forensic medicine department has written disinfection protocols in line with the national regulations (use of bactericidal disinfecting and deterging agent), insufficient cleaning/disinfection may be partly explained by a lack of housekeeping staff and an important workload (750 autopsies per year) that generate mispractices and neglects. In theory, cleaning standard operating procedures differed according to the surfaces considered. The instruments used for postmortem examination were placed in a plastic container with Hexanios G+R® solution (ANIOS, Lille, France) at the 0.5% dilution, before cleaning. The instruments that could be autoclaved were sterilized at 124°C for 50 min. All surfaces were cleaned using Cleaning Disinfecting ND 610 S LE

VRAI® (ACTION PIN, Dax, France), with either manual brushing (head supports, instrument trays, aprons, gloves) or pressure washer (necropsy tables, draining boards, and dissection boards), before rinsing and active drying.

Actually, mispractices were unveiled over the study month period. In-use disinfectant concentrations were often lower than those recommended by the manufacturer. Besides, active drying was almost always neglected and bacterial multiplication could continue on moist surfaces let to dry passively. Another cause could be the use of draining boards as dissection boards by some pathologists.

If hospital environment is rarely implicated in disease transmission except among immunocompromised patients, it is well known that health care–associated infections might occur in the case of inadvertent exposures to environmental or airborne pathogens (15,16). At hospital, environmental sampling strategies are commonly used to prevent nosocomial infections, evaluate infection control efforts, and to elaborate and update recommendations for cleaning/disinfection (16,17). In forensic laboratory environment, the objective is quite different and cannot be "zero tolerance" for bacterial contamination. However, bacteriology sampling is an easy method to check cleaning practices in postmortem rooms where various infectious agents are likely to contaminate the environment and increase occupational exposure. In our results, bacterial contamination of surfaces before autopsy was quantitatively low but human fecal flora was omnipresent whatever the surfaces, which revealed insufficient cleaning during the study period. This triggered actions to improve disinfection practices and checks were planned to assess their effectiveness.

In forensic medicine, occupational exposure to blood-borne microorganisms is well documented, but the objective of the present study was not to discuss the occupational risks but forensic sample contamination risks. Postmortem bacteriology is often ordered during autopsy, and its importance in forensic practices has been already demonstrated (18). If such procedure may be relevant in respect to epidemiological considerations (19), it can also be required in medico-legal expertises, to isolate pathogenic microorganisms and to establish or disprove with a high degree of reliability a causal relationship between infection and death. The diagnostic value of postmortem bacteriology is often discussed because various factors are known to generate biased results (18,19), especially sample contamination that may occur from body colonization before death (surfaces, organs, or fluids) but also from inappropriate techniques or transport media. Ensuring the validity of postmortem microbiological results requires great care about the conditions of sample collection and to systematically confront them to histological findings.

Conclusion

Bacteriological sampling appeared to be an easy way to monitor cleaning practices in postmortem rooms. The microorganisms that remain present in the environment after disinfection can potentially contaminate the samples taken from the cadaver. Knowing the environmental bacterial flora present at the moment of sample collection may help interpreting postmortem bacteriology when the isolated bacteria are opportunistic species. Bacteriological sampling of the environment should be considered an easy way to improve the value of medico-legal conclusions in case of infectious deaths.

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