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Original Article

Bacteria as Normal Flora in Postmortem Body Fluid Samples

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Bacterial culture and identification are both useful in the clinical and forensic fields, although the postmortem changes in human microbiology are poorly understood. This study aimed to identify bacteria that were considered normal flora in postmortem body fluid samples. Bacterial culture and identification testing were performed for 336 body fluid samples (e.g., cardiac blood, peripheral blood, pericardial fluid, pleural fluid, peritoneal fluid, cerebrospinal fluid, and urine) from 129 forensic autopsy cases. Bacteria were identified using both genetic and biochemical methods, and testing for C-reactive protein (CRP) was used to identify the presence of antemortem inflammation. Among the 129 autopsy cases, 79 cases (69.3%) were negative for CRP, and bacterial culture and identification testing were performed for 185 samples from those 79 cases. Bacteria that were considered both normal flora and pathogens were identified in the CRP-negative cases. Therefore, the results from postmortem bacterial culture and identification testing should be interpreted in the context of other postmortem examination, including CRP testing. Furthermore, case selection, postmortem testing, and interpretations of the results should be performed by both clinical bacteriologists and forensic pathologists. To best of our knowledge, this is the first study to examine normal flora in various postmortem body fluid samples form Korean autopsy cases.

Key Words: Bacteria; Body fluids; Postmortem changes; Autopsy

Introduction

Bacterial culture and identification are useful in the clinical and forensic field [1], although there are many concerns regarding postmortem bacterial culture and identification. Furthermore, there are no specific guidelines, recommendations, or checklists that address the standards for performing that type of testing [1]. The Human Microbiome Project has revealed that an adult body contains approximately ten times more microbial cells than human cells [2], although it is unclear what happens to these microbial cells after a person dies. During the antemortem period, the immune system ensures that bacteria are not present in most internal organs and body fluids (e.g., the blood and cerebrospinal fluid). However, the immune system fails

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after death, and bacterial proliferation starts in the ileocecal area and spreads to the liver and spleen and then continues to the heart and brain [3]. The present study aimed to identify bacteria that were considered normal flora in postmortem body fluid samples, which could help improve the interpretation of postmortem bacterial culture and identification results.

Materials and Methods

Bacterial culture and identification testing were performed using 336 body fluid samples (e.g., cardiac blood, peripheral blood, pericardial fluid, pleural fluid, peritoneal fluid, cerebrospinal fluid, and urine) from 129 forensic autopsy cases that were encountered between June 2016 and September 2017. All forensic autopsies were performed with a court-issued warrant that was requested by the public prosecutor. The authors and their institution have judged the study to be exempt from ethical approval.

All samples were obtained during autopsy using sterile syringes, with great care taken to avoid contamination. The samples were then inoculated into the transport medium as soon as possible and delivered to the laboratory. Cardiac blood was drawn from the inferior vena cava after opening the pericardium. Peripheral blood was drawn from the iliac vein after opening the peritoneal cavity. Pericardial fluid was obtained in the pericardial sac after lifting the apex of the heart. Pleural fluid was obtained in the peritoneal cavity (mainly from the paracolic gutters, parameseneric gutters, and Douglas pouch). Cerebrospinal fluid was drawn from the posterior cerebellomedulary cistern. Urine was drawn from the urinary bladder after opening the peritoneal cavity.

Genetic and biochemical methods were both used in the present study because they are used for postmortem bacterial culture and identification in South Korea. The genetic method involved subjecting the bacterial colonies to identification using the FAST MicroSEQ 500 16S rRNA Bacterial Identification Kit (Applied Biosystems, Foster City, CA, USA). The biochemical method involved subjecting the bacterial samples to identification using the Vitek2 system and Vitek-MS (BioMérieux, Marcy L'Etoile, France).

C-reactive protein (CRP) testing was also performed to identify antemortem inflammation using cardiac blood samples and a potable Alere Afinion AS100 Analyzer (Axis-Shield PoC AS, Oslo, Norway). This instrument can measure CRP values of 0.5–20 mg/dL within 4 minutues using a 1.5-μL sample of whole blood, which is automatically corrected for hematocrit levels of 20%–60%.

Results

Bacterial culture and identification were performed for 336 samples from 129 forensic autopsies, which involved 88 male individuals and 41 female individuals. The mean age was 50.9 years, with an age range from neonate to 111 years. The mean postmortem interval was 3.0 days. The 336 samples included 108 cardiac blood samples, eight peripheral blood samples, 74 pericardial fluid samples, 59 pleural samples, 38 peritoneal fluid samples, 27 cerebrospinal fluid samples, 10 urine samples, and 12 miscellaneous samples.

Among the 129 autopsy cases, 15 cases were not tested for CRP because of putrefaction or an insufficient cardiac blood volume. Among the 114 cases that were tested for CRP, 79 cases (69.3%) had negative results, and bacterial culture and identification were performed for 185 samples from those 79 cases (mean, 2.3 samples/case). The 185 samples included 71 cardiac blood samples, five peripheral blood samples, 41 pericardial fluid samples, 27 pleural fluid samples, 15 peritoneal fluid samples, 18 cerebrospinal fluid samples, three urine samples, and five miscellaneous samples. The general patient and sample characteristics are shown in Table 1.

Among the 71 cases that were negative for CRP in the cardiac blood, bacteria were identified in 52 cases (73.2%). *Streptococcus agalactiae* was identified in the peripheral blood from one case. Among the 41 pericardial fluid samples, bacteria were identified in 35 cases (85.4%). Bacteria were identified in all 27 pleural fluid samples. Among the 15 peritoneal fluid samples, bacterial were identified in 14 cases (93.3%). Among the 18 cerebrospinal fluid samples, bacteria were identified in 15 cases (83.3%). Among the three urine samples, bacteria were identified in two cases. Table 2 shows

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Table 1. General characteristics of the cases and samples

Characteristic	No.
Sex	129
Male	88
Female	41
Age, mean (range, yr)	50.9 (0–111)
Cases	129
Positive CRP cases	35
Negative CRP cases	79
Not tested for CRP	15
Samples	336
In positive CRP cases	151
In negative CRP cases	185
Cardiac blood	71
Peripheral blood	5
Pericardial fluid	41
Pleural fluid	27
Peritoneal fluid	15
Cerebrospinal fluid	18
Urine	3
Miscellaneous	5

CRP, C-reactive protein.

the results from the bacterial culture and identification testing in CRP-negative cases according to sample type and identification methods.

Discussion

Current knowledge regarding postmortem human microbiology has potential applications in the field of forensic microbiology [4]. For example, postmortem microbiology data may be useful as physical evidence during forensic death investigations. In addition, postmortem bacterial culture and identification tests could be used to estimate the postmortem interval [4], determine a cause of death [5], or detect micro-evidence [6]. Thus, the present study aimed to identify bacteria that could be considered normal flora in postmortem body fluid samples.

The study included 79 autopsy cases with negative results for CRP, which indicates that no antemortem inflammation was present. However, bacteria were identified in some of these cases, which suggest that these bacteria were normal flora. Although the mean postmortem interval was 3.0 days, bacteria were identified in cases with a postmortem interval of <1 day, which suggests that sampling for postmortem bacterial culture and identification should be performed as soon as possible during the 24–48 hours after death [1]. Nevertheless, the results of the postmortem bacterial culture and identification testing should be interpreted in the context of other postmortem examinations, including CRP testing. Furthermore, various bacteria were identified using genetic and biochemical methods, and different types of bacteria were occasionally identified in the same case. These differences and their significance have been addressed in a previous study [7].

A positive result could theoretically be categorized in several ways, such as true positive, agonal spread [8], postmortem transmigration, and contamination [9]. Although three cases with false-positive results could not be classified (except for possible antemortem infection), the present study revealed the presence of intestinal bacteria (e.g., *Escherichia coli* and *Enterococcus faecalis*) in cardiac blood samples, as well as skin flora (e.g., *Staphylococcus epidermidis*) in peripheral blood samples. Furthermore, some cases had positive results for bacteria that are generally considered pathogenic, such as *Staphylococcus aureus*, which was identified in the cardiac blood and pericardial fluid from a case of sudden infant death syndrome with a postmortem interval was 1 day. *Klebsiella pneumoniae* was also identified in the pleural fluid from a strangulation case with a postmortem interval of 1 day. Therefore, the results of postmortem bacterial culture and identification should be interpreted carefully and in combination with other postmortem examinations, including CRP tests. Interestingly, we also identified differences in the bacterial identification rate according to sample type in cases that were negative for CRP. In this context, pure growth of a pathogen in blood or cerebrospinal fluid is usually considered a factor that possibly contributed to the death [9]. Although the sample size was small, peripheral blood appears to be a useful sample type for performing postmortem bacterial culture and identification, although at least two sample types should be tested for each case.

In some CRP-negative cases with positive results from

Table 2. Representative postmortem bacteria in cases that were negative for C-reactive protein

	Cardiac blood	Pericardial fluid	Pleural fluid	Peritoneal fluid	Cerebrospinal fluid	Urine
By genetic method	*Acinetobacter* spp.	*Acinetobacter* spp.	*Aeromonas* spp.	*Citrobacter* spp.	*Aeromonas* spp.	*Acinetobacter* spp.
	Aeromonas spp.	*Aeromonas* spp.	*Aeromonas hydrophila*	*Citrobacter freundii*	*Comamonas* spp.	*Pseudomonas* spp.
	Citrobacter spp.	*Aeromonas hydrophila*	*Aeromonas veronii*	*Clostridium* spp.	*Enterobacter* spp.	
	Citrobacter freundii	*Citrobacter* spp.	*Citrobacter* spp.	*Enterobacter* spp.	*Escherichia* spp.	
	Escherichia spp.	*Citrobacter freundii*	*Citrobacter freundii*		*Lactococcus* spp.	
	Escherichia coli	*Enterobacter* spp.	*Citobacter braakii*		*Proteus* spp.	
	Enterococcus spp.	*Enterobacter cancerogenus*	*klebsiella* spp.		*Pseudomonas* spp.	
	Enterococcus faecalis	*Leuconostoc* spp.	*klebsiella pneumoniae*		*Serratia* spp.	
	Klebsiella spp.	*Leuconostoc lactis*	*Proteus* spp.		*Vibrio* spp.	
	Klebsiella pneumoniae	*Pseudomonas* spp.	*Proteus hauseri*			
	Lactococcus spp.	*Pseudomonas monteilii*	*Pseudomonas* spp.			
	Lactococcus lactis	*Pseudomonas fulva*	*Raoultella* spp.			
	Proteus spp.	*Staphylococcus* spp.	*Shewanella* spp.			
	Proteus hauseri	*Staphylococcus aureus*	*Shewanella algae*			
	Pseudomonas spp.	*Staphylococcus epidermidis*	*Vibrio* spp.			
	Pseudomonas monteilii					
	Pseudomonas fulva					
	Staphylococcus spp.					
	Streptococcus spp.					
	Streptococcus agalactiae					
	Vibrio spp.					
By biochemical method	*Acinetobacter* spp.	*Acinetobacter* spp.	*Aeromonas* spp.	*Acinetobacter* spp.	*Aeromonas* spp.	*Enterobacter* spp.
	Aeromonas spp.	*Acinetobacter baumannii*	*Chryseobacterium* spp.	*Citrobacter* spp.	*Enterobacter* spp.	*Lactobacillus* spp.
	Bacillus spp.	*Acinetobacter junii*	*Chryseobacterium indologenes*	*Enterobacter* spp.	*Enterobacter aerogenes*	*Pseudomonas* spp.
	Citrobacter spp.	*Aeromonas* spp.	*Citrobacter* spp.	*Enterococcus* spp.	*Lactococcus* spp.	*Streptococcus* spp.
	Citrobacter sordellii	*Aeromonas hydrophila*	*Citrobacter freundii*	*Escherichia* spp.	*Lactococcus lactis*	
	Escherichia spp.	*Aeromonas sobria*	*Enterococcus* spp.	*Escherichia coli*	*Staphylococcus* spp.	
	Escherichia coli	*Bacillus* spp.	*Escherichia* spp.	*Morganella* spp.	*Staphylococcus capitis*	
	Escherichia hermannii	*Bacillus pumilus*	*Escherichia* coli	*Morganella morganii*	*Staphylococcus epidermidis*	
	Enterobacter spp.	*Citrobacter* spp.	*Klebsiella* spp.	*Staphylococcus* spp.	*Vibrio* spp.	
	Enterobacter aerogenes	*Citrobacter freundii*	*Klebsiella pneumoniae*	*Streptococcus* spp.	*Vibrio parahaemolyticus*	

Continued

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Cardiac blood	Pericardial fluid	Pleural fluid	Peritoneal fluid	Cerebrospinal fluid	Urine
Enterobacter cloacae	*Enterobacter* spp.	*Lactobacillus* spp.			
Enterococcus spp.	*Enterobacter aerogenes*	*Lactococcus* spp.			
Enterococcus faecalis	*Enterococcus* spp.	*Lactococcus lactis*			
Enterococcus casseliflavus	*Enterococcus faecalis*	*Proteus* spp.			
Klebsiella spp.	*Escherichia* spp.	*Proteus penneri*			
Klebsiella pneumoniae	*Escherichia coli*	*Proteus vulgaris*			
Lactobacillus spp.	*Escherichia hermannii*	*Pseudomonas* spp.			
Lactococcus spp.	*Klebsiella* spp.	*Pseudomonas putida*			
Lactococcus lactis	*Lactobacillus* spp.	*Shewanella* spp.			
Proteus spp.	*Lactobacillus fermentum*	*Shewanella algae*			
Proteus vulgaris	*Lactococcus* spp.	*Shewanella putrefaciens*			
Pseudomonas spp.	*Lactococcus lactis*	*Streptocossus* spp.			
Pseudomonas putida	*Pantoea* spp.	*Streptococcus mitis*			
Staphylococcus spp.	*Photobacterium* spp.	*Streptococcus salivarius*			
Staphylococcus capitis	*Photobacterium damselae*	*Vibrio* spp.			
Streptococcus spp.	*Proteus* spp.				
Streptococcus mitis	*Proteus vulgaris*				
Streptococcus salivarius	*Pseudomonas* spp.				
Vibrio spp.	*Pseudomonas putida*				
	Staphylococcus spp.				
	Staphylococcus xylosus				
	Staphylococcus epidermidis				
	Stenotrophomonas spp.				
	Streptococcus spp.				
	Streptococcus mitis				
	Streptococcus parasanguinis				
	Streptococcus salivarius				
	Streptococcus agalactiae				
	Vibrio spp.				
	Vibrio parahaemolyticus				

the postmortem bacterial culture and identification, the identified bacteria seemed to be related to the cause of death. In this context, Kakizaki et al. [10] have demonstrated that marine species of *Vibrio* bacteria, which are bioluminescent, can be isolated from the blood in cases of seawater drowning. The present cases included eight cases of seawater drowning, and we identified *Vibrio* spp. in six of those cases. In addition, *Photobacterium* spp. was identified in five cases, and all of these cases involved bodies that were discovered in seawater. The postmortem intervals from these cases were 2–6 days, and the bodies were found between September and November. Interestingly, the *Vibrio* and *Photobacterium* spp. were only identified in cases where the body was discovered in seawater, and these bacteria were identified in the cerebrospinal fluid, cardiac blood, pericardial fluid, and pleural fluid. Thus, similar results may support a conclusion of seawater downing.

One patient died because of colon perforation during colonoscopy. In that case, peritoneal fluid without peritonitis was noted during autopsy and the postmortem bacteria culture and identification were performed using cardiac blood and peritoneal fluid. The postmortem CRP test revealed a negative result. *Enterococcus faecalis* was identified in the cardiac and peritoneal fluid, *Enterococcus faecium* and *Enterococcus casseliflavus* were identified in the cardiac blood, and *Enterococcus thailandicus* and *Enterobacter cloacae* were identified in the peritoneal fluid. The colon perforation likely explains the dissemination of normal intestinal flora to the peritoneal fluid and circulation. However, the cause of death in that case was not attributed to sepsis, but rather sudden cardiopulmonary arrest caused by massive pneumoperitoneum after air-filled colon rupture during the colonoscopy.

The present study included three cases of fire death. The bacterial culture and identification were performed using cardiac blood and pericardial fluid because the pericardial sac and heart were relatively preserved in those cases. *Enterococcus faecalis*, *Escherichia coli*, and *Klebsiella pneumoniae* were identified in the cardiac blood from one case, while *Pseudomonas* spp. was identified in the pericardial fluid from another case. The postmortem intervals were <2 days, and we were surprised to note the presence of bacterial in these cases, as we had assumed that the thermal effect of the burns would have killed any bacteria.

Although there are many concerns regarding the use of postmortem bacterial culture and identification, recent advances in postmortem bacteriology have highlighted its potential application in the forensic field. Furthermore, although the present study is limited by its relatively small sample size, it provides the first data regarding normal flora in various postmortem body fluids from Korean cases. Clinical and pathological data from after the autopsy should be combined with the results of postmortem bacterial culture and identification, and this process should be managed by clinical bacteriologists and forensic pathologists. Therefore, the present study may provide basic data to facilitate the interpret postmortem bacterial culture and identification results using postmortem body fluids.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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