The Value of Postmortem Microbiology Cultures

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Since the inception of evidence-based scientific concepts in medicine in the 19th century, the utility of postmortem microbiologic examinations has been a topic of controversy. For every study describing a lack of correlation between antemortem clinical and laboratory findings and postmortem culture results, there is equal evidence from other studies that indicates at least some limited utility in select cases. While the contributions of autopsies and postmortem microbiologic examinations in the discovery of novel infectious microorganisms are generally appreciated by the medical and scientific societies, the problems of implementing routine procedures in daily autopsy practice clearly relate to the lack of consensus on their broader utility as well as to a lack of regulatory guidelines. This review provides an overview of the literature-based evidence regarding the utility of postmortem microbiologic examinations together with some practical aspects and guidelines for those confronted with the issue of whether to allow or discourage the use of bacteriologic cultures obtained during autopsies.

Despite significant advances in the diagnosis and treatment of infectious diseases during the past century, infectious diseases continue to be a significant cause of mortality in the United States and worldwide (1). According to the U.S. National Vital Statistics Reports, deaths due to infections are among the leading 15 causes of death: influenza and pneumonia are ranked 8th and septicemia is ranked 11th as causes of death (1). Although there has been an interest in postmortem bacteriology since the beginning of scientific medical investigations in the 19th century, the utility of postmortem cultures has continuously been the subject of controversy and much confusion still exists to date regarding the interpretation of postmortem culture results. Interestingly, the topic of postmortem microbiology cultures has been addressed more frequently in pathology, autopsy, and forensic science literature than in the microbiology and infectious disease literature. Autopsy rates have continuously declined since the 1950s and according to some recent studies may be performed in less than 6% of all hospital deaths in the United States (2–4). At this time, accrediting agencies, such as the Joint Commission on Accreditation of Healthcare Organizations, do not hold hospitals accountable for performing autopsies and do not require minimum autopsy rates for accreditation purposes (4, 5). As a result, autopsies are now performed at higher frequencies only in academic settings as part of the requirements for physician and forensic science training programs and pathology residency/fellowship training (5, 6). At times, autopsy findings have contributed significantly to development of a better and more complete understanding of infectious diseases, including toxic shock syndrome related to infection by certain Gram-positive cocci, infections due to HIV and hantavirus, and severe acute respiratory syndrome (SARS) (7). In addition, the use of postmortem microbiologic cultures has been demonstrated to be of value in forensic autopsies and medicolegal investigations and as a diagnostic and epidemiologic tool in disease outbreak situations (7–10). While the College of American Pathologists (CAP) developed general checklist items for the performance of autopsies, there are no specific guidelines, recommendations, or checklist items that address performance standards for postmortem microbiology culture examinations (11). This review provides a brief historical perspective of autopsy microbiology, including a discussion of concerns regarding the postmortem spread of microorganisms followed by a description of methods for specimen procurement. The final discussion addresses current practices and the pros and cons of performing postmortem microbiology examinations.

HISTORICAL PERSPECTIVE AND CONCERNS REGARDING POSTMORTEM SPREAD OF ORGANISMS

Since 1895, when Achard and Phulpin published their work on determining the origin of microorganisms identified in postmortem examinations (12), the value of postmortem bacteriology cultures has continuously been a topic of debate (13). Over time, pathologists have repeatedly investigated the utility of postmortem blood and tissue cultures to aid in the diagnosis of bacteraemia/sepsis and pneumonia. In 1965, O’Toole and colleagues published a study demonstrating that postmortem bacteriologic tissue cultures were indicative of infection in only 44% of the cases examined (14). In their study, the investigators used spleen tissue cultures in lieu of blood cultures (BCs) to detect bacteremia. Despite other studies demonstrating some greater value of postmortem microbiologic examinations (15), the results presented by O’Toole, together with studies published during subsequent decades (16–18), led to and perhaps still support a prevailing belief among many pathologists and microbiologists that postmortem microbiologic examinations have no significant diagnostic utility and are in fact unreliable. One study in particular is worth a more detailed commentary: Wilson and colleagues (18) published a prospective review on the value of postmortem blood cultures in correlation with data available from antemortem blood cultures (BCs). In that thorough review of 111 patients, the authors found that postmortem blood cultures provided additional information not already known from antemortem blood cultures only rarely. In only 35% of all cases, results from antemortem and postmortem BCs were in agreement; of the 65% culture results with disagreement, no results of postmortem BCs were found to be clin-
cally relevant with regard to the cause of death (18). Furthermore, they found that a significant amount of cultures were affected by contamination due to the procurement process during the autopsy. Therefore, they indicated that postmortem BCs were of little value, should be interpreted with caution, and were indicated only in select cases when ante-mortem clinical and laboratory information, e.g., blood cultures, was not available (18). Lastly, it is remarkable that those authors also commented on the financial impact and cost of the autopsy and postmortem microbiologic cultures with respect to the greater fiscal issues in medicine, a concern as valid today as it was in 1993.

Two main theories explaining bacterial growth in postmortem blood and tissue cultures arose from the results of these earlier studies: (i) agonal spread and (ii) postmortem bacterial transmigration. In general, both of these concepts are concerned with bacterial invasion into the bloodstream. Agonal spread is understood as the invasion of bacteria into the bloodstream when systemic circulation is declining during the agonal period or is artificially maintained during resuscitation measures. This concept was first introduced by J.W. Fredette in 1916 (19) and was later supported by other investigators (16). However, other investigators argued against this concept; they published their findings on bacteremia and postmortem microbiology in children with burn wounds in 1975 (20). In contrast to “agonal spread,” the term “postmortem bacterial transmigration” describes a process by which bacteria migrate from mucosal surfaces and tissues into the bloodstream after circulation has ceased (21). This process was first described by Gradwohl in 1904 (22) and was subsequently supported by findings in other studies (23–25). Aside from the verification provided by clinical observational autopsy series, a few studies demonstrated evidence in support of bacterial transmigration in the form of animal as well as in vitro experiments (26–28). While the concept of agonal spread is theoretical in nature and only a few historic data exist in its support, the concept of bacterial transmigration has much stronger support in the scientific and medical literature (23–31). Kellerman and colleagues reported results from their in vitro experiments showing that bacteria could migrate through the intact intestinal wall of humans within 12 to 15 h after death (26). Carpenter and Wilkins reported results from a comprehensive retrospective review of more than 2,000 autopsy cases and showed that the rate of positive postmortem blood cultures increased from 20% to 40% in correlation with the length of the postmortem interval and the time of procurement of BCs during the first 18 h after death (24). Those authors also investigated the utility of lung cultures and found that the rate of positivity in postmortem lung cultures increased in correlation with the length of hospital stay and the postmortem interval before lung tissue was obtained for culture (24). The results from both of these studies are remarkable insofar as in both investigations the respective cultures were obtained using strict precautions to avoid external contamination during the procurement processes.

The studies referenced here further established the understanding of postmortem microbial spread that is still accepted to date by most pathologists and microbiologists: that the indigenous visceral microbial flora is the source of the organisms identified in the majority of postmortem blood and tissue cultures. In a more recent study investigating postmortem BCs obtained during and after the first 24 h postmortem among cadaveric tissue donors, the authors found that a significantly higher number of BCs were positive during the first 5 h postmortem than during the later time points of BC collection (32). In that study, blood cultures were obtained from the subclavian or femoral vessels, using aseptic techniques. The majority of the organisms, however, were found to be coagulase-negative staphylococci judged in most cases to be a contaminant. Overall, there was no significant increase or decrease in the BC positivity rate during the first 48 h after death. Despite the absence of a significant difference between the ≤24 h and >24 h postmortem intervals in the BC positivity rates, the authors observed a slight increase in the levels of Enterobacteriaceae recovered in postmortem BCs collected after the initial 24 h postmortem interval (19%) compared to BCs collected within the first 24 h after death (11%). Irrespective of this observation, the authors strongly argued against the concept of postmortem bacterial transmigration as a cause of this observation (32). Considering that bacteria may not have the necessary rapid mobility required to migrate in large numbers from the colonized intestinal sites to the larger vessels or heart after circulatory arrest, it seems unlikely that postmortem bacterial transmigration is of significant concern during the first 24 h after death. Such an understanding finds at least partial support from the results of earlier experimental studies on bacterial transmigration (23,27). In contrast, there is sufficient evidence from the same studies and elsewhere in the medical scientific literature in support of the notion that the recovery rate of bacteria from postmortem bacteriologic cultures of blood and tissue increases in proportion to the postmortem interval irrespective of whether one is more inclined to accept the possibility of agonal spread or embraces the concept of postmortem bacterial transmigration.

In addition to these two concepts, the possibility of iatrogenic contamination of postmortem cultures during the actual procurement of samples at the time of the autopsy procedure has to be considered as well (21). In similarity to issues related to contamination of clinical specimens such as blood cultures, the actual technique used to procure samples has a significant impact on the rate of contamination. Rates and measures to prevent contamination of clinical samples have been addressed in various microbiology guidelines, and algorithms to aid in BC result interpretation have been developed (33, 34). However, such evidence-based guidelines do not exist for postmortem blood and tissue culture interpretation, leaving the autopsy pathologist with a problem greater than that of his clinical colleagues in laboratory medicine when being confronted with potential contaminant microorganisms. Despite the lack of robust guidelines, some studies were published that investigated the problems related to contamination of postmortem bacteriologic cultures, suggesting various approaches to either minimize contamination or aid in the interpretation of culture results. While controversy still exists on whether the duration of the postmortem interval to performing the autopsy has any significant impact on recovery of microorganisms, a greater level of consensus exists that both prompt cooling and limited mobilization of the deceased body decrease the possibility of postmortem spread of microorganisms (9, 21, 24, 35). More importantly, the use of an aseptic technique to reduce specimen contamination has been emphasized in numerous autopsy studies (9,14,21). Among several recommended techniques, the most frequently cited approach is that of searing the organ’s surface with a hot spatula before obtaining the blood and/or tissue samples, using sterile instrumentation (24, 25, 35, 36). From a review of these studies, it appears that use of this technique together with
adherence to obtaining both blood and tissue cultures prior to the main evisceration of the body would likely result in significant reduction of contamination. For the completeness of this review, a brief mention of the study by O’Toole and colleagues (14) is warranted. Those investigators obtained 440 tissue cultures in 54 autopsy cases without antemortem evidence of infection. They used a rigorous aseptic protocol, including a whole-body iodine scrub of the corpse, use of separate sterile instruments (e.g., scalpels, blades, needles, syringes, forceps), and a complete “surgical scrub and gowning” of all personnel involved in the autopsies. In addition, all autopsy procedures were carried out in an air-controlled morgue. Under these conditions, the investigators found 324 of all cultures (n = 440; 74%) to be negative; most of the positive cultures yielded organisms considered to be external contaminants. A small number of cultures were positive for Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, and Escherichia coli; all considered significant. Considering that all of the autopsies were performed within the first 20 h postmortem, the investigators argued against the concept of agonal spread but emphasized that despite the rigorous aseptic technique, external contamination is a serious consideration when obtaining postmortem blood and tissue cultures (14). Using a similar approach to performing a “sterile autopsy,” Minckler and colleagues (37) found 45% of lung and 75% of kidney specimens to be negative, with the remainder of postmortem culture specimens yielding contaminant organisms more often than significant pathogens, hence supporting the earlier conclusions by O’Toole and colleagues. Considering the need to avoid bacterial contamination in addition to minimizing postmortem migration of bacterial organisms and decomposition of the body itself, one may conclude from the studies referenced in this paragraph that the deceased body should be moved to a locker cooled to 4 to 6°C as soon as possible. In addition, unnecessary movements should be avoided, and autopsies should be performed within 24 h of death. Performing the autopsy in a clean environment and using aseptic techniques to procure blood and tissue samples are both measures to limit the possibility of external contamination of postmortem bacteriologic cultures. However, those measures do not eliminate or decrease contamination rates to levels acceptable in most antemortem clinical cultures. The following paragraphs address aspects of sample procurement and interpretation of culture results within the context of clinical-pathological postmortem correlations.

CONSIDERATIONS FOR THE PROCUREMENT OF SPECIMENS
For the reasons discussed in the prior paragraph, tissue and blood cultures as part of the postmortem examination should be obtained within 24 to 48 h of death. Furthermore, the body sites selected for culture must be sterilized prior to obtaining the sample, e.g., using a soldering spatula or iron. All samples should be collected prior to the evisceration of the body; blood and other fluid samples for culture should be collected first, followed by tissue samples. All specimens collected for culture should be transported immediately to the microbiology laboratory, using appropriate collection and transport media, as indicated in clinical specimen collection guidelines (9, 21, 34, 38, 39). Blood cultures obtained from heart blood, spleen cultures, or peripheral venous sites are commonly applied during postmortem examinations; such cultures have a reported positivity rate between 7% and 69% (24, 40–42). With the exception of a study by Fredette (19), there is little evidence allowing for an estimation of sensitivity and/or specificity of postmortem blood cultures (21). In most adult autopsies, the isolation of a typical pathogen in a monomicrobial culture (e.g., S. aureus, Streptococcus pneumoniae, E. coli) is likely to represent a true pathogen; the positive predictive value increases even further with supportive evidence of inflammation and other pathological findings in tissue sections (e.g., lungs).

Cerebrospinal fluid (CSF) can be obtained either by lumbar puncture, applying the same sterile technique as used in antemortem clinical settings, or by cisternal puncture. It is of utmost importance to apply a strict sterile technique in both circumstances to avoid contamination of the specimen. CSF for culture is typically not obtained during adult autopsies but has been proven useful in the investigation of forensic autopsies and cases of sudden infant death (21, 43). As is true for the interpretation of postmortem BC results, the isolation of a single potential pathogen such as S. pneumoniae, S. aureus, or E. coli should be considered a significant finding and a possible explanation of or cause contributing to the patient’s death.

Among the various sources for postmortem tissue culture, sections from the lungs are probably the most controversial of specimens and the associated results certainly the most difficult to interpret. The lower respiratory tract is usually a sterile body site; however, bacteria present in upper respiratory tract and bronchial secretions could ultimately multiply in number after death and migrate further into the lower respiratory tract (21). Despite this possibility of postmortem bacterial migration, the lungs should remain (mostly) sterile in the absence of a true infection. Lung tissue biopsy specimens should be obtained with the organs being in situ, i.e., prior to evisceration, and the organ surface should be sterilized by a hot spatula. Tissue samples should then be obtained using sterile forceps and scalpel. Even when applying a strict aseptic technique during the procurement process, lung tissue cultures are frequently prone to contamination. Some studies have demonstrated that almost 50% of the lung specimens obtained for culture are contaminated, i.e., are positive for some organism(s), despite the lack of any further pathological evidence of a pulmonary infection (15, 44, 45). While the isolation of typical pathogenic bacteria (e.g., S. pneumoniae) from lung tissue sections would be indicative of pneumonia, in many other situations, such cultures demonstrate polymicrobial growth of contaminant organisms. Careful consideration must be taken when interpreting results from postmortem lung tissue cultures. Cultures from other solid organs such as liver and kidney are even more prone to the problems of contamination encountered with lung cultures (15, 21).

VALUE OF POSTMORTEM CULTURES AND CLINICAL CORRELATION
Conceptually, there are two primary reasons to obtain postmortem blood and/or tissue cultures: (i) identification of the etiologic agent as the cause of a previously undiagnosed infection and (ii) confirmation of the antemortem diagnosis. Occasionally, the postmortem culture may be invaluable in establishing the diagnosis of an infectious disease as the cause of death (46). In some cases, an infection may be discovered only during the postmortem examination of the organs, e.g., heart valve vegetations in endocarditis. In such instances, cultures of the actual tissue are inappropriate due to issues of contamination, and blood cultures obtained at the beginning of the autopsy may be invaluable to determine the exact etiology of the infection. In other cases, postmortem blood
and tissue cultures may provide additional information regarding the extent and severity of an already suspected or known antemortem infectious disease process that resulted, however, in a rapid decline and death of the patient (47, 48). Procurement of postmortem cultures in order to confirm the suspicion of an antemortem diagnosis may appear somewhat counterintuitive. However, in rare instances, postmortem cultures, particularly, BC and spleen cultures, may be useful to determine the etiology of a fulminant infectious disease process when the patient’s death occurred prior to obtaining adequate clinical (antemortem) cultures. Lastly, results from postmortem cultures can also be useful in determining the effectiveness of antimicrobial therapy for a known antemortem diagnosis of bacteremia/sepsis (49). The utilization of BCs and/or spleen cultures appears particularly beneficial in determining the efficacy of antimicrobial therapy as the potential contaminants. Such information could ultimately prove to be invaluable in the investigation of patients who died from infectious causes related to presumed failure of the prescribed antimicrobial therapy.

For the reasons of contamination and postmortem bacterial transmigration, cultures obtained during the autopsy procedure certainly have some limited value and interpretation of their results requires special consideration with respect to data obtained from antemortem cultures and other clinical-pathological correlations. In this review, I have outlined some studies that questioned the utility of postmortem cultures (17, 18), while others demonstrated some utility (50). In my opinion, the majority of routine and broadly applied postmortem cultures as part of all postmortem examinations provide little new information, and the additional cost to the laboratory and the autopsy may not be justified. Admittedly, there is a lack of studies for assessing the cost-benefit ratio of postmortem cultures; perhaps the overall low rate of autopsies performed at this time is significantly limiting the opportunities for such analyses. Considering this fact, together with the lack of regulatory guidelines, there might be a need and opportunity to develop more specific guidelines based on prospective data from autopsies; regulatory agencies and organizations such as the CAP may be able to further support additional research in this area.

CONCLUSIONS

In similarity to other clinical microbiology cultures, postmortem cultures are of value in only a limited number of patients. Submission of the most appropriate specimens (e.g., blood) in preselected cases should be limited to cases which have a high likelihood of positive results being of value to the autopsy. The ability to differentiate between true-positive culture results and postmortem transmigration and/or contamination remains a major challenge to microbiologists and pathologists. From the literature reviewed here, it seems reasonable to state that there is very little evidence to support the theory of agonal spread and that postmortem bacterial transmigration may have little influence on postmortem microbiologic cultures if the autopsy is performed within the first 24 to 48 h after death. Furthermore, it appears that monomicrobial growth of a typical opportunistic and/or pathogenic microorganism identified in postmortem blood or tissue cultures can be considered a true indicator of infection. However, polymicrobial growth and/or the presence of typical contaminant organisms, such as coagulase-negative staphylococci and mixed intestinal flora, appear more likely to be results of iatrogenic contamination during procurement of the specimen or due to postmortem bacterial transmigration. In all cases, a thorough clinical-pathological correlation is necessary as part of the interpretation of postmortem bacteriologic cultures. Effective communication between the pathologist and microbiologist would provide the greatest assurance that specimens would be collected in appropriate cases, without contamination, and would be interpreted in the context of all available information. The engaged microbiologist will ultimately be rewarded for this effort by recognizing that laboratory work and cost will be diminished by avoiding the processing of contaminated and inappropriate postmortem specimens, while providing expertise and guidance in the assessment of postmortem diagnoses related to infectious diseases. The interest and willingness of the autopsy pathologist and the microbiologist to carefully review and correlate the clinical, laboratory, and pathological information will ultimately determine the real value of postmortem cultures.

REFERENCES


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Minireview

Stefan Riedel received his M.D. and Ph.D. degrees in 1996 and 1999, respectively, from the Johann Wolfgang Goethe University in Frankfurt, Germany. After initial clinical training in general and orthopedic surgery in England, Germany, and Switzerland, he went on to complete residency training in anatomic and clinical pathology at Baylor University Medical Center in Dallas in 2005. Following a 2-year fellowship in medical and public health microbiology at the University of Iowa Hospitals & Clinics in Iowa City, Dr. Riedel was appointed Director of the Clinical Pathology Laboratories at Johns Hopkins Bayview Medical Center. He holds an academic appointment as Assistant Professor in Pathology at The Johns Hopkins University School of Medicine. Since 2007, Dr. Riedel has developed a very active research program at the Bayview Medical Center, where his research is focused on the role of biomarkers and traditional blood culture technology for the diagnosis and management of sepsis. In addition, his research laboratory is involved in studies relating to diagnosis, management, and epidemiology of emerging bacterial antimicrobial resistance. He is an active member of the American Society of Microbiology and a diplomate (active status) of the American Board of Medical Microbiology (ABMM); he served until 2013 on the ABMM examination validation committee. Dr. Riedel is a Fellow of the College of American Pathologists (CAP) and is currently a member of the CAP Microbiology Resource Committee; he also serves as an associate editor for BMC Infectious Diseases and is a member of the editorial board of the Journal of Clinical Microbiology.