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Importance of microbiology in autopsy procedure

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Abstract---The importance of bacterial cultures in autopsy has been controversial since Filippo Pacini and Robert Koch's early investigation into the etiology of cholera. Death-war and / or post-mortem invasion by the patient's native flora poses a problem with respect to post-mortem intervals, selection of anatomical location of cultures containing blood, CSF, and number of specimens required to be bought and collected in Microbial Laboratory for processing. Additionally, importance of postmortem virus research is significant. An increasingly important aspect of occupational safety is involved in handling of patient samples by laboratory staff. During the AIDS epidemic, regulatory bodies were urged to reconsider the importance of postmortem microbiological tests. This editorial is used to determine the exact cause of death without sacrificing the logistical and human resources of the Institute of Microbiology Practical sampling guidelines. The American University of Pathologists (CAP) are known to have developed a general checklist item for performing autopsy, but there are no specific guidelines, recommendations, or checklist items to address performance criteria for postmortem microbial culture studies. This review provides a brief historical perspective on autopsy microbiology, including a discussion of concerns about the postmortem spread of microorganisms, followed by a description of how to sample. The final discussion concerns current practice and the strengths and weaknesses of post-mortem microbiology research.

Keywords---microorganisms, autopsy procedure, microbiology.

Historical Perspective

The clinical relevance of postmortem microbial cultures is a controversial issue, at least in 1895, since Achard and Phulpin attempted to identify the origin of a microorganism found during autopsy. Recently, O'Toole et al. found that positive postmortem bacterial cultures indicated infection in 13 of 29 patients. In 9 of the 29 patients, positive culture was considered to be associated with contamination. In the other 7 patients, the importance of postmortem positive cultures was unclear. In a classic article, Koneman and Davis (14) reported similar results in a routine autopsy study of 396. In, 71.5% of patients cultured at least one organism from blood, lungs, or other organs were found. However, there was a low correlation between the microorganisms recovered by the postmortem test and the clinical status of the patient's vestibule. In 42% of cases, no correlation was found between premortal (sputum) and postmortem culture of lung tissue. Similarly, Wilson et al. (25) Their findings included in prenatal clinical reports with postmortem cultures of lung, liver, spleen, and heart. At necropsy, the clinically diagnosed infectious agent could be reliably identified in only 2.8% of patients. A positive postmortem culture, had no obvious diagnostic significance and eventually led to the general belief that postmortem bacteriological data was unreliable. Two theories that try to explain these positive cultures have emerged from this belief. Invasion after death and invasion during the death war.

Microbiological autopsy is the examination of microbial samples from a corpse to determine the cause and manner of death. Postmortem bacterial migration as a phenomenon occurs when bacteria from the mucosal surface or the indigenous flora of the body invade tissues and organs of a deceased person. This can affect the results of microbiological autopsy. Organisms obtained from tissue from postmortem microbiological examination may be derived from migration of indigenous flora. This process is known as postmortem invasion. Numerous factors, including time since death and autopsy, environmental temperature and humidity, existence of wounds or injuries, use of antimicrobial medications before death, might affect bacterial invasion after death. Postmortem alterations in the body, such as putrefaction, gas production, tissue liquefaction, and colour changes, can be observed due to bacterial invasion after death, which in turn affect the results of microbiological autopsy. Postmortem bacteriology is the study of microorganisms that remain in the body after death. Particularly in situations involving infection, sepsis, or poisoning, it might be useful in identifying the reason and manner of death. It can also reveal details on the effectiveness of antibiotic therapy, the detection of new pathogens, and medical mistakes. Postmortem bacteriology needs precise sample methods, suitable storage and transit conditions, and accurate result interpretation to overcome obstacles such as contamination and bacterial postmortem translocation which refers to the movement of bacteria from densely colonized area, such the intestines, to other tissues after death. To standardize postmortem bacteriology in various contexts, including minimally invasive autopsy (MIA) and prenatal autopsy, several recommendations have been developed.

The results of several studies support the theory of postmortem invasion. In a retrospective study of with 2,033 autopsies, Carpenter and Wilkins reported that the percentage of positive cardiac blood cultures increased in proportion to the

interval between death and blood draw. Rose and Hockett used membrane filtration to reveal exponential growth of microorganisms that developed within 6-8 hours of the death of a patient who died from a cause other than infection. Another study examined the relationship between postmortem cardiac blood cultures and pathological cause of death (7). After death, normal oropharyngeal and gastrointestinal flora appeared to have invaded the blood. In support of this claim, these findings directly indicates that oropharyngeal organisms can invade the lungs at the time of death. After death, Norris and Pappenheimer introduced microorganisms (*Bacillus prodigiosus*) into the human mouth and showed that at least 50% of the cases in their experiment obtained microorganisms from the lungs. Nehring et al. (17) used an inoculum consisting of 106 colony forming units (CFU) / ml *Bacillus subtilis* and 109 CFU / ml *Staphylococcus epidermidis*. This inoculation was injected into the liver of 4,444,33 corpses within 10 minutes of the 4,444 deaths. At necropsy, tracer organisms were found in the corpse's right atrium blood (45.6%), lungs (23.3%), and left atrium (19.8%). Their findings showed that Bacteria present in the liver can "move from right to left into the systemic circulation, without a viable circulatory system." Kellerman et al. showed in vitro bacterial reincarnation of post mortem samples. Other studies show that anaerobic bacilli rapidly infiltrate the tissues of animals without refrigeration after death.

While several studies support the post hoc theory, other studies have provided strong evidence to the contrary (10,15). Many of the studies supporting post-invasion were conducted at 37 °C (2, 12). However, cooling to 10 °C can prevent post-invasion of anaerobes for at least 96 hours after death (2). In addition, another study showed that if the cadaver was immediately cooled and an autopsy was performed within 48 hours, the proportion of positive cultures obtained from postmortem samples did not increase with the interval between death and sample collection. (10, 15). Therefore, it is not considered to have a significant effect on post-culture results. A large proportion of the positive cultures found at post-mortem examination may be related to bacterial involvement that occurs during painful periods: anguish involvement or terminal sepsis. Fredette (8) found that people shortly before death had "local tissue resisted". Viability decreases until bacteria cannot be prevented from moving into the body." To support 4,444 of these hypotheses, Fredette presented 4,444 of 119 autopsies (8). 42 (35.3%) of 119 blood cultures from people collected within 10 minutes after death of people were positive. Of these 119 patients, had blood samples taken before death. Post-mortem examination of microorganisms found microorganisms in 6 of 14 patients (43%).

Contamination of Clinical Specimens

The results of early studies have yielded two major theories that explain bacterial growth in postmortem blood and tissue culture: (i) Agonal respiration and (ii) Postmortem bacterial migration. In general, these two concepts relate to the invasion of bacteria into the bloodstream. Agonal respiration is best understood as the entry of bacteria into the bloodstream when systemic circulation is reduced during death or when it is artificially maintained during resuscitation. This concept was studied by Fredette in 1916, later supported by other researchers. However, other researchers opposed this concept. In 1975, they published results

on bacteremia and postmortem microbiology in burned children. In contrast to "agonal respiration," the term "postmortem bacterial migration" refers to the process by which bacteria migrate from mucosal surfaces and tissues into the bloodstream after cessation of circulation. This process was first described by Grathwohl in 1904 and later supported by discoveries in other studies. Apart from validation by clinical autopsy series, some studies have shown evidence of bacterial reincarnation in both animal and in vitro experiments. Although the concept of Agonal respiration is theoretical and there is little historical data to support it, the concept of bacterial reincarnation is much more strongly supported in the scientific and medical literature (23–31). Kellerman et al. Reported the results of an in vitro experiment showing that bacteria can migrate through the intact intestinal wall of humans within 12 to 15 hours after death. Carpenter and Wilkins reported the results of a comprehensive retrospective review of more than 2,000 autopsy cases, with a correlation between length and time of autopsy intervals to increase postmortem blood culture (BC) positive rates from 20% to 40%. The study involved blood culture collection in the first 18 hours after death. These authors also examined the usefulness of lung cultures and found that the positive rate in postmortem lung cultures increased in proportion to the length of hospital stay and the postmortem interval until lung tissue was obtained for the culture. The results of these two studies are noteworthy in that in both studies, each culture was obtained under strict precautions to avoid external contamination during the procurement process.

In addition to these two concepts, the potential for toxicity of post-mortem cultures should also be taken into account during actual sample collection at the time of the autopsy. Similar to problems associated with contamination of clinical samples such as blood cultures, the actual technique used to obtain a sample has a significant impact on the rate of contamination. Regulations and measures to prevent contamination of clinical specimens are described in various microbiological guidelines, and algorithms have been developed to help interpret BC results. However, such evidence-based guidelines do not exist for post-mortem interpretation of blood and tissue cultures, leaving autopsy pathologists with greater problems than physicians. Despite the lack of firm guidelines, several published studies have investigated the problems associated with contamination of post-mortem bacterial cultures, suggesting different approaches to minimize contamination or to facilitate interpretation of culture results. Although there is still controversy as to whether the length of the post-mortem interval until an autopsy is performed has a significant effect on microbial recovery, the extent of consensus higher exists that rapid cooling and restriction of bodily movement of the dead reduce the likelihood of consequential cadaveric spread of microorganisms. More importantly, the use of aseptic techniques to reduce sample contamination has been emphasized in numerous autopsy studies. Among several recommended techniques, the most commonly cited approach is to grasp the surface of the organ with a hot spoon prior to blood and/or tissue sampling, using sterile measuring equipment. A review of these studies suggests that the use of this technique, along with adherence to blood and tissue culture prior to major evisceration, can significantly reduce contamination. Obtaining both blood and tissue cultures prior to removal from the primary body can significantly reduce contamination. For the completeness of this review, we need to briefly mention the study by O'Toole and colleagues. These investigators

obtained 440 cultures in 54 autopsies with no evidence of infection prior to autopsies. They used a strict aseptic procedure, including a full body scrub with iodine, using separate sterile instruments (e.g., scalpel blades, needles, syringes, forceps), 'exfoliators' and full gowns for all staff involved in autopsies. In addition, all autopsy procedures are performed in an airborne controlled morgue. Under these conditions, the investigators found 324 of all cultures (n 440; 74%) negative; most active cultures produce organisms that are considered external contaminants. A small number of cultures were positive for *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa* and *Escherichia coli*, all of which were considered significant. Considering that all autopsies were performed within the first 20 hours after autopsies, investigators objected to the concept of dynamic transmission but emphasized that although the technique is for strict infection, extrinsic contamination is a matter of serious consideration when blood collection and post-mortem tissue culture are taken. Using a similar approach to performing a 'sterile autopsy', Minckler and colleagues found that 45% of lung samples and 75% of kidney samples were negative, with the rest of the culture samples being negative. Cadaveric cultures often produce blood samples that contaminate the organism agent findings by O'Toole. The study can draw conclusions given the need to avoid microbial contamination in addition to minimizing post-mortem migration of microorganisms that degrade the body itself. The study also mentions that the body of the dead person should be moved to a locker and refrigerated to 4 to 6°C as soon as possible. In addition, unnecessary travel and autopsies should be avoided within 24 hours of death. Performing autopsies in a clean environment and using aseptic techniques to obtain blood and tissue samples are two measures to limit the possibility of foreign contamination of post-mortem bacterial cultures. However, these measures do not eliminate or reduce the contamination rate to an acceptable level in most clinical pre-mortem cultures. The following paragraphs discuss aspects of specimen collection and interpretation of culture results in the context of postmortem clinical correlation.

In contrast, there is ample evidence from the same study and elsewhere in the medical scientific literature, suggesting that the rate of bacterial recovery from postmortem blood and tissue culture increases in proportion to postmortem intervals. This tends to do so to accept the potential for the spread of distress, or involves the concept of postmortem bacterial migration. It can be concluded from previous studies that contamination can introduce bacteria from external sources, such as the environment, instruments, personnel which can drastically affect accuracy and reliability of the microbiological results leading to false-positive diagnoses of infection. Contamination during agonal phase or after death, when bacteria from highly colonized sites, such as the gastrointestinal tract or the oral cavity, spread to other organs or body fluids can interfere with identification of the primary site of infection or the type of bacteria involved. Contamination also can cause postmortem changes in the body, such as color changes, putrefaction, gas formation, tissue liquefaction. These changes can alter morphology and appearance of tissues and organs and making diagnosis procedures difficult to distinguish between infection and postmortem colonization.

Things to Consider While Procuring Specimens

Human cadaver tissue is available for transplantation from tissue banks. A 24-hour postmortem time restriction has been established for tissue retrieval. From a microbiological standpoint, this research intended to assess the evidence supporting this limit. Among 100 possible tissue donors, the delay in growth in postmortem blood cultures, identification of the isolated species, and clinical/environmental variables were evaluated. Within (25/65=38 percent) and after (24/65=37 percent) 24 hours after death, there was no significant difference in the rate of donors with growing blood cultures. Within and after 24 hours after death, coagulase-negative staphylococci and gastro-intestinal microbes were identified. The blood culture findings of donors were substantially negatively associated to two factors: antimicrobial medication and "delay before body chilling." There is no justification for avoiding tissue retrieval in donors after 24 hours of death from a microbiological standpoint. Prior to the obliteration of the corpse, all samples should be gathered; blood and other fluid samples for culture should be taken first, accompanied by tissue samples. As fast as feasible, the specimen should be transported along with complete blood culture request to the clinical microbiology laboratory, ideally within 2 hours per CLSI. Any delay in testing the infected bottles might increase the likelihood of misleading false findings. If delays are anticipated, it is critical to consult the manufacturer's Instructions for Use (IFU). The CDC recommendations for "General Blood and Body Fluid Precautions" have been applied at the University of Illinois Hospital & Health Sciences System. As a result, all specimens should be handled with the same care. This guideline must be properly observed at all times since the safety of laboratory staff is a primary issue. Biohazard bags must be used to transport all specimens. In the outer pocket, place the demand. Requisitions should not be stapled to the bag. Do not put the demand in the specimen bag. To avoid specimen leakage, each bag must be well sealed. The pathologist should be able to assess the optimum place to culture the lungs based on their appearance. Parenchymal consolidation and fibrinous pleuritis are the most typical symptoms of infectious pneumonia. The best technique for identifying these alterations is to observe and palpate the lungs while they are still in place. By burning the surface of the lungs with a hot spatula, the surface of the lungs may be disinfected. A cube of tissue may be mobilized by four 90° stabbing movements with a sterile blade, which can then be pulled up using sterile forceps. After that, the blade may make the last incision needed to liberate the tissue block. If the tip of the forceps is excessively heated, the tissue will cling and it will be difficult to place the specimen into the container. After the lung has been dissected, portions of consolidation may still be cultured if it has not been perfused with formalin. The surface should be sterilized once again by searing. When analyzing the findings of postmortem lung tissue cultures, care must be exercised. Pneumonia may be more difficult to see on a gross level in people with moderate to severe emphysema. As a result, the prosecutor's index of suspicion should be increased in these patients. The prosecutor may need to get Cerebrospinal Fluid (CSF) if infectious meningitis is suspected but not proven prior to death. A cisternal tap is the most straightforward way to achieve this. The treatment involves laying the body in a prone posture and ensuring sure there is enough cushioning beneath the face to prevent deformity. Insert a 12-gauge needle at the midline at the base of the occipital bone and slightly superiorly, toward the eyes, after vigorously

cleaning the skin with iodine and then alcohol. Slowly and cautiously advance the needle, aspirating fluid as often as possible. In order to reduce bleeding, avoid moving the syringe around unnecessarily. Even if no or just blood-tinged CSF is aspirated, a good specimen may be collected once the calvarium is removed. In the subarachnoid space, a syringe may be placed. If this does not work, a tissue sample of the meninges and a tiny piece of the underlying brain may be obtained. Pus tends to form in the inferior part of the brain, allowing for material collecting.

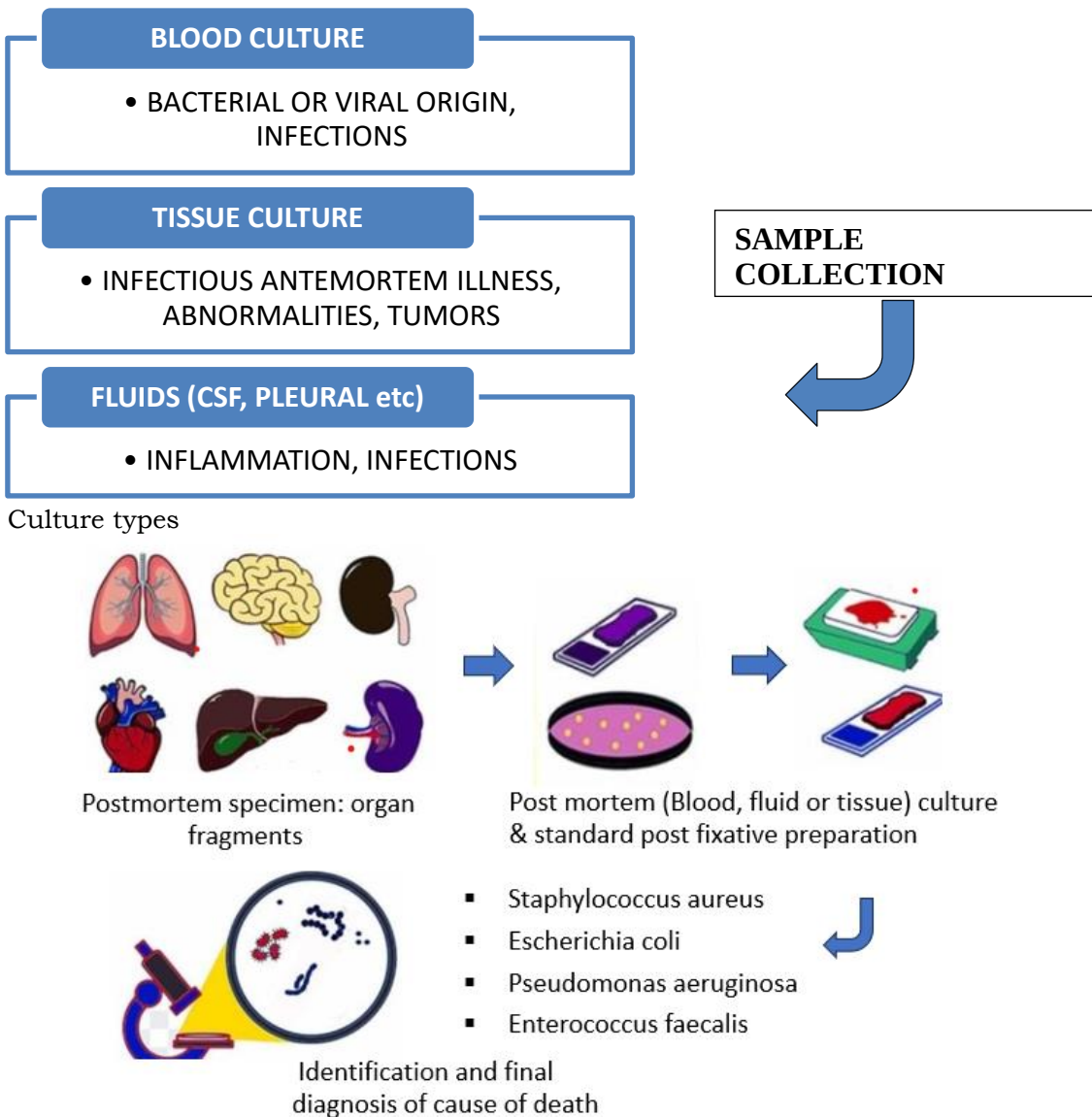


Figure 1. Postmortem culturing

Need of Postmortem Cultures and Clinical Correlation

The key reasons for obtaining postmortem blood and/or tissue cultures are: identification of the etiologic agent as the reason of a previously undetected illness and validation of the antemortem diagnosis.

In certain cases, a postmortem culture may be very helpful in determining the reason of death to be an infectious condition. In rare circumstances, such as heart valve vegetations in endocarditis, an illness may only be diagnosed after the organs have been removed from the body. Blood cultures acquired at the start of the autopsy may be essential in determining the specific origin of the illness in such cases, since cultures of the real tissue are unsuitable owing to contamination concerns. Tissue cultures may reveal more about the scope and seriousness of an antemortem infectious illness process that was previously recognized or known yet resulted in the patient's fast deterioration and death. It may seem contradictory to get postmortem cultures in order to validate an antemortem diagnostic suspect. When a patient's death occurs prior to getting decent clinical (antemortem) cultures, postmortem cultures, especially BC and spleen cultures, may be valuable to pinpoint the genesis of a fulminant infectious illness process. Finally, postmortem culture data may be used to assess the efficacy of antibiotic treatment. Cultures acquired during the autopsy process have limited relevance due to contamination and postmortem bacterial transmigration, and their interpretation requires careful examination of data from antemortem cultures and other clinical-pathological correlations.

The presence of a slightly enlarged, mushy, or diffuent spleen during postmortem examination is frequently linked with systemic infection, typically septicemia. A dependable sampling strategy for bacteriological culture is required to evaluate the probable association of this postmortem result with the existence of broad clinical syndrome. The collection and evaluation of postmortem bacteriology specimens is riddled with difficulties. Direct splenic culture, has been demonstrated to be reliable, especially when just one organism was isolated. There also exists the chance of a hybrid culture emergence during investigation. It's worth noting that there was no link found in the period between death and necropsy and the spleen consistency. Because all of the postmortem investigations in this series were done within four days after death, autolysis seems to have had no impact on splenic consistency during this period. The enzymes released by the enormous number of neutrophil cells that are claimed to be present may explain the spleen's mushy consistency. No linkage between the consistency of the spleen and the quantity of neutrophil cells used in this examination. Given this, and the absence of regulatory requirements, there may be a need and potential to produce more precise criteria based on potential data from autopsy; regulatory bodies and organizations like the CAP may be able to fund future study in this area.

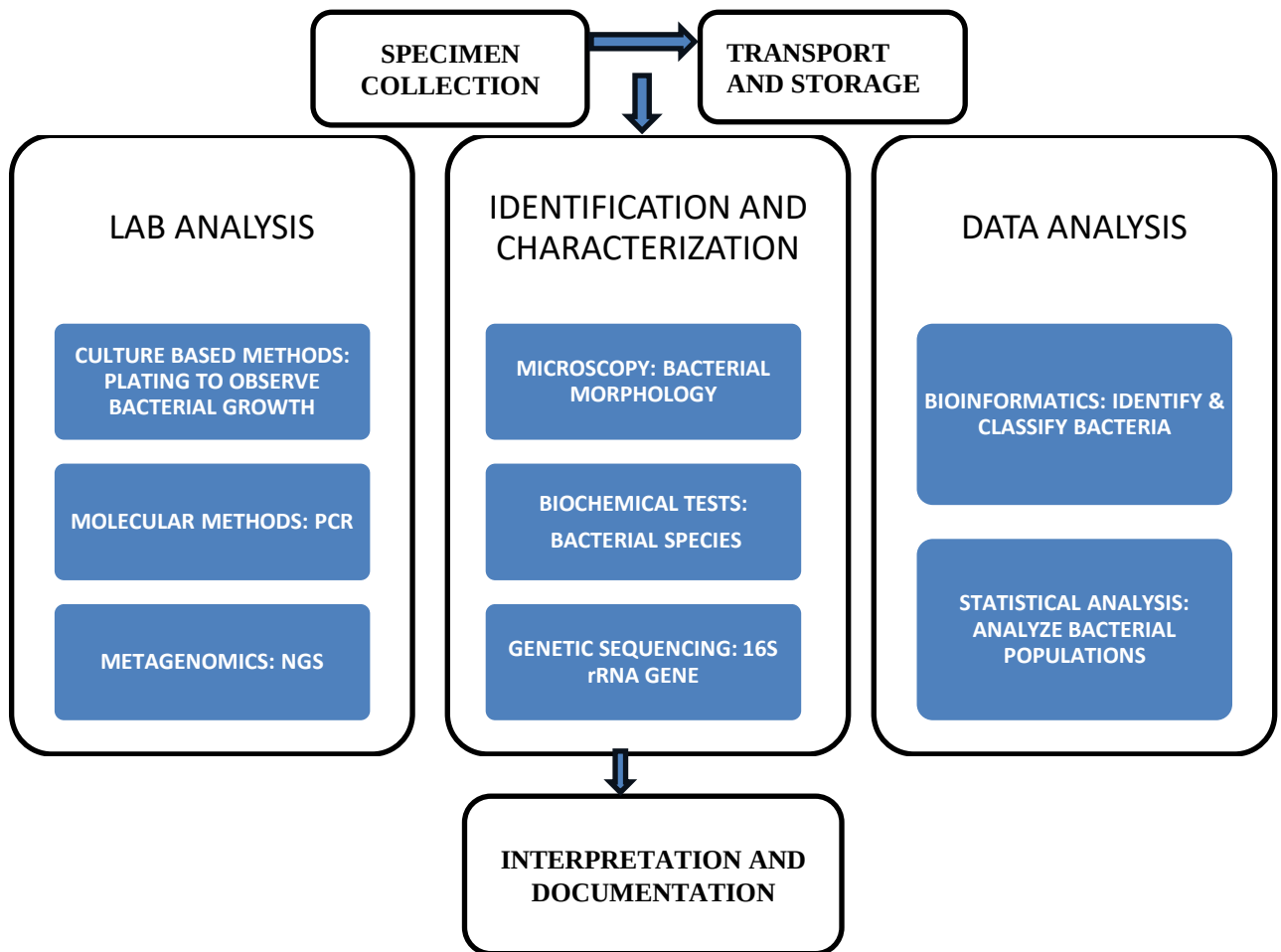


Figure 2. Collection and evaluation of postmortem bacteriological specimen

Conclusions

Postmortem cultures are useful in a small group of patients, comparable to other clinical microbiology cultures. In preselected instances, the reporting of the most relevant specimens (e.g., blood) should be confined to cases with a high possibility of positive findings being useful to the autopsy. Microbiologists and pathologist continue to have a significant issue in distinguishing between true-positive culture findings and postmortem transmigration and/or contamination. Contamination is a serious issue with postmortem samples, although it may be minimized to levels comparable to those seen in living samples if proper measures are followed. Monomicrobial growth of a common parasitic and/or pathogenic bacteria discovered in postmortem blood or tissue cultures seems to be a reliable serious illness. Polymicrobial development and/or the involvement of common contaminant species such coagulase-negative staphylococci and mixed intestinal flora, on the other hand, seem to be more likely the consequence of iatrogenic contamination during specimen collection or postmortem bacterial transmigration.

It's crucial to know whether or not a certain isolate is noteworthy. This includes reviewing the patient's medical history and examining the tissues for evidence of infection. Cell count, differentiation, and protein assessment should all be performed on CSF specimens, just as they would in life; however, this will only be relevant if the culture is obtained promptly after demise. Advanced scientific approaches must be used to aid the diagnosing process. In the event that a bacterial isolate has caused acute sickness they will most likely be dead microscopic organisms and live microbes in the flow, and this can be found out by enhancing microorganisms' explicit ribosomal RNA utilizing the polymerase chain response.

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Agonal spread into the circulation is uncommon, and it's much more uncommon in the CSF When death is otherwise unknown, postmortem microbiology has the most to contribute, but interpretation is the most challenging. Sudden Unexpected Death in Infancy (SUDI) is a unique condition in that if death occurs quickly, inflammation may not be visible to confirm a bacterial isolate. Samples should be gathered as soon as feasible after death and before necropsy in these cases. They should be obtained in a completely aseptic manner, just as they should be in life. The CSF should be submitted for testing as soon as possible, just as in life. Modern molecular approaches should be used to screen for bacterial toxins in blood and CSF. Few examples of such approaches that could help further this field include Polymerase Chain Reaction (PCR) and its variants for toxin gene detection, DNA sequencing techniques for toxin identification. Proteomics and mass spectrometry for toxin protein analysis, Microarray technology for simultaneous toxin screening. Bioinformatics tools and databases for toxin prediction. Notwithstanding, the presence of a microorganism in the blood doesn't generally demonstrate critical infection—this is valid throughout everyday life and evidently also in death—however if bacteremia occurs before death in a patient whose reason for death is generally obscure, the organism should be thought to be a considered causative element of the demise and not let go this factor away. This review aims to highlight the importance of post mortem culture data as the information collected and analysed from microbial samples of a corpse to determine the aspects of death such as cause and manner of death for an accurate and reliable diagnosis which can have social, legal or medical implications. The data can help identify presence and type of bacteria that may have caused or contributed to the death, especially in cases of infection, sepsis. Additionally, it can reveal the efficacy of antimicrobial therapy, identification of emerging pathogens, and the detection of medical errors. It can help in prevention or control procedures in the spread of infectious diseases by identifying the source, transmission, or outbreak of bacteria. Most importantly it can aid in improving quality and safety of health care by detecting and correcting medical errors, such as misdiagnosis, infection, inappropriate treatment, or control breaches.

The best guarantee that specimens would be gathered in relevant instances, without contaminants, and evaluated in the context of all available relevant information would come from effective communication between the pathologist and microbiologist. The committed microbiologist will be honored for their efforts in the long run by acknowledging that mitigating the computation of contaminated and inadequate postmortem specimens will reduce laboratory work and costs, while also offering assistance and guidance in the evaluation of postmortem diagnoses caused by infectious diseases. The genuine utility of postmortem cultures will be determined by the autopsy pathologist's and microbiologist's willingness to meticulously evaluate and integrate clinical, laboratory, and pathological data.

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