



# The Underutilization of Forensic Microbiology: A Narrative Review

Rumpa Saha<sup>1</sup> Stuti Kaushik<sup>2</sup> Arvind Kumar<sup>3</sup> Swapnil Choudhary<sup>3</sup>

<sup>1</sup>Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India

<sup>2</sup>Department of Microbiology, Hamdard Institute of Medical Sciences and Research, Delhi, India

<sup>3</sup>Department of Forensic Medicine and Toxicology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India

Address for correspondence Rumpa Saha, MD, Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi 110095, India (e-mail: rumpachatterjee@yahoo.co.in).

Ann Natl Acad Med Sci (India) 2023;59:139–146.

## Abstract

One of the main reasons of death in India is infection. At many centers, determining the infectious cause of death during autopsy is not regularly done. Although it is still a neglected field, postmortem microbiology has the potential to be a crucial tool for identifying the reason and circumstances of unexpected death. In addition to its use in forensic autopsies and medicolegal investigations, this tool can help with the detection of novel pathogen presentations, estimation of drug resistance, identification of bioterrorism agents, and a better understanding of infectious diseases like toxic shock syndrome, Human Immunodeficiency Virus (HIV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

## Keywords

- ▶ forensic microbiology
- ▶ postmortem microbiology
- ▶ postmortem transmigration
- ▶ agonal spread
- ▶ COVID-19

In India, there has not been much use of microbiology in postmortem, and there is a dearth of specific guidelines or recommendations by regulatory agencies. In an effort to highlight the value of microbiology in postmortem, this narrative review focusses on suggestions made by a group of academicians from Europe in February 2016 and how they may be used in an Indian context. Based on the Indian Council of Medical Research and the Centers for Disease Control and Prevention standards, we have briefly discussed about postmortem in coronavirus disease 2019 related deaths.

## Introduction

One of the main reasons of death in India is infection.<sup>1</sup> At many centers, determining the infectious cause of death during autopsy is not regularly done. Although it is still a neglected field, postmortem microbiology has the potential to be a crucial tool for identifying the reason and circumstances of unexpected death. In addition to its use in forensic autopsies and medicolegal investigations, this tool is useful for identifying emerging pathogens, novel presentations of well-known pathogens, estimating drug resistance, identifying bioterror-

ism agents, and gaining a better understanding of infectious diseases like toxic shock syndrome and Human Immunodeficiency Virus (HIV).<sup>2</sup> In context of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which caused an unprecedented pandemic, the importance of postmortem microbiology was unmatched. It assisted in not only figuring out the cause of sudden death (SD) but also in comprehending the etiopathogenesis of this epidemic.<sup>3</sup> It can also be used as an epidemiological and diagnostic tool in outbreak situations. Prior to coronavirus disease 2019 (COVID-19), informed fetal

article published online  
September 12, 2023

DOI <https://doi.org/10.1055/s-0043-1771030>.  
ISSN 0379-038X.

© 2023. National Academy of Medical Sciences (India). All rights reserved.

This is an open access article published by Thieme under the terms of the Creative Commons Attribution-NonDerivative-NonCommercial-License, permitting copying and reproduction so long as the original work is given appropriate credit. Contents may not be used for commercial purposes, or adapted, remixed, transformed or built upon. (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

autopsy contributed to the growing body of information demonstrating a connection between the Zika virus and microcephaly.<sup>4</sup> One of the main reasons for our diminished understanding of the Middle East respiratory Syndrome compared with Severe Acute Respiratory Syndrome is the dearth of autopsy investigations.

It is impossible to overstate its educational advantages for medical professionals and trainees, and a quality assurance tool for diagnostic and therapeutic procedures is its added advantage. Compared with autopsy, pathology, and forensic science, this topic has been mentioned less frequently in the microbiological literature. In India, postmortem microbiology has not been widely used, and regulatory organizations have not issued any precise recommendations or standards.

In an effort to highlight the value of microbiological investigations postmortem, this narrative review focusses on suggestions made by a group of academicians from Europe in February 2016 and how they may be used in an Indian context. Based on the Indian Council of Medical Research (ICMR) and the Centers for Disease Control and Prevention (CDC) recommendations, the authors have also attempted to add a brief explanation of postmortem in COVID-19 instances.

Only 130 studies on sterile autopsy were found in a search of the PubMed database between the years of 1994 and 2022.<sup>5</sup> These investigations lacked standardized sample collection and culture procedures as well as a “gold standard” for identifying antemortem infectious diseases that were already present.

However, the aforementioned research did draw attention to a few theoretical and practical problems of postmortem microbiology. The pace of microbial multiplication, the availability of nutrients, and the concentration of oxygen available to the microbe all affect how quickly organisms multiply in the body after death. Some challenges to tracking pathogen cultures in postmortem samples are: in at least 50% of cases, saliva drains into the lung after death; about 12 to 15 hours after death, an experimental organism can pass through the human intestinal wall; and postmortem cooling of the body prevents bacterial development.

## The Concept of Sterile Autopsy

O'Toole et al hypothesized this process in 1965, and Minckler et al confirmed it in 1966.<sup>6,7</sup> They started using “sterile mortuaries” with “surgical scrub and gowning” for the autopsy personnel, surgically cleaning the body, and performing dissection in the autopsy room with controlled air flow and using sterile surgical tools. Samples were cultured on common microbiological media during the postmortem interval, which was maintained at <20 hours. It was emphasized that single positive cultures for usual pathogens are likely to indicate actual infection and that these sterile techniques can prevent contamination.

Following these suggestions may help to reduce postmortem iatrogenic contamination<sup>6,7</sup>:

- Use of aseptic technique.
- Sealing organ surface with hot spatula before sampling.
- Obtain blood/tissue samples prior to evisceration.

- Body should be moved to a 4 to 6°C refrigerated locker as soon after death as possible.
- Avoid making needless body movements.
- Clean autopsy room with good ventilation (at least 20 air changes per hour).
- Consider autopsy to be performed as a sterile procedure.
- All postmortem samples to be sent on ice to the microbiology laboratory without any delay in transport.

Postmortem blood cultures can be taken from the heart and spleen to investigate for bacteremia. By pushing a sterile cotton swab deeply through the burned region, tissue cultures can be obtained. Swabs can be cultured using trypticase soy broth, sheep blood agar, and MacConkey agar. Collection of five regular cultures can detect undiagnosed bacteremia, with the spleen being the most trustworthy organ. Due to contamination from upper respiratory microorganisms, lung cultures are unreliable for detecting pneumonia.<sup>7</sup>

## Selection of Appropriate Type of Culture at Postmortem

Usually, standard bacteriological cultures are carried out. Nonetheless, as and when necessary, studies on viruses, mycobacteria, and fungi may be conducted.

Here, it's crucial to look at the four most typical scenarios—highlighted in recent recommendations made by a European Committee—where the use of microbiological methods at autopsy is necessary.<sup>2</sup>

These groups consist of:

- Sudden death.
- Bioterrorism.
- Tissue and cell transplantation.
- Paleomicrobiology.

### Sudden Death

The cases of SD are further divided into four subgroups:

- (a) Group 1: SD in infancy and childhood (0–16 y) without clinical symptoms. Appropriate samples to be collected: nasopharyngeal (NP) swab, blood, cerebrospinal fluid (CSF), feces, and portions of lung, spleen, myocardium.
- (b) Group 2: SD in the young (17–35 y) without clinical symptoms. Blood, myocardium, and spleen are preferably collected.
- (c) Group 3: SD at any age with clinical symptoms. Collection of spleen and blood are recommended while complementary samples can be collected according to the system involved in suspected underlying infection.
- (d) Group 4: Trauma-related or iatrogenic deaths. Blood, tracheobronchial swabs, lung, spleen are recommended to be collected. According to medical and surgical procedures prior to SD, it is necessary to add tissue/fluid from the involved organ, 2 to 3 cm of distal end of central venous catheter tip and pus from deep wounds using a syringe or a sterile swab for bacteriology culture once the wound surroundings/skin surface are cleaned of debris.

When doing virological studies, samples should be collected using sterile saline or an RNA stabilizing solution. An indication of an immunosuppressive condition should prompt the microbiologist to conduct mycological search as well. The choice to conduct molecular investigations depends on the clinical information obtained prior to death and the results of the autopsy.

### Death due to Bioterrorism

The discovery of such biocrimes is a special area of microbial forensics where intentional or threatened use of bacteria, fungus, or poisons from live organisms is used to cause death or sickness in people, animals, or plants. Laboratory investigations are conducted in accordance with the patient's clinical background prior to death and the results of the autopsy.

### Cell and Tissue Transplantation

To lower the risk of transmitting infectious diseases to the recipient during cell and tissue transplantation from the deceased, it is important to assess the possible donor's medical background and physical status, to exclude donor infection with *Treponema pallidum*, HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-cell lymphotropic virus. Serological and molecular assays are conducted for evaluation of cell/tissue transplant final product for yeast, filamentous fungus, aerobic and anaerobic bacterial presence. The Transplantation Society of Australia and New Zealand has issued guidelines (April 2016) regarding these viruses.<sup>8</sup> There are other recommended investigations in addition to these.

### Paleomicrobiology

Paleomicrobiology is a growing subject dedicated to finding, identifying, and characterizing microorganisms in prehistoric remains where microbial DNA connected with the host can endure for approximately 20,000 years. In between sampling, instruments should always be decontaminated with 10% sodium hypochlorite. The samples should be put in sterile tubes or containers at once or kept at 4°C, shielded from light, and brought to the laboratory as soon as possible. Mummified bodies, skeletal remains, tooth powder, coprolites, and dental calculus can all be used to obtain samples. In Peru, the remains of humans were used to date a 9,000-year-old Chagas disease case.<sup>9</sup>

### Specific Indications for Postmortem Microbiological Cultures

- Confirm presence of unproven infection.
- When cause of death is unknown.
- Holds relevance in public health—fatal notifiable diseases and emerging infections.
- Evaluation of efficacy of antimicrobial therapy.
- Investigation of malpractice related to treatment of infectious disease.

- Identification of infectious agents in human skeletal remains.
- Identification of microbial growth after death.
- Investigation of infectious agents in hospital acquired infections.<sup>2</sup>

### Specimen Procurement for Postmortem Microbiology

Precautions to be taken in each autopsy are as follows<sup>10</sup>:

- Body should be placed in sealed body bag at 4°C as soon as possible until autopsy starts.
- Conducted within 24 hours following death, microbiological sample taken as soon as possible.
- Skin disinfected with 0.05% chlorhexidine with 0.5% cetrimonium bromide in water.
- Organ surfaces seared with red-hot spatula or soldering iron before sampling.
- Blood samples, body fluids, and NP exudates best collected at beginning.
- Tissue specimens obtained prior to evisceration.
- Tubes with heparin or oxalate or fluoride avoided, toxic to many microorganisms.
- Exudate collected using a syringe or flocked swab.
- Retrieved samples should arrive within 2 hours when stored at room temperature and within 48 hours when stored at 2 to 8°C in adequate transport media.

Recommendations for specimens to be collected are<sup>10</sup>:

- A total of 3 to 5 mL blood/fluid is to be collected from interior of heart/other organs by searing surface with hot spatula and withdrawing through syringe/pipette. Preferential order of places to take blood in situ: peripheral femoral → subclavian → carotid → jugular → left ventricle.
- More than 1 mL of CSF by lumbar puncture/cisternal puncture as in antemortem collection, indicated in sudden infant death.
- More than 1 to 2 cm<sup>2</sup> solid tissues to be cut with sterile scalpel/scissor through seared surface.
- Vegetations on cardiac valves cultured by picking small portion with sterile forceps.
- If heart opened aseptically vegetations to be washed in several batches of sterile saline, grinded, and plated immediately.
- Swabs if indicated, should be two in number.
- Smears to be prepared from all lesions subjected to culture.

### Interpretation of Positive Bacterial Cultures

Positive bacterial cultures may be interpreted as one of the following<sup>10</sup>:

- Indicative of true infection.
- Culture contamination.
- Postmortem transmigration of bacteria.
- Agonal spread of bacteria.

A typical pathogenic organism isolated in monomicrobial culture is likely to be a true pathogen. Polymicrobial growth is suggestive of contamination.

Postmortem transmigration of bacteria was first described by Gradwohl in 1904.<sup>11</sup> Bacteria migrate from mucosal surfaces and tissues into bloodstream after circulation has ceased. Reviews of clinical autopsy cases indicate:

- Blood culture positivity rates increased from 20 to 40% in correlation with length of postmortem interval.<sup>12</sup>
- Postmortem lung culture positivity correlates with increased length of hospital stay and postmortem interval.<sup>12</sup>
- Transmigration of bacteria through intact intestinal walls in humans occurs in 12 to 15 hours.<sup>13</sup>
- Comparison of conventional culture with real time polymerase chain reaction (PCR) for evaluation of transmigration is suggestive of:
  - Transmigration from intestine to blood, liver, portal vein, mesenteric lymph nodes, and pericardium,
  - Skin/oral cavity/respiratory tract also potential sources of transmigration,
  - Detection rates better by PCR.
  - Blood cultures unreliable (high rate of contamination/polymicrobial/enteric organisms)
  - Liver/pericardium better samples (higher/stable rates of sterility)
  - Relative amounts of intestinal bacterial DNA (*Bifidobacteria*, *Bacteroides*, *Enterobacter*, *Clostridia*) increased with time.

The concept of agonal spread of bacteria, given by Fredette in 1916, states invasion of bacteria into the bloodstream when systemic circulation is artificially maintained during resuscitation efforts or is dropping during the agonal period.<sup>14</sup> On the basis of the relationship between antemortem and postmortem cultures, this has continued to be a contentious issue and has shed light on the potential existence of “terminal sepsis.”

When compared with agonal spread, which has little support in the literature and is a theoretical idea, postmortem transmigration of bacteria is widely accepted by pathologists and microbiologists.

### Importance of Postmortem Virology Including Severe Acute Respiratory Syndrome Coronavirus 2

In certain viral infections postmortem analysis is important:

- Postmortem testing in certain circumstances maybe the only option to confirm a diagnosis of novel influenza A virus infection. For immunohistochemistry staining, a minimum of eight blocks and preserved tissue specimens from pulmonary locations are advised. For culture and molecular analyses, fresh or frozen samples can be used as recommend by the CDC.<sup>15</sup>
- The World Health Organization advises using the fluorescent antibody technique to examine the impressions or

smears of tissue samples taken from the brain stem and Ammon's horn.<sup>16</sup>

- For suspected Ebola virus infections, oral swabs can be collected following all standard safety precautions and placed in viral transport medium, along with postmortem tissue samples (liver, spleen, bone marrow, kidney, lung, and skin snips) and handled in Biosafety Level-4 laboratories.
- Siliguri saw an acute encephalitis outbreak between January 31 and February 23, 2001. A group of scientists from four prominent institutions collected necropsy samples for investigations. The results were later approved by the CDC.<sup>17</sup>

### Severe Acute Respiratory Syndrome Coronavirus 2 (Indian Council of Medical Research)

#### Precautions for Packing and Transport of Body to the Mortuary

For COVID-19 positive or suspected instances, the Ministry of Health and Family Welfare of the Government of India has published instructions on dead body handling. At the mortuary, negative pressure must be maintained. Dissection of a body cavity should only be done one at a time. Use the proper recommended practices to reduce the production of aerosols during autopsies, particularly when handling lung tissue.<sup>18,19</sup>

Prior to transferring a cadaver to the mortuary, the NP swab for reverse transcription-PCR is advised by the ICMR in all suspect cases. All catheters, drains, and tubes should be taken out, and any wounds or holes created by their removal should be disinfected with 1% sodium hypochlorite solution (Hypo) and application of impermeable tape.

According to the Biomedical Waste Disposal recommendations, any associated sharp objects should be disposed of in sharp containers. The nasal and oral orifices should be sealed, and the body should be double-packed in a clear, leak-proof body bag. Hypochlorite is to be used to clean the body bag's outside. Together with other information about the patient, the COVID status of the patient should be prominently displayed on the label. The health care worker moving the body to the mortuary should be outfitted in appropriate personal protective equipment (PPE).

Before and after the transfer, the trolley should be cleaned with hypochlorite solution. It is recommended that the cold chamber used to store bodies be divided into sections specifically designated for COVID-19 and non-COVID-19 bodies. With COVID-19 patient bodies, lower spaces should be chosen to prevent spilling of bodily fluids while handling the body. After touching a COVID-19-infected body, all surfaces, including high-risk touch sites, must be decontaminated with hypochlorite.

Considering the need for strict biosafety procedures, no forensic science or virology laboratory in India has been designated for the examination of specimens from COVID-19 infected patients. Nonetheless, the CDC has provided guidelines for completing autopsies in COVID suspect cases.

**Table 1** Specimens to be collected during postmortem in suspect/confirmed coronavirus disease 2019 patients

Covid status	Autopsy	Specimen to be collected
Suspect	Yes	PM swab for COVID: •URT: NP swab •LRT: Lung swab from each lung
		Separate swab specimens for testing other respiratory pathogens
		Formalin-fixed autopsy tissues from lungs, upper airway, and major organs
Suspect	No	NP swab for COVID Separate NP swab for other respiratory pathogens
Confirmed	Yes	NP swab specimens for testing of other respiratory pathogens •Other postmortem microbiologic and infectious disease testing, as indicated •Formalin-fixed autopsy tissues from lung, upper airway, and other major organs

Abbreviations: COVID, coronavirus disease; NP, nasopharyngeal; PM, post mortem ; URT, upper respiratory tract; LRT, lower respiratory tract.

► **Table 1** summarizes the specimens to be collected during postmortem in suspect/confirmed COVID-19 patients.<sup>20</sup> ► **Table 2** outlines the CDC recommendations for engineering control and PPE.<sup>20</sup> ► **Table 3** outlines the CDC guidelines for specimen collection.<sup>20</sup>

Swabs should be collected and put in a transport medium. To increase test sensitivity and reduce the consumption of testing resources, it is best to combine both NP and oropharyngeal swabs when they are collected in a single tube.

In addition to basic bacterial cultures, toxicological tests and other studies as necessary should be guided by the

decedent's clinical and exposure history, scene investigation, and gross autopsy results.

Samples from these tissues should be taken if the clinical history or laboratory results acquired before to death indicate the involvement of other organs.

For the best fixation, it is advised to collect tissue samples that are around 5 mm thick and placed in 10% buffered formalin at 10 times greater than the volume of tissue.

Another option is to send original blocks of formalin-fixed, paraffin-embedded tissues from autopsies for examination.

**Table 2** Engineering control and personal protective equipment recommendations (Centers for Disease Control and Prevention) for autopsy in suspect coronavirus disease 2019 patients

Specimen to be collected	Engineering control recommendations	PPE recommendations
Postmortem, NP swab only	A negative pressure room is not required	<ul style="list-style-type: none"> <li>• Nonsterile, nitrile gloves.</li> <li>• Heavy-duty gloves over the nitrile gloves if there is a risk of cuts, puncture wounds, or other injuries that break the skin,</li> <li>• Clean, long-sleeved fluid-resistant or impermeable gown</li> <li>• 4. Plastic face shield or a face mask and goggles</li> </ul>
Autopsy (COVID suspect/positive)	<p>Conducted in airborne infection isolation rooms (AIIRs)</p> <ul style="list-style-type: none"> <li>• Are at negative pressure to surrounding areas</li> <li>• Have minimum of 6 air changes per hour (ACH) for existing structures and 12 ACH for renovated or new structures</li> <li>• Have air exhausted directly outside or through a high-efficiency particulate aerosol (HEPA) filter</li> </ul> <p>Doors should be kept closed except during entry and egress. If AIIR is not available, ensure the room is negative pressure with no air recirculation to adjacent spaces. A portable HEPA recirculation unit could also be placed in the room to provide further reduction in aerosols. Local airflow control (i.e., laminar flow systems) can be used to direct aerosols away from personnel. If use of an AIIR or HEPA unit is not possible, the procedure should be performed in the most protective environment possible. AIIR room air should never be recirculated in the building, but directly exhausted outdoors, away from windows, doors, areas of human traffic or gathering spaces, and from other building air intake systems</p>	<ul style="list-style-type: none"> <li>• Double surgical gloves interposed with a layer of cut-proof synthetic mesh gloves</li> <li>• Fluid-resistant or impermeable isolation gown</li> <li>• Waterproof apron</li> <li>• Goggles or face shield</li> <li>• NIOSH-certified disposable N-95 respirator or higher</li> </ul>

Abbreviations: COVID, coronavirus disease; NIOSH, National Institute for Occupational Safety and Health; PPE, personal protective equipment.

**Table 3** Centers for Disease Control and Prevention guidelines for specimen collection in autopsy of suspect coronavirus disease 2019 patients

Specimen	Alternate specimen	Steps of collection	Storage
Nasopharyngeal (NP) swab	Oropharyngeal (OP) specimen nasal midturbinate (NMT) swab anterior nares (nasal swab; NS) specimen NP wash/aspirate or nasal aspirate (NA) specimen	Insert flexible wire shaft minitip swab through the nares parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. For NS, a single polyester swab with a plastic shaft should be used to sample both nares	2–8°C for up to 72 h after collection. If a delay in testing or shipping is expected, store specimens at –70° C or below
Lung swab		Collect one swab from each lung (left and right) by one of the two methods: During internal exam, after heart–lung block is removed, insert one swab as far down into the tracheobronchial tree as possible on either side (left and right) or first wipe the surface of each lung with an iodine-containing disinfectant clean and dry the surface, then use a sterile scalpel to cut a slit of the lung and insert the swab to collect sample on either side	2–8°C for up to 72 h after collection. If a delay in testing or shipping is expected, store specimens at –70° C or below
Autopsy tissue specimens		<ul style="list-style-type: none"> <li>• A minimum of three representative sections of lung parenchyma, preferably from different locations</li> <li>• A minimum of two sections of airway, to include trachea, bronchi, or both airways</li> </ul>	

Recently, a potential technique in forensic microbiology has been described: the use of the V4 hypervariable region of bacterial 16S rRNA gene sequences for taxonomic classification (“barcoding”) and phylogenetic analysis of human post-mortem microbiota.<sup>21</sup>

### Microbiology in Minimally Invasive Autopsy

In nations where the body cavities are not accessed, minimally invasive autopsy (MIA) is an alternative to traditional autopsy. Needles are used to access the primary organs via the percutaneous route. The history leading up to the death and a thorough external examination are combined with the findings. It is believed that using needle aspirations and biopsies alongside multislice computed tomography or magnetic resonance imaging scans will boost the sensitivity of MIA.<sup>22</sup> In environments with limited resources, MIA can be used to determine the cause of death in order to improve public health in general. The MIA protocol calls for taking postmortem biopsies from important organs and doing in-depth histopathological and microbiological analyses. Blood, CSF, liver, brain, lungs, heart, spleen, kidneys, bone marrow, and uterus from women of childbearing age are among the specimens gathered.<sup>23–25</sup> Histopathological hints guide the selection of the necessary microbiological testing. Testing for HIV, HBV, HCV, malaria, and bacteriological and mycological culture are a few of the investigations conducted. The use of

molecular methods is also an option depending on the circumstances. This method has been shown very effective at several institutions and is believed to be able to pinpoint an infectious cause of death in about 84% of cases.<sup>22</sup>

### Underutilization of Postmortem Microbiology

Full diagnostic autopsies are rarely performed when they are not legally necessary, despite the fact that they might reveal crucial information concerning the cause of death. Postmortem microbiology thus continues to be a subject that is neglected, primarily due to issues with religious and local cultural beliefs, technical limitations, a lack of skilled labor, and infrastructure.<sup>26,27</sup> Postmortem cultures and MIB are extremely important in everyday settings but are underutilized as a result of doctors' lack of understanding of growing medicolegal concerns and clarity about consent. Organ retention, poor communication, and a lack of comprehension were some other problems related the inability to get consent that were brought to light by a review of the factors influencing the uptake of postmortem examination in the pediatric population.<sup>26</sup> This can be overcome by utilizing more modern methods, like MIA, which are more palatable to the staff, patients, and bereaved family members.

The specific purposes for which postmortem microbiology is used can explain its usefulness.

The following are examples of its significance:

- Undiagnosed infection—A recent German case report detailed the detection of multidrug-resistant *Klebsiella pneumoniae* in a patient with sepsis superimposed on SLE (Systemic Lupus Erythematosus). This was not diagnosed antemortem, despite blood culture being collected 4 to 5 times.<sup>28</sup>
- New pathogen—A 16-year-old healthy child developed spontaneous nontraumatic lethal myonecrosis, which was caused by a new bacterium called *Clostridium fallax*.<sup>29</sup>
- Novel presentation—In Canada, a novel case of herpes simplex virus hepatitis that killed an immunocompetent man was reported.<sup>30</sup>
- A 53-year-old patient in Romania lost his life after having intestinal TB that was mistakenly identified as Crohn's disease.<sup>31</sup>
- Isolation of *Candida albicans* from heart blood at autopsy indicated disseminated disease.<sup>32</sup>
- *Exserohilum* in steroid solutions was discovered, which caused a fatal neurological illness outbreak in the United States.<sup>33</sup>
- Detection of coinfections with increased mortality in COVID-19. These include secondary disseminated mucormycosis in nine patients between March 1 and April 30, 2020.<sup>34</sup>
- A 58-year-old lady with flu-like symptoms with COVID-19 in her blood was found dead.<sup>35</sup>
- A pilot project was started in a tertiary care hospital in Delhi to find out about the perception of health care professionals regarding the role MIA played in identifying the causes of neonatal deaths.<sup>36</sup>

## Medicolegal Aspects of Forensic Microbiology

Forensic microbiology still remains an underutilized entity in this era of modern medicine. It has a huge potential to be utilized for the purpose of administration of justice because of its medicolegal ramifications. The applied aspects of postmortem microbiology are immensely diversified including the estimation of postmortem interval, dwelling into the cause of death, especially in cases of sudden natural deaths, drowning, identification of emergent pathogens, novel presentation of known pathogens, biocrimes involving humans, agroterrorism, cases of sexual assault, medical malpractice, nosocomial infection control, estimation of antibiotic drug resistance, food safety, and environmental contamination, and investigation of food borne illnesses, etc.

Identifying the etiological agent as the origin of an infection that had previously gone undetected and validating the antemortem diagnosis are the two main conceptual justifications for obtaining postmortem blood and/or tissue cultures. In some cases, an infection may only be identified during the postmortem examination, as in the case of endocarditis and heart valve vegetations. Blood cultures acquired at the start of the autopsy may be extremely helpful in these situations to pinpoint the precise origin of the

infection since cultures of the real tissue are inappropriate due to contamination concerns. In other instances, postmortem blood and tissue cultures may offer additional vital details on the extent and severity of a previously suspected or diagnosed antemortem infectious disease but caused the patient's condition to deteriorate rapidly and lead to death. However, in rare instances, postmortem cultures, particularly, blood cultures 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 antemortem cultures. Lastly, the efficacy of antimicrobial therapy could be determined by the results from postmortem cultures. Differentiating between true-positive culture results and postmortem transmigration and/or contamination remains a major challenge to microbiologists and pathologists. Moreover, monomicrobial development of a typical opportunistic and/or pathogenic microbe seen in postmortem blood or tissue cultures seems to be a reliable sign of infection. However, it appears more likely that polymicrobial growth and/or the presence of typical contaminant organisms, like mixed intestinal flora and coagulase-negative Staphylococci, are the result of iatrogenic contamination during the collection of the specimen or because of postmortem bacterial transmigration.<sup>10</sup>

## Conclusions

Postmortem cultures are indicated in a limited number of scenarios. It is of utmost important to safeguard cultures from contamination by following aseptic techniques. Cultures are spleen and heart blood are suggested as the best autopsy specimens for bacteriology. Whenever terminal infection is suspected, sample collection from at least two if not more different sites should be the standard practice as it increases the possibility to detect the causative agent of antemortem infection. Isolation of a single common organism is more suggestive of a true infection as compared with contamination in cases of rare polymicrobial isolation. Postmortem culture results must be interpreted along with clinical, other laboratory, and pathological findings to assess its actual weightage. Bacteriological examinations performed after death can be viewed as a consistent investigative device for postmortem quality check of the diagnostic and therapeutic procedures done antemortem and serve as an added supplement to health care-associated infections within a specific hospital environment. The recent pandemic of SARS-CoV-2 highlights the role postmortem microbiology will play in describing etiology and confirming the cause in SD. Techniques like MIA are promising tools to supplement application of this specialty.

### Authors' Contribution

R.S.: Analyzed the data and revised the manuscript critically for important intellectual content (microbiology), final approval. S.K.: Analyzed the data and drafted the manuscript. A.K.: Revised the manuscript critically for important intellectual content (forensic medicine) and final approval. S.C.: Data collection and analysis of the data. All authors critically revised the manuscript,

approved the final version to be published, and agree to be accountable for all aspects of the work.

#### Conflict of Interest

None declared.

#### References

- Vos TGBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020;396(10258):1204–1222
- Fernández-Rodríguez A, Cohen MC, Lucena J, et al. How to optimise the yield of forensic and clinical post-mortem microbiology with an adequate sampling: a proposal for standardisation. *Eur J Clin Microbiol Infect Dis* 2015;34(05):1045–1057
- Nimir M, Tank A, Snead D. COVID-19 post-mortem findings: how the departed can teach us. *Diagn Histopathol* 2021;27(10):422–424
- Schwartz DA. Autopsy and postmortem studies are concordant: pathology of zika virus infection is neurotropic in fetuses and infants with microcephaly following transplacental transmission. *Arch Pathol Lab Med* 2017;141(01):68–72
- Sterile autopsy - Search Results - PubMed (nih.gov). Accessed February 24, 2022
- O'Toole WF, Saxena HM, Golden A, Ritts RE. Studies of post-mortem microbiology using sterile autopsy technique. *Arch Pathol* 1965;80(05):540–547
- Minckler TM, Newell GR, O'Toole WF, Niwayama G, Levine PH. Microbiology experience in collection of human tissue. *Am J Clin Pathol* 1966;45(01):85–92
- The Transplantation Society of Australia and New Zealand. Clinical guidelines for organ transplantation from deceased donors. Version 1.0–April 2016 TSANZ Clinical guidelines. Accessed January 23, 2022 at: TSANZ - Document Library - Transplantation Society of Australia & New Zealand
- Aufderheide AC, Salo W, Madden M, et al. A 9,000-year record of Chagas' disease. *Proc Natl Acad Sci U S A* 2004;101(07):2034–2039
- Riedel S. The value of postmortem microbiology cultures. *J Clin Microbiol* 2014;52(04):1028–1033
- Gradwohl RBH. Importance de l'examen bactériologique pratiqué sur les cadavres. *Ann Inst Pasteur (Paris)* 1904;18:767–773
- Carpenter HM, Wilkins RM. Autopsy bacteriology: review of 2,033 cases. *Arch Pathol* 1964;77:73–81
- Kellerman GD, Waterman NG, Scharefenberger LF. Demonstration in vitro of postmortem bacterial transmigration. *Am J Clin Pathol* 1976;66(05):911–915
- Fredette JW. Bacteremias in the agonal period. *J Lab Clin Med* 1916;2:180–188
- Centers for Disease Control and Prevention, Coordinating Center for Infectious Diseases. 2010 Guidelines for the submission of tissue specimens for the pathologic evaluation of influenza virus infections. Accessed February 2022 at: <http://www.cdc.gov/h1n1flu/tissuesubmission.htm>
- World Health Organization. Laboratory techniques in rabies. 5th edition, Volume 1. Accessed December 2021 at: untitled (who.int)
- Aditi SM, Shariff M. Nipah virus infection: a review. *Epidemiol Infect* 2019;147:e95
- Indian Council of Medical Research. 2019 Standard guidelines for medicolegal autopsy in COVID-19 deaths in India. Accessed March 2021 at: Standard-guidelines-for-Medico-legal-autopsy-in-COVID-19-deaths-in-India-10.05.2020-32-Pages-English-1.07-MB.pdf (tn.gov.in)
- Ministry of Health & Family Welfare. 2020 Government of India. EMR Division. Directorate General of Health Services. COVID-19: guidelines on dead body management. Accessed March 2021 at: [https://www.mohfw.gov.in/pdf/1584423700568\\_COVID19\\_GuidelinesonDeadbodymanagement.pdf](https://www.mohfw.gov.in/pdf/1584423700568_COVID19_GuidelinesonDeadbodymanagement.pdf)
- Centers for Disease control and Prevention. Collection and submission of postmortem specimens from deceased persons with confirmed or suspected COVID-19. Accessed March 10, 2021 at: COVID-19 Guidance Postmortem Specimens | CDC
- Lutz H, Vangelatos A, Gottel N, et al. Effects of extended postmortem interval on microbial communities in organs of the human cadaver. *Front Microbiol* 2020;11:569630
- Saegeman V, Cohen MC, Burton JL, et al. Microbiology in minimally invasive autopsy: best techniques to detect infection. ESGFOR (ESCMID study group of forensic and post-mortem microbiology) guidelines. *Forensic Sci Med Pathol* 2021;17(01):87–100
- Addison S, Arthurs OJ, Thayyil S. Post-mortem MRI as an alternative to non-forensic autopsy in fetuses and children: from research into clinical practice. *Br J Radiol* 2014;87(1036):20130621
- Bassat Q, Castillo P, Martínez MJ, et al. Validity of a minimally invasive autopsy tool for cause of death determination in pediatric deaths in Mozambique: an observational study. *PLoS Med* 2017;14(06):e1002317
- Menendez C, Castillo P, Martínez MJ, et al. Validity of a minimally invasive autopsy for cause of death determination in stillborn babies and neonates in Mozambique: an observational study. *PLoS Med* 2017;14(06):e1002318
- Lewis C, Hill M, Arthurs OJ, Hutchinson C, Chitty LS, Sebire NJ. Factors affecting uptake of postmortem examination in the prenatal, perinatal and paediatric setting. *BJOG* 2018;125(02):172–181
- Lishimpi K, Chintu C, Lucas S, et al. Necropsies in African children: consent dilemmas for parents and guardians. *Arch Dis Child* 2001;84(06):463–467
- D'Ovidio C, Pompilio A, Crocetta V, Gherardi G, Carnevale A, Di Bonaventura G. Fatal sepsis by *Klebsiella pneumoniae* in a patient with systemic lupus erythematosus: the importance of postmortem microbiological examination for the ex post diagnosis of infection. *Int J Legal Med* 2015;129(05):1097–1101
- Hausmann R, Albert F, Geißdörfer W, Betz P. *Clostridium fallax* associated with sudden death in a 16-year-old boy. *J Med Microbiol* 2004;53(Pt 6):581–583
- Poley RA, Snowdon JF, Howes DW. Herpes simplex virus hepatitis in an immunocompetent adult: a fatal outcome due to liver failure. *Case Rep Crit Care* 2011;2011:138341
- Gurzu S, Molnar C, Contac AO, Fetyko A, Jung I. Tuberculosis terminal ileitis: a forgotten entity mimicking Crohn's disease. *World J Clin Cases* 2016;4(09):273–280
- Thorn JL, Gilchrist KB, Sobonya RE, Gaur NK, Lipke PN, Klotz SA. Postmortem candidaemia: marker of disseminated disease. *J Clin Pathol* 2010;63(04):337–340
- Casadevall A, Pirofski LA. Exserohilum rostratum fungal meningitis associated with methylprednisolone injections. *Future Microbiol* 2013;8(02):135–137
- Hanley B, Naresh KN, Roufousse C, et al. Histopathological findings and viral tropism in UK patients with severe fatal COVID-19: a post-mortem study. *Lancet Microbe* 2020;1(06):e245–e253
- Lacy JM, Brooks EG, Akers J, et al. COVID-19: postmortem diagnostic and biosafety considerations. *Am J Forensic Med Pathol* 2020;41(03):143–151
- Das MK, Arora NK, Rasaily R, et al. Perceptions of the healthcare providers regarding acceptability and conduct of minimal invasive tissue sampling (MITS) to identify the cause of death in under-five deaths and stillbirths in North India: a qualitative study. *BMC Health Serv Res* 2020;20(01):833