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Postmortem Bacteriology in Nosocomial Bronchopneumonia: A Comparison Between Cultures and Molecular Analysis

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Abstract

Nosocomial bronchopneumonia is a severe lung infection that develops more than 48 h after hospital admission and is frequently caused by antibiotic-resistant bacteria. It is often identified postmortem in forensic practice, particularly in patients with severe traumatic injuries requiring prolonged hospitalization and immobilization. Diagnosis is typically based on macroscopic findings and histopathological examination of lung tissue. This study aimed to evaluate the diagnostic value of postmortem microbiological testing by comparison with antemortem microbiological data. Ten patients with a clinical diagnosis of nosocomial bronchopneumonia were selected from forensic cases. During autopsy, tracheal swabs and lung tissue samples were collected and subjected to culture-based and molecular analyses. The results were compared with those obtained from antemortem microbiological investigations. Pathogens characteristic of nosocomial infections were identified; however, concordance with in-hospital microbiological data was highest when using next-generation sequencing (NGS) metagenomic analysis. Tracheal swab culture appears to have limited reliability for postmortem identification of bacterial agents in healthcare-associated bronchopneumonia. In contrast, metagenomic next-generation sequencing (mNGS) of lung tissue obtained at autopsy showed the highest concordance with antemortem microbiological findings and may provide valuable complementary diagnostic information, particularly in polymicrobial infections.

Keywords: healthcare-associated bronchopneumonia; postmortem bacteriology; bacterial culture; molecular analysis; 16S rRNA gene sequencing; next-generation sequencing (NGS); metagenomics; traumatic injuries

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1. Introduction

Healthcare-associated infections (HAIs), also known as nosocomial infections, represent a major global public health concern, significantly affecting morbidity, mortality, and healthcare costs. According to the most recent World Health Organization (WHO) Global

Report on Infection Prevention and Control (2024), HAIs are among the most frequent adverse events associated with health care delivery worldwide. On average, 7 out of every 100 patients in acute-care hospitals in high-income countries and 15 out of every 100 patients in low- and middle-income countries acquire at least one HAI during their hospital stay. Approximately one in ten affected patients dies as a consequence of these infections. Patients admitted to intensive care units are at particularly high risk of acquiring HAIs [1].

Although current Romanian legislation on HAIs is fully aligned with European standards [2], HAIs remain significantly underestimated in Romania, with official prevalence rates ranging from 0.2% to 0.25%. This underestimation is attributable to several factors contributing to underreporting [3]. One of the main obstacles to diagnosing and preventing HAIs is the lack of rapid and reliable techniques for identifying the causative microorganisms [4].

Respiratory tract infections, surgical site infections, urinary tract infections, bloodstream infections, and gastrointestinal infections—of which *Clostridioides difficile* infections account for nearly half—are the most commonly reported types of HAIs [5].

Among the bacterial pathogens responsible for healthcare-associated infections, several belong to the ESKAPE group, including *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *and Enterobacter* spp. These organisms are recognized as major multidrug-resistant pathogens because of their remarkable ability to evade the effects of antimicrobial therapy and are responsible for a substantial proportion of severe hospital-acquired infections worldwide. In patients with prolonged hospitalization, particularly those admitted to intensive care units, ESKAPE pathogens are among the leading causes of healthcare-associated pneumonia and are associated with increased morbidity, mortality, and healthcare costs [6,7].

Due to the nature of forensic practice, healthcare-associated bronchopneumonia is frequently encountered, particularly in patients who have suffered severe traumatic injuries requiring prolonged hospitalization and immobilization.

From a conceptual standpoint, postmortem microbiological examinations play an important role in confirming antemortem infectious diagnoses, supporting the identification of etiological agents, and contributing to the determination of the cause of death in medico-legal investigations [8].

Antemortem microbiological findings can be further evaluated using autopsy data. When interpreted together with macroscopic and histopathological findings, postmortem microbiological analysis may confirm the infectious process and provide additional evidence supporting the determination of the cause of death [9,10]. Furthermore, the identification of pathogenic microorganisms may have considerable medico-legal value by supporting the assessment of the role of healthcare-associated infection in thanatogenesis and the establishment of the causal relationship between infection and death [11].

Although postmortem bacteriology has been controversial, several studies indicate that postmortem cultures retain diagnostic value. Correlating autopsy and histological findings with postmortem culture results from different anatomical sampling sites may help forensic pathologists identify the etiological agent of antemortem infections and contribute to establishing the precise cause of death [12].

Polymerase chain reaction (PCR), broad-range 16S rRNA gene sequencing, and metagenomic approaches have enabled culture-independent identification of microorganisms, thereby broadening the spectrum of detectable pathogens and allowing a more comprehensive characterization of postmortem microbial communities [10].

PCR is a highly sensitive molecular technique used for postmortem bacterial identification, particularly when traditional culture methods fail due to prior antibiotic adminis-

tration, fastidious growth requirements, or prolonged postmortem interval (PMI). PCR can detect microbial DNA from both viable and non-viable organisms and is not significantly affected by prior antimicrobial use.

Among molecular approaches, broad-range 16S rRNA gene-based PCR is one of the most widely used methods. The 16S rRNA gene is a major component of the 30S small ribosomal subunit present in all prokaryotes [13]. It contains highly conserved regions that serve as universal primer binding sites, together with nine hypervariable regions (V1–V9) that are important for phylogenetic analysis and taxonomic classification. The presence of these highly conserved regions enables amplification of the 16S rRNA gene from a wide range of bacterial species using a limited set of universal primers [14].

In cases where identification of pneumonia-causing bacteria by postmortem lung tissue culture is impractical—due to unculturable organisms or difficulties in selecting appropriate culture conditions—16S rRNA gene-based metagenomic analysis enables identification of the bacterial genera involved in infection [15].

Metagenomic next-generation sequencing (mNGS), which combines high-throughput sequencing with bioinformatic analysis, is an emerging and promising diagnostic approach. mNGS is less affected by prior antibiotic use, enables simultaneous detection of a broad range of pathogens (including bacteria, fungi, viruses, and parasites), requires shorter analysis time, and provides semi-quantitative data useful for interpretation [16].

The aim of this study was to evaluate the ability of postmortem microbiological methods to identify the clinically relevant pathogen detected during hospitalization by comparing postmortem conventional culture, PCR/Sanger sequencing, and metagenomic next-generation sequencing (mNGS) results with antemortem microbiological findings. Furthermore, we compared the diagnostic performance, advantages, and limitations of each method in the postmortem investigation of healthcare-associated bronchopneumonia.

2. Materials and Methods

2.1. Study Design and Case Selection

Between 2024 and 2025, we conducted an experimental study on autopsied patients at the Institute of Forensic Medicine Timișoara.

Case selection was guided by relevance to forensic practice, with particular emphasis on cause-of-death attribution, sampling techniques, methodological constraints, and interpretative challenges.

Patients were included if they underwent autopsy at the Institute of Forensic Medicine Timișoara and met the following inclusion criteria:

(i) sustained a traumatic injury requiring hospitalization;
(ii) were hospitalized for more than 48 h;
(iii) had a clinical diagnosis of healthcare-associated bacterial bronchopneumonia supported by microbiological testing;
(iv) died during hospitalization; and
(v) had a postmortem interval of less than 48 h.

A total of ten cases met all inclusion criteria and were included in the study.

This study was designed as a pilot exploratory study to assess the feasibility and comparative performance of different postmortem microbiological methods in a highly selected forensic population. Because of the rarity of cases fulfilling all inclusion criteria, the primary objective was to generate preliminary evidence and identify methodological strengths and limitations rather than to establish definitive diagnostic accuracy.

Written informed consent was obtained from the legal representatives of the deceased patients included in the study. All procedures were conducted in accordance with the

Declaration of Helsinki and approved by the Ethics Committee of Victor Babeș University of Medicine and Pharmacy Timișoara (approval no. 61/20 December 2019).

2.2. Sample Collection

During the forensic autopsy, two tracheal swabs and one lung tissue sample were collected from each patient.

The anterior chest wall was disinfected with alcohol and povidone–iodine to minimize sample contamination, after which the thoracic cavity was opened using instruments disinfected with formaldehyde. Using sterile gloves and a sterile surgical blade, an incision was made in the anterior wall of the trachea. An eSwab (COPAN Diagnostics, Brescia, Italy) was inserted into the tracheal lumen and advanced into each main bronchus as far as the swab length permitted. The procedure was repeated using a second sterile swab.

Subsequently, the thoracic cavity and lungs were examined, and a region of the lung surface was aseptically dissected. A lung tissue sample (parenchymal fragment) was then collected under sterile conditions.

Quality assurance during postmortem sampling relied on strict aseptic procedures, including disinfection of the chest wall, the use of sterile gloves, sterile swabs and surgical blades, aseptic collection of lung parenchyma, and immediate preservation of samples intended for molecular testing. Dedicated negative collection controls, such as simultaneously processed sterile saline samples, were not included.

The first tracheal swab was placed in liquid Amies transport medium and transported within 1–2 h to the microbiology laboratory (Bioclinica Laboratory, Timișoara, Romania). Liquid Amies is a phosphate-buffered, non-nutritive transport medium designed to preserve the viability of aerobic, anaerobic, and fastidious bacteria during transport. It is commonly used with flocked swabs (e.g., eSwab) to facilitate sample elution and enable multiple downstream analyses, including culture, Gram staining, and molecular testing. The selection of the culture media was based on the validated standard operating procedures routinely implemented by the accredited clinical microbiology laboratory where the conventional culture analyses were performed. The selected media are routinely used for the isolation of bacterial pathogens commonly associated with respiratory tract infections and healthcare-associated pneumonia.

The second tracheal swab was placed in DNA/RNA Shield (Zymo Research, Irvine, CA, USA). The lung tissue sample was also preserved in DNA/RNA Shield and, together with the second swab, was transported to the Molecular Epidemiology Laboratory of the National Institute for Medical-Military Research and Development "Cantacuzino", Bucharest, Romania.

The postmortem sampling protocol was developed by the authors based on published recommendations and established principles of forensic and clinical microbiology to ensure standardized and aseptic sample collection [17–20].

2.3. Sample Analysis

All microbiological and molecular analyses were performed according to the validated standard operating procedures routinely implemented in the participating accredited laboratories.

At the Bioclinica Laboratory (Timișoara, Romania), samples were incubated under aerobic and microaerophilic conditions at 37 °C and inoculated onto Columbia agar supplemented with 5% sheep blood, chocolate agar with Vitox (for microaerophilic cultures), and Chapman, Drigalski, and Sabouraud agar (for aerobic cultures). Subcultures were performed after 24 h of incubation, and final results were recorded after 72 h. According to the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines, bacterial identi-

fication was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and the VITEK® 2 Compact system (bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing (AST) was performed using the VITEK® 2 Compact automated system with the appropriate AST cards routinely used by the accredited clinical microbiology laboratory, and the results were interpreted according to CLSI 2023 criteria.

Molecular analyses were performed at the Molecular Epidemiology Laboratory of the National Institute for Medical-Military Research and Development "Cantacuzino", using the laboratory's validated standard operating procedures, and included polymerase chain reaction (PCR), 16S rRNA gene Sanger sequencing, and metagenomic next-generation sequencing (mNGS). For bacterial identification by Sanger sequencing, the approximately 1500 bp 16S rRNA gene was amplified by PCR using universal bacterial primers, and the resulting PCR products were subjected to Sanger sequencing.

Quality assurance procedures included the use of a nuclease-free water extraction blank as a negative control during DNA extraction. Each PCR run included a negative control (nuclease-free water) and a positive control consisting of bacterial DNA from a Gram-negative organism. PCR control samples were subsequently subjected to Sanger sequencing to verify amplification specificity and exclude contamination-related artifacts.

For mNGS analysis, DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Sequencing was performed using MiSeq v3 reagent kits (600 cycles) on Illumina MiSeq or NovaSeq 6000 platforms (Illumina, San Diego, CA, USA).

Raw sequencing data were assembled using the metaSPAdes software (v3.15.5). Contigs longer than 500 base pairs were aligned against the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST, v2.16.0.) for taxonomic identification.

mNGS analysis was preferentially performed on lung tissue samples because they were considered higher-quality specimens, collected directly from the site of infection and less influenced by airway colonization or contamination than tracheal swabs.

Postmortem samples were collected under strict aseptic conditions, and molecular findings were interpreted together with antemortem microbiological findings, conventional culture results, histopathological examination, and the clinical context.

In patients with prolonged hospitalization, only antemortem bacterial culture results obtained within 10 days prior to death were included in the analysis. This time window was selected to ensure that the antemortem microbiological findings remained representative of the infectious process present at the time of death and to allow a meaningful comparison with postmortem diagnostic methods, including conventional culture, which may be affected by changes in bacterial viability over time.

2.4. Statistical Analysis

All data were recorded and analyzed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and SPSS Statistics (IBM SPSS Statistics version 29) (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as absolute frequencies and percentages. Continuous variables, including age and length of hospitalization, were summarized as mean ± standard deviation (SD) or median and interquartile range (IQR), as appropriate.

For the analysis of microbiological results, concordance between antemortem and postmortem bacterial identification was assessed separately for each diagnostic method. Concordance was defined as the detection of at least one antemortem pathogen in the corresponding postmortem sample, even when additional microorganisms were identified. This

operational definition was selected to evaluate whether postmortem diagnostic methods were able to recover the clinically relevant pathogen previously identified during hospitalization rather than to assess complete microbiological agreement between antemortem and postmortem findings. Results classified as "not tested", "non-compliant sample", or technically non-interpretable were treated as missing in the valid-only analysis. In a secondary failure-inclusive analysis, non-tested or technically failed results were considered diagnostic failures.

Concordance rates were calculated as n/N and percentages. Because the same cases were evaluated using multiple postmortem diagnostic methods, global differences in concordance rates were assessed using Cochran's Q test. Pairwise comparisons between diagnostic methods were performed using exact McNemar tests. Bonferroni correction was applied for multiple pairwise comparisons; for six comparisons, the adjusted significance threshold was $\alpha = 0.0083$.

To explore the potential influence of antibiotic therapy on postmortem culture concordance, empirical treatment adequacy was classified as discordant, potentially adequate, or non-evaluable based on comparison with the postmortem antimicrobial susceptibility profile. The association between empirical antibiotic adequacy and postmortem culture concordance was assessed using Fisher's exact test. Given the small sample size, all inferential results were interpreted cautiously, with emphasis on descriptive trends and effect direction.

3. Results

3.1. Patient Demographic and Clinical Data

Relevant patient data were collected, including sex, age, trauma circumstances, hospital and ward of admission, duration of hospitalization, and type of traumatic injuries.

In-hospital diagnosis of nosocomial bronchopneumonia was established in accordance with Order No. 1106/2016 of the Romanian Ministry of Health and Decision 2012/506/EU, based on a combination of imaging findings, clinical signs and symptoms, and microbiological test results.

The study group consisted of 10 patients, predominantly male (7/10), with ages ranging from 29 to 82 years. Most cases were associated with traumatic injuries resulting from falls (from height or same level), followed by burns and road traffic accidents. All patients required hospitalization, with a duration ranging from 7 to 197 days. The majority were admitted to neurosurgery or polytraumatology departments, reflecting the severity of traumatic injuries. Traumatic brain injury was the most frequent diagnosis, often associated with additional lesions such as rib fractures, pneumothorax, or limb fractures. Two cases involved severe burn injuries affecting a significant percentage of the total body surface area.

Demographic and clinical data are presented in Table S1 (Supplementary Materials).

3.2. Clinical Diagnosis of Hospital-Acquired Bronchopneumonia

The clinical course of each case during hospitalization is summarized in Table S2 (Supplementary Materials), with particular emphasis on risk factors for the development of bronchopneumonia, respiratory symptoms, imaging investigations, microbiological findings, and administered treatments.

In all cases, nosocomial bronchopneumonia was included among the discharge diagnoses.

For patients with prolonged hospitalization, only microbiological test results obtained within a maximum of 10 days prior to death were considered.

3.3. Macroscopic and Microscopic Lung Examination

During forensic autopsy, macroscopic pulmonary changes consistent with bronchopneumonia were identified. These findings were further confirmed by histopathological examination of the collected lung tissue samples.

Macroscopic and histopathological lung findings are summarized in Table S3 (Supplementary Materials).

3.4. Results of Microbiological Testing

To facilitate comparison between diagnostic methods, microbiological findings are first presented descriptively, followed by an assessment of concordance with the corresponding antemortem microbiological results.

The results of microbiological analyses performed during hospitalization (antemortem) and after death (postmortem) are presented in Table 1.

Table 1. Comparison of antemortem microbiological findings with postmortem microbiological results obtained by conventional culture, PCR/16S rRNA gene Sanger sequencing, and metagenomic next-generation sequencing (mNGS).

Case	Antemortem Culture	Postmortem Culture	PCR + 16S rRNA Gene Sanger Sequencing (Tracheal Swab)	PCR + 16S rRNA Gene Sanger Sequencing (Lung Tissue)	mNGS (Tracheal Swab)	mNGS (Lung Tissue)
1	*Pseudomonas aeruginosa*	*Proteus mirabilis*; *Klebsiella pneumoniae* subsp. *pneumoniae*	Mixed respiratory microbiota	Mixed respiratory microbiota	Not tested	*Pseudomonas aeruginosa*; *Proteus mirabilis*; *Klebsiella pneumoniae*; *Acinetobacter baumannii*
2	*Acinetobacter baumannii*	*Staphylococcus aureus*; *Klebsiella pneumoniae* subsp. *pneumoniae*; *Acinetobacter baumannii*	Poor amplification	*Acinetobacter baumannii*	Not tested	*Acinetobacter baumannii*; *Serratia* sp.
3	*Escherichia coli*	*Escherichia coli*	Poor amplification	*Escherichia coli*	Not tested	*Escherichia coli*
4	*Acinetobacter baumannii*; *Citrobacter* sp.	*Klebsiella pneumoniae* subsp. *pneumoniae*	Mixed respiratory microbiota	Mixed respiratory microbiota	Not tested	*Acinetobacter baumannii*; *Citrobacter* sp.; *Klebsiella pneumoniae*
5	*Klebsiella pneumoniae*	*Klebsiella pneumoniae* subsp. *ozaenae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	Not tested	*Acinetobacter baumannii*; *Klebsiella pneumoniae*
6	*Klebsiella pneumoniae*	*Citrobacter freundii*; *Proteus mirabilis*	Mixed respiratory microbiota	Mixed respiratory microbiota	Not tested	*Klebsiella pneumoniae*; *Corynebacterium striatum*
7	*Pseudomonas aeruginosa*	*Serratia marcescens*	*Escherichia coli*	Poor amplification	Not tested	*Pseudomonas aeruginosa*; *Klebsiella pneumoniae*; *Escherichia coli*
8	*Klebsiella pneumoniae*	*Klebsiella pneumoniae* subsp. *pneumoniae*	Non-compliant sample	*Klebsiella pneumoniae*	Non-compliant sample	*Klebsiella pneumoniae*
9	*Pseudomonas aeruginosa*	*Proteus mirabilis*; *Klebsiella pneumoniae* subsp. *ozaenae*	*Klebsiella pneumoniae*	Poor amplification	*Klebsiella pneumoniae*	Not tested
10	*Acinetobacter baumannii*	*Acinetobacter baumannii*; *Enterobacter cloacae* complex	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	Not tested	*Pseudomonas aeruginosa*; *Acinetobacter baumannii*

Notes: Mixed respiratory microbiota = polymicrobial detection without a dominant species. Poor amplification = insufficient DNA yield for reliable sequencing. Non-compliant sample = sample unsuitable for analysis. Not tested = analysis not performed.

Accordingly, the comparative analysis included only antemortem bacterial culture results obtained within 10 days prior to death.

Overall, the concordance between antemortem and postmortem microbiological findings varied depending on the diagnostic method used. Conventional postmortem culture of tracheal swabs showed partial agreement with antemortem results, with concordant findings observed in approximately half of the cases.

Molecular analysis using polymerase chain reaction (PCR) with 16S rRNA gene Sanger sequencing yielded inconsistent results, frequently limited by poor amplification or the presence of mixed respiratory microbiota, which reduced its diagnostic utility, particularly in polymicrobial infections.

In contrast, metagenomic next-generation sequencing (mNGS), especially when performed on lung tissue samples, demonstrated the highest level of concordance with antemortem microbiological findings. mNGS enabled the identification of multiple bacterial species, including those not detected by conventional culture methods, and appeared less affected by prior antibiotic therapy.

3.5. Antimicrobial Susceptibility Profiles of Postmortem Isolates

The antimicrobial susceptibility profiles of bacterial strains isolated from postmortem cultures are presented in Table 2.

Table 2. Antimicrobial susceptibility profiles of bacterial isolates identified by postmortem conventional culture.

Case	Bacterial Strain	MDR Profile	Key Resistance	Key Susceptibility
1	*Proteus mirabilis*	No	AMP, AMC	TZP, CAZ, IPM, MEM, CIP
	Klebsiella pneumoniae	Yes	Multiple β-lactams, quinolones	CXM, CPD
2	*Staphylococcus aureus*	No	Penicillins	Limited susceptibility
	Klebsiella pneumoniae	Yes	Multiple β-lactams	FOX, CFM
	Acinetobacter baumannii	Yes	Broad resistance	IPM, MEM
3	*Escherichia coli*	No	AMP, AMC	FOX, CFM, CTX
4	*Klebsiella pneumoniae*	Yes	Broad resistance	—
5	*Klebsiella pneumoniae* subsp. *ozaenae*	No	AMP, AMC	CXM, CTX, CAZ
6	*Citrobacter freundii*	No	Cephalosporins	ETP, MEM
	Proteus mirabilis (MDR)	Yes	Broad resistance	—
7	*Serratia marcescens*	No	Early β-lactams	IPM, MEM, CIP
8	*Klebsiella pneumoniae*	No	Partial resistance	AMC, CAZ
9	*Proteus mirabilis*	No	Limited resistance	Broad susceptibility
	Klebsiella pneumoniae	Yes	Broad resistance	—
10	*Acinetobacter baumannii*	Yes	Broad resistance	—
	Enterobacter cloacae complex	Yes	Broad resistance	—

Abbreviations: MDR = multidrug-resistant (resistant to ≥3 antimicrobial classes). AMP = ampicillin; AMC = amoxicillin/clavulanic acid; TZP = piperacillin/tazobactam; CF = cephalothin/cephalexin; CXM = cefuroxime; FOX = cefoxitin; CFM = cefixime; CPD = cefpodoxime; CTX = cefotaxime; CAZ = ceftazidime; ETP = ertapenem; IPM = imipenem; MEM = meropenem; CIP = ciprofloxacin.

A high prevalence of multidrug-resistant (MDR) organisms was observed, particularly among *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter cloacae* complex isolates.

Comparison with the antibiotic therapy administered during hospitalization (Table S2) revealed frequent discordance between empirical treatment and susceptibility profiles. Several patients received antibiotics to which the isolated pathogens demonstrated resistance, particularly in cases involving broad-spectrum β-lactam antibiotics.

Notably, *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates frequently exhibited resistance to multiple antibiotic classes, including penicillins and cephalosporins, while retaining susceptibility mainly to carbapenems in some cases. This finding may partly explain the reduced concordance observed in postmortem culture results.

Overall, the data suggest that prior antibiotic therapy may have contributed both to the selection of resistant strains and to the decreased sensitivity of culture-based postmortem microbiological methods.

3.6. Statistical Analysis of Microbiological Concordance

Concordance with antemortem microbiological findings varied substantially across the evaluated postmortem diagnostic methods (Table 3). Postmortem tracheal swab culture showed concordance in 5/10 cases (50.0%). PCR/16S rRNA gene Sanger sequencing performed on tracheal swab samples showed limited concordance, with only 1 concordant result among 9 valid cases (11.1%; 10.0% when calculated against the total cohort). PCR/Sanger gene sequencing of lung tissue samples showed concordance in 4/10 cases (40.0%).

Table 3. Concordance of postmortem diagnostic methods with antemortem microbiological findings for the detection of the clinically relevant pathogen.

Postmortem Diagnostic Method	Valid Cases	Missing/Non-Analyzable Cases	Concordance, n/N	Concordance (%)	Interpretation
Postmortem tracheal swab culture	10	0	5/10	50.0%	Moderate concordance with antemortem microbiological findings.
PCR/16S rRNA gene Sanger sequencing from tracheal swab	9	1	1/9	11.1% valid-only; 10.0% overall	Low diagnostic concordance; one case was missing/non-compliant.
PCR/16S rRNA gene Sanger sequencing from lung tissue	10	0	4/10	40.0%	Higher concordance than tracheal swab PCR/Sanger, but lower than lung tissue mNGS.
mNGS from tracheal swab	1	9	0/1	0.0% valid-only	Not suitable for inferential analysis because most samples were not tested or were non-compliant.
mNGS from lung tissue, valid-only analysis	9	1	9/9	100.0% valid-only	All analyzable lung tissue mNGS samples were concordant.
mNGS from lung tissue, failure-inclusive analysis	10	0	9/10	90.0%	Highest concordance among all evaluated methods.
Any available mNGS result	10	0	9/10	90.0%	Overall concordance when any evaluable mNGS result was considered.

The SPSS frequency tables showed that postmortem culture was concordant in 5 cases and discordant in 5 cases. Tracheal swab PCR/16S rRNA gene Sanger sequencing was concordant in 1 of 9 valid cases, while lung tissue PCR/16S rRNA gene Sanger sequencing was concordant in 4 of 10 cases. Lung tissue mNGS was concordant in 9 of 9 analyzable cases in the valid-only analysis and in 9 of 10 cases in the failure-inclusive analysis.

mNGS performed on tracheal swab samples could not be reliably evaluated statistically because only one case yielded a valid result, while nine cases were either not tested or non-compliant. In contrast, mNGS performed on lung tissue showed the highest concordance. In the valid-only analysis, all analyzable lung tissue mNGS samples were concordant

with antemortem microbiological findings (9/9, 100.0%). In the failure-inclusive analysis, where the non-tested case was considered a diagnostic failure, lung tissue mNGS remained concordant in 9 out of 10 cases (90.0%).

A global comparison of the failure-inclusive diagnostic methods showed a statistically significant difference in concordance rates across methods, Cochran's Q(3) = 15.720, $p = 0.001$ (Table 4). A sensitivity analysis restricted to valid lung tissue mNGS results also remained statistically significant, Cochran's Q(2) = 8.400, $p = 0.015$, supporting the consistency of the observed differences between diagnostic methods.

Table 4. Overall and pairwise statistical comparison of concordance rates among postmortem diagnostic methods.

Analysis	Diagnostic Methods Compared	N	Test	Test Statistic	df	*p*-Value	Interpretation
Global comparison, failure-inclusive analysis	Culture; tracheal swab PCR/Sanger failure-inclusive; lung tissue PCR/Sanger; lung tissue mNGS failure-inclusive	10	Cochran's Q	15.720	3	0.001	Concordance rates differed significantly across diagnostic methods
Sensitivity analysis, valid-only mNGS lung tissue	Culture; lung tissue PCR/Sanger; lung tissue mNGS valid-only	9	Cochran's Q	8.400	2	0.015	Differences remained significant after excluding the non-tested mNGS lung tissue case
Culture vs. lung tissue mNGS failure-inclusive	50.0% vs. 90.0%	10	Exact McNemar	—	—	0.125	mNGS showed higher numerical concordance, but the difference was not statistically significant
Culture vs. lung tissue PCR/Sanger	50.0% vs. 40.0%	10	Exact McNemar	—	—	1.000	No significant difference between methods
Culture vs. tracheal swab PCR/Sanger failure-inclusive	50.0% vs. 10.0%	10	Exact McNemar	—	—	0.125	Culture showed higher numerical concordance, but the difference was not statistically significant
Tracheal swab PCR/Sanger failure-inclusive vs. lung tissue PCR/Sanger	10.0% vs. 40.0%	10	Exact McNemar	—	—	0.250	Lung tissue PCR/Sanger showed higher numerical concordance, but the difference was not statistically significant
Tracheal swab PCR/Sanger failure-inclusive vs. lung tissue mNGS failure-inclusive	10.0% vs. 90.0%	10	Exact McNemar	—	—	0.008	Lung tissue mNGS showed significantly higher concordance after Bonferroni correction
Lung tissue PCR/Sanger vs. lung tissue mNGS failure-inclusive	40.0% vs. 90.0%	10	Exact McNemar	—	—	0.063	mNGS showed higher numerical concordance, but the difference was not statistically significant

Bonferroni-adjusted α for six pairwise comparisons was 0.0083. *p*-values are two-sided exact McNemar *p*-values. The global Cochran's Q analysis showed a significant difference among methods, Q(3) = 15.720, $p = 0.001$; the valid-only sensitivity analysis also remained significant, Q(2) = 8.400, $p = 0.015$.

Pairwise exact McNemar tests with Bonferroni correction showed that lung tissue mNGS had significantly higher concordance than tracheal swab PCR/Sanger sequencing in the failure-inclusive analysis (90.0% vs. 10.0%; $p = 0.008$; Bonferroni-adjusted $\alpha = 0.0083$). Other pairwise comparisons did not reach statistical significance after correction for multi-

ple comparisons, although lung tissue mNGS showed higher descriptive concordance than both postmortem culture and lung tissue PCR/16S rRNA gene Sanger sequencing.

The exploratory analysis of empirical antibiotic adequacy included seven evaluable cases, while three cases were classified as non-evaluable. No statistically significant association was observed between empirical antibiotic adequacy and postmortem culture concordance (Fisher's exact test, $p = 1.000$) (Table 5). Accordingly, the antimicrobial susceptibility findings should be interpreted as descriptive observations rather than evidence of a causal relationship between multidrug resistance and microbiological concordance.

Table 5. Association between empirical antibiotic adequacy and postmortem culture concordance with antemortem microbiological findings.

Empirical Antibiotic Adequacy	Postmortem Culture Discordant, n (%)	Postmortem Culture Concordant, n (%)	Total, n	Fisher Exact *p*-Value	Interpretation
Discordant with postmortem susceptibility profile	3 (60.0)	2 (40.0)	5	1.000	No statistically significant association with culture concordance
Potentially adequate	1 (50.0)	1 (50.0)	2	1.000	Exploratory result because of the very small number of evaluable cases
Non-evaluable	—	—	3	—	Excluded from Fisher's exact test
Total evaluable cases	4 (57.1)	3 (42.9)	7	1.000	No evidence of association; interpretation limited by low statistical power

Three cases were classified as non-evaluable and were excluded from Fisher's exact test. All expected cell counts were below 5; therefore, this analysis should be considered exploratory. The SPSS output showed 7 valid cases, 3 missing cases, and Fisher's exact $p = 1.000$.

Overall, lung tissue mNGS showed the highest descriptive concordance with antemortem microbiological findings, whereas PCR/16S rRNA gene Sanger sequencing performed on tracheal swab samples demonstrated the lowest concordance. Conventional culture demonstrated intermediate concordance among the evaluated postmortem diagnostic methods.

4. Discussion

Nosocomial bronchopneumonia remains a significant complication in patients with severe traumatic injuries, particularly in those requiring prolonged hospitalization, intensive care, and invasive procedures. In such cases, multiple risk factors—including mechanical ventilation, prolonged immobilization, and prior antibiotic exposure—contribute to the development of complex and often polymicrobial infections [21].

In forensic practice, establishing the diagnosis of nosocomial bronchopneumonia requires a multidisciplinary approach that integrates clinical data with postmortem macroscopic, histopathological, and microbiological findings. In the present study, all cases demonstrated characteristic macroscopic and histopathological features consistent with bronchopneumonia, supporting the clinical diagnosis and highlighting the importance of autopsy in validating antemortem findings [9].

The identification of the etiological agent in postmortem settings is particularly relevant in medico-legal contexts, where determining causality and potential healthcare-associated liability is essential [22]. However, postmortem bacteriology has traditionally been regarded with caution due to the risk of contamination, bacterial transloca-

tion, and postmortem microbial overgrowth [10,23]. Despite these concerns, previous studies have shown that, when appropriate sampling techniques are applied and postmortem intervals are controlled, microbiological findings can provide valuable diagnostic information [12,17,18].

In our study, postmortem culture demonstrated moderate concordance with antemortem microbiological results (50%). This finding is consistent with existing literature, which emphasizes the limitations of culture-based methods in postmortem settings, particularly in patients who have received prolonged antibiotic therapy or in whom fastidious or non-culturable organisms are involved [8]. Moreover, distinguishing between true infection and contamination remains a significant challenge in interpreting postmortem culture results.

Molecular approaches, particularly PCR with 16S rRNA gene Sanger sequencing, showed variable and generally limited diagnostic performance. While this method is valuable for detecting specific bacterial DNA, its applicability is restricted in polymicrobial infections, as mixed bacterial populations cannot be reliably resolved using conventional Sanger sequencing [24]. This limitation was reflected in our results, where a substantial proportion of samples yielded mixed respiratory microbiota or poor amplification.

In contrast, metagenomic next-generation sequencing (mNGS) demonstrated the highest concordance with antemortem findings (90%). Importantly, concordance was defined as the detection of the antemortem pathogen in postmortem samples, even in the presence of additional bacterial species. This definition was intentionally adopted because the primary objective of the study was to determine whether postmortem diagnostic methods could recover the clinically relevant pathogen identified during hospitalization, rather than to assess complete microbiological agreement between antemortem and postmortem findings. The frequent identification of multiple microorganisms likely reflects polymicrobial infections or secondary colonization processes, which are common in patients with prolonged hospitalization and extensive antibiotic exposure. These findings are in agreement with recent studies highlighting the superior diagnostic performance of mNGS in detecting a broad spectrum of pathogens, including bacteria that are difficult or impossible to culture [15,16,25].

Each microbiological method employed in this study presents specific advantages and limitations that should be considered in the interpretation of results. Conventional culture remains widely available and allows antimicrobial susceptibility testing, which is essential for clinical and epidemiological purposes. However, its diagnostic yield is significantly influenced by prior antibiotic therapy, postmortem interval, and the presence of fastidious or non-culturable organisms, limiting its reliability in postmortem settings [8,23].

PCR-based methods targeting the 16S rRNA gene offer increased sensitivity and the ability to detect bacterial DNA in both viable and non-viable organisms, making them less affected by prior antimicrobial treatment [10,13]. Nevertheless, conventional Sanger sequencing has important limitations in polymicrobial infections, as mixed bacterial populations often result in uninterpretable sequences or poor amplification, reducing its diagnostic value in complex infections [24].

Metagenomic next-generation sequencing (mNGS) represents a powerful culture-independent approach that enables comprehensive identification of microbial communities, including bacteria, viruses, fungi, and parasites, within a single assay [16,25]. Its high sensitivity and broad detection spectrum make it particularly useful in polymicrobial infections and in cases where prior antibiotic therapy has compromised culture results. However, mNGS also has notable limitations, including higher costs, longer turnaround times, the need for specialized bioinformatics analysis, and challenges in distinguishing between true pathogens, colonizers, and contaminants [15,16]. Some additional microorganisms detected

by mNGS may represent opportunistic pathogens, colonizing organisms, or contaminants, depending on the clinical and pathological context [26].

The antimicrobial susceptibility profiles of postmortem isolates further support the complexity of these infections. A high prevalence of multidrug-resistant (MDR) organisms was observed, particularly among *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter cloacae* complex isolates. Comparison with the antibiotic therapy administered during hospitalization revealed frequent discrepancies between empirical treatment and susceptibility patterns, suggesting that antibiotic pressure may have contributed to both the selection of resistant strains and the reduced sensitivity of culture-based methods. This observation is consistent with previous reports indicating that prior antibiotic exposure can significantly impact microbiological results, particularly in postmortem settings [8]. However, the present study was not powered to quantitatively assess the relationship between antimicrobial resistance, prior antibiotic exposure, and microbiological concordance.

The present statistical analysis provides preliminary evidence that the diagnostic concordance of the evaluated postmortem microbiological methods differed across techniques. Specifically, Cochran's Q test suggested an overall difference in concordance among the evaluated postmortem diagnostic methods. Lung tissue mNGS showed the highest concordance with antemortem microbiological findings, reaching 90.0% in the failure-inclusive analysis and 100.0% among analyzable samples. These findings suggest the potential value of mNGS as a complementary diagnostic approach in postmortem evaluation of nosocomial bronchopneumonia, especially in cases with polymicrobial findings or prior antibiotic exposure.

However, pairwise analyses should be interpreted cautiously. After Bonferroni correction, the only statistically significant pairwise difference was observed between lung tissue mNGS and tracheal swab PCR/Sanger sequencing. Comparisons between lung tissue mNGS and conventional culture or lung tissue PCR/Sanger sequencing showed higher numerical concordance for mNGS, but did not reach statistical significance, most likely because of the very small sample size. Therefore, the results showed higher descriptive concordance for lung tissue mNGS, while a statistically significant pairwise difference was demonstrated only in comparison with tracheal swab PCR/16S rRNA gene Sanger sequencing.

The analysis of empirical antibiotic adequacy did not show a significant association with postmortem culture concordance. Nevertheless, this finding should be interpreted as exploratory, because only seven cases were evaluable and all expected cell counts were low. The absence of statistical significance should therefore not be interpreted as evidence that antibiotic therapy had no influence on postmortem culture results.

Taken together, these results suggest that conventional culture remains a valuable diagnostic tool, although its performance may be reduced in complex, polymicrobial, and antibiotic-exposed cases. Among the evaluated methods, lung tissue mNGS showed the highest concordance with antemortem microbiological findings and may provide valuable complementary information, particularly in healthcare-associated infections. However, because of its high analytical sensitivity, microorganisms detected exclusively by mNGS should be interpreted cautiously and always in the context of the patient's clinical history, histopathological findings, and conventional microbiological evidence. Therefore, mNGS should be considered a complementary diagnostic method rather than a replacement for conventional microbiological techniques.

4.1. Study Limitations

The study has several limitations that should be acknowledged. First, the relatively small sample size limits the statistical power of the analysis and the generalizability of the

findings. This is partly explained by the intentional selection of a homogeneous study population, restricted to patients with traumatic injuries and nosocomial bronchopneumonia—a combination frequently encountered in forensic practice—in order to ensure greater comparability of cases.

Second, the inclusion of only patients with traumatic injuries, although representative of forensic practice, may limit the generalizability of the findings to other clinical populations. Third, prior antibiotic therapy could have influenced postmortem microbiological results by reducing bacterial viability and promoting the selection of resistant strains.

In addition, technical issues, including inadequate samples and poor amplification in some PCR analyses, may have affected the accuracy of molecular testing. Another limitation of this study is the absence of dedicated negative controls during postmortem sample collection and the lack of documented dedicated negative controls for the mNGS workflow. Strict aseptic procedures were implemented throughout autopsy sampling to minimize the risk of contamination, including skin disinfection before specimen collection and the use of sterile instruments and collection materials. In addition, molecular analyses included quality control measures during DNA extraction and PCR, including a nuclease-free water extraction blank, a PCR negative control (nuclease-free water), and a positive control consisting of DNA from a Gram-negative bacterium. Despite these precautions, contamination from skin microbiota, environmental microorganisms, or postmortem bacterial translocation cannot be completely excluded. Therefore, molecular findings, particularly those obtained by mNGS, should always be interpreted in conjunction with the clinical history, histopathological findings, conventional microbiological results, and the overall forensic context.

Finally, another limitation of this study is that strain-level comparison or molecular typing of the identified microorganisms was not performed. Consequently, it was not possible to evaluate the potential clonal relatedness of the isolates or to investigate possible cross-transmission events or common environmental sources of infection. Future studies incorporating molecular typing techniques and genomic epidemiology may provide additional insights into the transmission dynamics of healthcare-associated pathogens.

A further limitation concerns the statistical power of the comparative analyses. Although a significant global difference between diagnostic methods was observed, the small number of cases limited the ability of pairwise McNemar tests to detect statistically significant differences between individual methods. In addition, some molecular results were non-evaluable because of non-compliant samples, poor amplification, or lack of testing. For this reason, both valid-only and failure-inclusive analyses were performed. Finally, the exploratory analysis of antibiotic adequacy was limited by the small number of evaluable cases and should be interpreted only as a descriptive trend rather than as definitive evidence of association.

4.2. Future Directions

Future studies should include larger cohorts and a broader range of clinical conditions to validate these findings. Additionally, the integration of mNGS into routine forensic practice warrants further investigation, particularly in terms of cost-effectiveness, standardization, and interpretation of polymicrobial results.

5. Conclusions

In this pilot exploratory study, lung tissue mNGS showed the highest concordance with antemortem microbiological findings among the evaluated diagnostic methods.

These findings suggest that mNGS may represent a valuable complementary tool in the postmortem investigation of healthcare-associated bronchopneumonia, particularly in complex or polymicrobial infections.

However, larger prospective studies are required to validate these preliminary findings before definitive conclusions regarding diagnostic performance can be drawn.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/microbiolres17080142/s1. Table S1: Demographic and clinical characteristics of the study population; Table S2: Clinical course, microbiological findings, and antimicrobial treatment during hospitalization; Table S3: Macroscopic and histopathological findings of the lungs at autopsy.

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Abbreviations

The following abbreviations are used in this manuscript:

HAIs	healthcare-associated infections
PCR	polymerase chain reaction
mNGS	metagenomic next-generation sequencing
ICU	intensive care unit
TBSA	total body surface area

References

1. World Health Organization. *Global Report on Infection Prevention and Control*; WHO: Geneva, Switzerland, 2024.
2. Coman, A.; Pop, D.; Mureșan, F.; Oprescu, F.; Fjaagesund, S. Surveillance and reporting of hospital-associated infections—A document analysis of Romanian healthcare legislation evolution over 20 years. *Healthcare* **2025**, *13*, 229. [CrossRef] [PubMed]
3. Voidăzan, S.; Albu, S.; Toth, R.; Grigorescu, B.; Răchiță, A.; Moldovan, I. Healthcare-associated infections—A new pathology in medical practice? *Int. J. Environ. Res. Public Health* **2020**, *17*, 760. [CrossRef] [PubMed]
4. Szabó, S.; Feier, B.; Căpățână, D.; Tertiș, M.; Cristea, C.; Popa, A. An overview of healthcare-associated infections and their detection methods caused by pathogenic bacteria in Romania and Europe. *J. Clin. Med.* **2022**, *11*, 3204. [CrossRef] [PubMed]
5. European Centre for Disease Prevention and Control. Healthcare-Associated Infections. Available online: https://www.ecdc.europa.eu/en/healthcare-associated-infections (accessed on 12 March 2026).
6. Denissen, J.; Reyneke, B.; Waso-Reyneke, M.; Havenga, B.; Barnard, T.; Khan, S.; Khan, W. Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. *Int. J. Hyg. Environ. Health* **2022**, *244*, 114006. [CrossRef] [PubMed]

7. Kumar, P.B.P.; Jacob, E.S.; Bhuvaneswari, C.K. *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* (ESKAPE) pathogens and colistin resistance in ventilator-associated pneumonia: A prospective cross-sectional study. *Cureus* **2025**, *17*, e98097. [CrossRef] [PubMed]
8. Riedel, S. The value of postmortem microbiology cultures. *J. Clin. Microbiol.* **2014**, *52*, 1028–1033. [CrossRef] [PubMed]
9. Turner, G.D.; Bunthi, C.; Wonodi, C.B.; Morpeth, S.C.; Molyneux, C.S.; Zaki, S.R.; Levine, O.S.; Murdoch, D.R.; Scott, J.A. The role of postmortem studies in pneumonia etiology research. *Clin. Infect. Dis.* **2012**, *54*, S165–S171. [CrossRef] [PubMed]
10. Camatti, J.; Bonasoni, M.P.; Santunione, A.L.; Cecchi, R.; Radheshi, E.; Carretto, E. Postmortem microbiology in forensic diagnostics: Interpretation of infectious causes of death and emerging applications. *Diagnostics* **2026**, *16*, 325. [CrossRef] [PubMed]
11. Tsokos, M.; Püschel, K. Postmortem bacteriology in forensic pathology: Diagnostic value and interpretation. *Leg. Med.* **2001**, *3*, 15–22. [CrossRef] [PubMed]
12. Diac, I.; Keresztesi, A.A.; Cerghizan, A.M.; Negrea, M.; Dogăroiu, C. Postmortem bacteriology in forensic autopsies—A single-center retrospective study in Romania. *Diagnostics* **2022**, *12*, 2024. [CrossRef] [PubMed]
13. Drevinek, P.; Hollweck, R.; Lorenz, M.G.; Lustig, M.; Bjarnsholt, T. Direct 16S rRNA gene PCR followed by Sanger sequencing as a clinical diagnostic tool for detection of bacterial and fungal infections: A systematic review and meta-analysis. *J. Clin. Microbiol.* **2023**, *61*, e00338-23. [CrossRef] [PubMed]
14. Kurniawan, F.D.; Alia, D.; Shiraishi, M.; Higo, M.; Inoue, Y.; Hagiwara, K. A systematic algorithm using 16S ribosomal RNA for accurate diagnosis of pneumonia pathogens. *Sci. Rep.* **2025**, *15*, 29253. Correction in *Sci. Rep.* **2025**, *15*, 35557. https://doi.org/10.1038/s41598-025-22878-3. [CrossRef] [PubMed]
15. Yoshizawa, H.; Motooka, D.; Nakamura, S.; Yoshida, K. Metagenomic identification of pathogenic bacteria for pneumonia from postmortem lung tissue. *Hum. Pathol. Rep.* **2022**, *28*, 300621. [CrossRef]
16. Xie, G.; Zhao, B.; Wang, X.; Bao, L.; Xu, Y.; Ren, X.; Ji, J.; He, T.; Zhao, H. Exploring the clinical utility of metagenomic next-generation sequencing in the diagnosis of pulmonary infection. *Infect. Dis. Ther.* **2021**, *10*, 1419–1435. [CrossRef] [PubMed]
17. Fernández-Rodríguez, A.; Burton, J.L.; Andreoletti, L.; Alberola, J.; Fornes, P.; Merino, I.; Martínez, M.J.; Castillo, P.; Sampaio-Maia, B.; Caldas, I.M.; et al. Postmortem microbiology in sudden death: Sampling protocols proposed in different clinical settings. *Clin. Microbiol. Infect.* **2019**, *25*, 570–579. [CrossRef] [PubMed]
18. Fernández-Rodríguez, A.; Cohen, M.C.; Lucena, J.; Van de Voorde, W.; Angelini, A.; Ziyade, N.; Saegeman, V. 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*, 1045–1057. [CrossRef] [PubMed]
19. Chambers, M.; Hooper, J.E. Infection: Postmortem Cultures. *The Autopsy Book*. Available online: https://theautopsybook.com/postmortem-cultures/ (accessed on 12 March 2026).
20. Burton, J.L.; Saegeman, V.; Arribi, A.; Rello, J.; Andreoletti, L.; Cohen, M.C.; Fernandez-Rodriguez, A.; ESGFOR Joint Working Group of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group of Forensic and Postmortem Microbiology and the European Society of Pathology. Postmortem microbiology sampling following death in hospital: An ESGFOR task force consensus statement. *J. Clin. Pathol.* **2019**, *72*, 329–336. [CrossRef] [PubMed]
21. O'Toole, R.F. The interface between COVID-19 and bacterial healthcare-associated infections. *Clin. Microbiol. Infect.* **2021**, *27*, 1772–1776. [CrossRef] [PubMed]
22. Treglia, M.; Pallocci, M.; Passalacqua, P.; Sabatelli, G.; De Luca, L.; Zanovello, C.; Messineo, A.; Quintavalle, G.; Cisterna, A.M.; Marsella, L.T. Medico-legal aspects of hospital-acquired infections: Five years of judgments of the Civil Court of Rome. *Healthcare* **2022**, *10*, 1336. [CrossRef] [PubMed]
23. Palmiere, C.; Egger, C.; Prod'Hom, G.; Greub, G. Bacterial translocation and sample contamination in postmortem microbiological analyses. *J. Forensic Sci.* **2016**, *61*, 367–374. [CrossRef] [PubMed]
24. Janda, J.M.; Abbott, S.L. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *J. Clin. Microbiol.* **2007**, *45*, 2761–2764. [CrossRef] [PubMed]
25. Yu, Z.; Xie, Q.; Zhao, Y.; Duan, L.; Qiu, P.; Fan, H. NGS plus bacterial culture: A more accurate method for diagnosing forensic-related nosocomial infections. *Leg. Med.* **2021**, *52*, 101910. [CrossRef] [PubMed]
26. Rong, R.; Long, Y.; Li, Y.; Lin, L.; Yang, J.; Hu, Z.; Liu, D.; Chen, P. Metagenomic and targeted next-generation sequencing in infectious disease diagnostics: Current applications, challenges, and future perspectives. *Diagnostics* **2026**, *16*, 991. [CrossRef] [PubMed]