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Systematic Review

Microbial Colonization Patterns in Antemortem Wounds versus Postmortem Injuries: A Systematic Review

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Abstract

Background: Distinguishing antemortem (vital) wounds from postmortem injuries remains one of the most persistent challenges in forensic pathology, particularly once decomposition has obscured classical morphological vital reactions. Over the past two decades, microbial community profiling has emerged as a candidate adjunct to immunohistochemical vitality markers, since living tissue, dying tissue, and decomposing tissue host distinguishable bacterial ecologies.

Objective: This systematic review synthesizes evidence on microbial colonization patterns that differentiate antemortem wounds from postmortem injuries, and situates these findings against the broader postmortem (thanatomicrobiome) and clinical wound-colonization literature.

Methods: A structured literature search was conducted across PubMed-, Scopus-, Web of Science-, and Google Scholar-indexed sources for English-language studies published between July 2000 and March 2026, using combinations of terms relating to antemortem injury, postmortem wounds, thanatomicrobiome, wound vitality markers, and forensic microbiology. Animal models, human cadaveric studies, and clinical wound-colonization studies were eligible; case reports lacking comparative data and non-peer-reviewed sources were excluded.

Key findings: Twenty-six studies met inclusion criteria. Antemortem wounds are characterized by an early, host-driven, aerobic Gram-positive-dominant flora (Staphylococcus, Streptococcus, Enterococcus) reflecting an intact circulatory and immune response, whereas postmortem injuries in decomposing tissue show a delayed, environmentally driven shift toward Acinetobacter, Enterobacteriaceae, and anaerobic putrefactive taxa. Genus-level differences become statistically robust only several days after wounding, limiting utility in the early postmortem interval.

Conclusion: Microbial community analysis offers a promising, decomposition-resistant complement to immunohistochemical vitality markers, but standardization of sampling, sequencing depth, and statistical thresholds is needed before forensic validation. Implications for casework interpretation and future research priorities are discussed.

Keywords: Forensic Microbiology; Thanatomicrobiome; Wound Vitality; Postmortem Interval; Antemortem Injury; Bacterial Succession; Forensic Pathology; Necrobiome.

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Introduction

Determining whether a wound was inflicted before or after death is a foundational task in forensic pathology, with direct bearing on cause-of-death determination, manner-of-death classification, and the medicolegal reconstruction of events surrounding a death. Traditionally, this distinction has relied on macroscopic and microscopic vital reactionsred blood cell extravasation, fibrin deposition, neutrophil margination, and immunohistochemical markers such as CD15, tryptase, and FVIIIrathat develop in living, perfused tissue but are absent or markedly attenuated in tissue injured after circulatory arrest [1,3]. However, these vital reactions have a finite diagnostic window: as autolysis and putrefaction progress, classical histological and immunohistochemical signs degrade or become confounded by postmortem artefact, and in advanced decomposition they may be unobtainable altogether [3]. Microorganisms occupy a different

temporal niche. The human body carries a dense, site-specific commensal microbiota throughout life, and following death this microbiota undergoes a predictable, directional succession driven by the cessation of immune surveillance, circulatory clearance, and oxygen deliverya process now described under the umbrella terms thanatomicrobiome (internal, endogenous communities) and necrobiome (the broader assemblage of internal and external decomposer organisms) [6-7]. Because microbial succession continues well beyond the point at which classical vital reactions become unreadable, several groups have proposed that comparing the bacterial ecology of a wound margin against the surrounding unwounded tissue, or tracking community divergence over the days following injury, could provide a decomposition-resistant marker of wound vitalitythat is, a way of inferring whether a wound was sustained while the host immune and circulatory systems were still functional [1].

The rationale for this review is threefold. First, the wound-vitality literature has historically developed in parallel with, rather than integrated into, the broader postmortem-interval (PMI) microbiome literature, despite substantial conceptual overlap. Second, several primary studies and adjacent systematic reviews addressing PMI estimation, thanatomicrobiome succession, and immunohistochemical wound dating have appeared in the past five years, warranting a synthesis focused specifically on the antemortem-versus-postmortem injury question rather than PMI estimation in general [10,14-16]. Third, forensic practitioners and laboratories increasingly request guidance on whether microbial sampling of injuries has matured enough to support casework interpretation, or whether it remains an investigational tool.

Accordingly, this review asks: What microbial colonization patterns reliably distinguish antemortem wounds from postmortem injuries, across animal models, human cadaveric studies, and the adjacent clinical wound-microbiology literature, and what is the evidentiary maturity of these patterns for forensic application? The scope is bounded to studies published between July 2000 and March 2026; to bacterial (and where reported, fungal) colonization of cutaneous and soft-tissue wounds in animal and human subjects; and to studies reporting a direct or indirect antemortem/postmortem (or living/decomposing) comparison. Studies addressing PMI estimation from intact, unwounded cadaveric sites (skin, organs, gravesoil) were included only where they informed interpretation of background postmortem microbial succession against which wound-specific signatures must be distinguished; pure entomological, environmental-soil, or skeletal PMI studies without a wound-vitality component were considered contextual rather than core evidence.

Literature Search Strategy: This review followed a PRISMA-informed search and screening process adapted for a narrative-systematic hybrid synthesis. Searches were conducted iteratively across PubMed-, Scopus-, Web of Science-, MDPI-, Frontiers-, and Google Scholar-indexed literature, supplemented by hand-searching the reference lists of retrieved systematic reviews (a recognized supplementary strategy for topics, such as this one, where indexing terms are inconsistent across disciplines spanning forensic pathology, microbiology, and wound care).

Search terms combined three concept blocks using Boolean AND/OR logic: (1) injury-timing terms“antemortem wound”, “postmortem injury”, “wound vitality”, “wound age estimation”, “perimortem”; (2) microbiology terms “microbiome”, “microbial community”, “bacterial succession”, “thanatomicrobiome”, “necrobiome”, “16S rRNA”, “wound colonization”; and (3) context terms “forensic pathology”, “forensic microbiology”, “postmortem interval”, “cadaver”. Searches were restricted to records with a publication date between July 2000 and March 2026 and to English-language full texts.

Inclusion criteria were: (a) original research (animal or human, in vivo, ex vivo, or cadaveric) or systematic/scoping review reporting bacterial or fungal colonization data; (b) an explicit or inferable comparison between antemortem (vital, living, or pre-decomposition) and postmortem (decomposing or post-circulatory-arrest) states, or, for clinical wound studies, a temporal colonization sequence in living patients suitable as an antemortem comparator; (c) peer-reviewed publication.

Exclusion criteria were: case reports without comparative microbial data; studies confined to gravesoil, entomological succession, or skeletal/bone PMI without a soft-tissue wound component; conference abstracts without full text; and studies not available in English.

Across the search iterations, approximately 110 unique records were identified. Title and abstract screening against the eligibility criteria removed records addressing unrelated topics (e.g., clinical diagnostic discordance between antemortem and postmortem disease diagnoses, veterinary toxicology, unrelated omics methods reviews). Approximately 40 full texts were assessed in detail, of which 26 met all inclusion criteria and form the evidence base for this review.

The included set comprises one animal-model wound microbiome study, four human cadaveric/thanatomicrobiome primary studies, five systematic or scoping reviews (covering

immunohistochemical wound-vitality markers, wound omics, and PMI microbiome), eleven postmortem-microbiology and bacterial-translocation studies providing mechanistic and methodological context, and two clinical wound-colonization studies anchoring the antemortem comparator. No formal quality-scoring instrument (e.g., AMSTAR-2, Newcastle-Ottawa) was applied uniformly across this heterogeneous study-design mix; instead, study design, sample size, and methodological transparency were tabulated narratively (Table 1) to allow qualitative weighting of evidence strength, consistent with the scoping-review precedent set by comparable forensic-microbiology syntheses [2,14].

Study Selection Flow (PRISMA-informed)

- Records identified through database and supplementary searching: ~110
- Records after duplicate removal and title/ abstract screening: ~40 assessed in full text
- Full texts excluded (no antemortem/ postmortem comparator, wrong outcome, non-English, abstract only): ~14
- Studies included in qualitative synthesis: 26

Type of Review: This article is a systematic review with elements of a scoping synthesis. A purely systematic, meta-analytic design pooling effect sizes such as relative abundance differences or diagnostic sensitivity/specificity across studies was judged premature given the heterogeneity of the underlying evidence base: included studies span animal models (porcine wound microbiomes), human cadaveric thanatomicrobiome studies, immunohistochemical vitality-marker studies, and clinical chronic-wound bacteriology, each using different sampling sites, sequencing depths, taxonomic resolution, and outcome metrics. A strict meta-analysis would force statistically inappropriate pooling across non-commensurable measures (e.g., relative 16S abundance versus immunohistochemical staining sensitivity).

Instead, this review adopts a systematic search and screening methodology (explicit search strategy, inclusion/exclusion criteria, PRISMA-informed selection flow, as detailed in Section 2) combined with a thematic narrative synthesis (Section 4), which is the design recommended by methodological guidance such as the Cochrane Handbook and the PRISMA statement when meta-analysis is not feasible due to clinical or methodological heterogeneity.

This hybrid approach mirrors the design used by directly comparable recent reviews in this spacefor example, the PRISMA-registered systematic reviews of postmortem-interval microbiology by Moitaset al. and by Tozzo et al., and the PROSPERO-registered systematic review of wound omics by Ros et al.each of which combined systematic search methodology with narrative rather than quantitative synthesis [10,14,15]. The review is systematic rather than purely narrative in that: (i) the search strategy, databases, date range, and search terms are fully specified and reproducible (Section 2); (ii) inclusion and exclusion criteria were defined prospectively rather than studies being selected opportunistically; (iii) a defined screening and selection flow is reported; and (iv) included studies are tabulated with study design, sample size, methodology, and findings (Table 1) to allow the reader to weigh evidence strength independently of the narrative interpretation. It departs from a meta-analysis in that no pooled quantitative effect estimate (e.g., a pooled odds ratio for a given taxon's diagnostic value) is calculated, and from a pure scoping review in that explicit eligibility criteria, rather than broad topic mapping, governed study selection.

This design choice has direct implications for how findings should be interpreted: conclusions in this review describe consistent qualitative directions of effect (e.g., "Staphylococcus and Acinetobacter were consistently enriched in postmortem rather than antemortem wound samples across the identified comparative studies") rather than quantitative pooled estimates of diagnostic accuracy, and readers seeking validated sensitivity/specificity values for forensic casework should consult the primary immunohistochemical-marker validation studies cited directly (e.g., Gauchotteet al.) rather than this synthesis.

Main Body

Antemortem wound colonization: the living-host baseline:

Wounds sustained while host physiology remains intact are colonized in a predictable, immunologically constrained sequence. Clinical wound-microbiology studies, though developed independently of forensic questions, provide the necessary living-host baseline against which postmortem deviation can be measured. Nahid et al. followed 47 chronic wounds in 28 patients over 30 days and documented an early phase dominated by Gram-positive cocci of normal skin flora (Staphylococcus aureus, beta-hemolytic streptococci), with Gram-negative rods (Proteus, Escherichia coli, Klebsiella) and, eventually, obligate anaerobes (Bacteroides, Clostridium, Actinomyces) emerging only as the wound persisted and tissue oxygenation fell [23]. This staged patternaerobic Gram-positive skin commensals first, anaerobic and environmental organisms laterrecurs across the acute and chronic wound-colonization literature and reflects active local perfusion, an intact inflammatory cascade,

and host immune containment of deeper anaerobic invasion.

Postmortem injury colonization: decomposition-driven succession

Once circulatory and immune function cease, this host-constrained pattern breaks down. Hyde et al. demonstrated a clear aerobic-to-anaerobic community shift across the bloat stage of decomposition in cadaveric tissue, a transition driven by oxygen depletion and putrefactive gas accumulation rather than by any localized injury response [23].

Javan et al. and subsequent thanatomicrobiome work showed Clostridium spp. proliferation and organ-specific bacterial signatures developing in a time-dependent manner after death, independent of any antemortem wounding [6,7]. Critically, Heimesaatet al.'s murine model demonstrated that intestinal bacterial translocation to extra-intestinal sites and blood begins within minutes of death and intensifies by several log-orders within 72 hours meaning that microbial findings at a postmortem wound site can reflect generalized postmortem dissemination rather than anything specific to the wound itself, a major interpretive confound that any forensic application must control for [17].

The differential signature: what distinguishes antemortem from postmortem wounds

The single study directly modelling this comparison Xiang et al.'s porcine wound model found that antemortem and postmortem wounds were indistinguishable by gross morphology once decomposition had progressed, yet 16S rDNA community analysis showed Enterococcus and Enterobacter enriched specifically in antemortem wounds, while Staphylococcus and Acinetobacter were specific to postmortem wounds, with this divergence becoming statistically robust only by post-injury day 6–9 [1]. This finding is broadly consistent with, though not identical to, the immunohistochemical literature: Gauchotteet al. found CD15 and tryptase reliably distinguished vital from postmortem stab wounds in the minutes-to-hours range, but lost reliability once putrefaction set inprecisely the time window in which a complementary microbial signature appears to strengthen [3]. Ros et al.'s systematic review of wound omics similarly concluded that cytokine and protein-level vitality markers (IL-6, IL-1β, TNF-α) are most informative in the early post-injury interval, reinforcing a picture in which immunohistochemical and microbial markers occupy complementary rather than overlapping temporal windows [5]. Agreement across studies is strongest on the general direction of effect (host-driven Gram-positive/anaerobic-contained flora in antemortem wounds versus environmentally driven, Acinetobacter/ Enterobacteriaceae-enriched flora in postmortem injury); agreement is weakest, and contradictions most evident, on the precise time threshold at which this divergence becomes detectable, which ranges from under an hour in immunohistochemical studies to several days in the sole direct microbial comparison study available.

Table 1: Summary of Key Included Studies

Author, Year	Design / n	Substrate / Method	Key Finding Relevant to Antemortem vs Postmortem Colonization
Xiang et al., 2023	Animal model, swine wounds	16S rDNA, wound swabs, days 1–9 post-wounding	Bacterial community divergence between antemortem and postmortem wounds became significant by post-injury day 6–9; Enterococcus and Enterobacter enriched in antemortem wounds, Staphylococcus and Acinetobacter in postmortem wounds.
Gauchotteet al., 2013	Human autopsy + ex vivo model, n = 102	Immunohistochemistry (FVIIIra, CD15, tryptase)	CD15 and tryptase, not FVIIIra, reliably distinguished antemortem from postmortem stab wounds; markers unreliable once putrefaction/desiccation set in.
Casse et al., 2016	Narrative review	Synthesis of IHC/biochemical vitality markers	Confirms red-cell extravasation alone is unreliable; supports CD15, TNF-α, IL-6, IL-1β as promising vitality markers that indirectly track with early antemortem inflammatory/microbial response.
Ros et al., 2022	Systematic review (PRISMA), 1992–2021	Omics biomarkers across published wound studies	Cytokine and protein expression differ consistently between vital and postmortem wounds; supports a biological (not purely morphological) basis for ante/postmortem differentiation that parallels microbial findings.
Tomassini et	Scoping review,	IHC/immunofluorescence	No single gold-standard marker; combined

al., 2024	1973–2022	dating methods	panels perform better than single markers, mirroring the multi-taxon approach needed in microbial differentiation.
Hyde et al., 2013	Field cadaver study, n = 2	16S rRNA pyrosequencing, bloat stage	Aerobic-to-anaerobic community shift documented across the bloat stage, establishing baseline postmortem succession against which antemortem signatures must be distinguished.
Javan et al., 2016 (Sci Rep)	Cross-sectional, n = 27 corpses	Thanatomicrobiome, internal organs, PMI 3.5–240 h	Clostridium spp. dominance increased with PMI; organ-specific signatures proposed as PMI markers, distinct from wound-localized antemortem flora.
Johnson et al., 2016	Field cadaver study, n = 17 (21 sampled)	16S sequencing, ear/nasal skin, machine learning	k-NN regressor predicted PMI to within ±55 accumulated degree days from skin microbiome data alone.
Heimesaatet al., 2012	Murine model	Culture + molecular, gut and extra-intestinal sites	Intestinal bacterial translocation to blood and organs began within 5 minutes of death, with enterobacteria/enterococci rising 3–5 log-orders by 72 ha major confound for interpreting postmortem wound or blood cultures.
Nahid et al., 2021	Longitudinal clinical cohort, 28 patients / 47 wounds	Culture, VITEK-2, WGS over 30 days	Living chronic wounds showed an early Gram-positive (S. aureus)-dominant phase shifting toward Enterobacteriaceae and resistant Gram-negative organisms over timethe antemortem comparator series.

Discussion

Synthesizing across the included evidence, three trends emerge. First, microbial community divergence between antemortem and postmortem injury appears to be real and reproducible at the genus level, but its time-course is currently characterized in only one direct comparative model (a porcine wound model), creating a substantial gap between mechanistic plausibility and forensic validation in human casework [1]. Second, the wound-vitality literature (immunohistochemistry, omics biomarkers) and the postmortem-microbiome literature (thanatomicrobiome, necrobiome, PMI estimation) have developed largely in parallel, with limited cross-citation despite addressing temporally adjacent and mechanistically linked phenomenaa gap this review's synthesis attempts to bridge.

Inconsistencies across the literature concern threshold timing (hours for immunohistochemical markers versus days for the available microbial comparison data) and the confounding effect of postmortem bacterial translocation, which several studies show begins within minutes of death and can mimic or mask a wound-specific signature if sampling and controls are not rigorous [17,18,20].

Clinically and forensically, these findings suggest that microbial sampling of injuries is not yet ready to stand alone as a vitality marker, but may have particular value in the specific scenario where classical vital reactions and immunohistochemistry have been degraded by decomposition precisely the scenario in which current diagnostic tools are weakest [3]. Compared with existing forensic guidance, which still relies primarily on histology and immunohistochemistry as the first-line approach to wound dating, this review supports microbial profiling as an adjunct rather than a replacement, to be deployed specifically once decomposition precludes reliable IHC interpretation.

Limitations of the Review

Several limitations should be considered when interpreting this synthesis. The evidence base directly addressing antemortem-versus-postmortem wound microbial differentiation is small, and the central comparative finding rests substantially on a single animal-model study, limiting generalizability to human forensic casework. Publication bias likely favors studies reporting positive, statistically significant taxonomic differences over null results, inflating apparent diagnostic promise. Considerable heterogeneity exists across studies in sampling site (skin, organ, blood, gravesoil), sequencing platform and depth (culture-based versus 16S rRNA amplicon versus metagenomic), and statistical approach, which precluded quantitative pooling and limits direct comparability of magnitude-of-effect across studies.

This review was restricted to English-language, peer-reviewed sources, which may have excluded relevant non-English forensic literature,

particularly from East Asian and European forensic-pathology groups active in this area. The search, while extensive across PubMed-, Scopus-, and Web of Science-indexed content via systematic search engines, did not involve direct querying of proprietary database interfaces (e.g., native Embase or Scopus search syntax), and a small number of relevant records may therefore have been missed. Finally, several included sources were themselves narrative or systematic reviews rather than primary data, meaning some original findings are represented here at one remove; where possible, the primary study was traced and cited directly, but this was not always feasible within the time and access constraints of this review.

Conclusion

This systematic review synthesized 26 studies addressing microbial colonization patterns in antemortem wounds and postmortem injuries. The available evidence indicates that antemortem wounds, while host physiology remains intact, are colonized in a staged, immunologically constrained sequence dominated initially by aerobic Gram-positive skin commensals, whereas postmortem injuries are progressively overtaken by an environmentally driven, putrefaction-associated flora enriched in Acinetobacter and Enterobacteriaceae, with this divergence from antemortem patterns becoming statistically detectable within roughly six to nine days post-injury in the single available direct comparative model. This pattern is broadly congruent with, and temporally complementary to, the established immunohistochemical and omics-based vitality-marker literature, which is most reliable in the earlier, less-decomposed interval where microbial signatures have not yet had time to diverge.

In answer to the review question, current evidence supports microbial community profiling as a biologically plausible and decomposition-resistant adjunct for distinguishing antemortem from postmortem injury, but not yet as a independently validated, standalone forensic diagnostic tool. For practice, forensic pathologists should consider wound microbial sampling as a supplementary line of evidence specifically in cases where decomposition has degraded classical vital-reaction and immunohistochemical findings, while continuing to rely on established IHC panels (CD15, tryptase) as first-line markers in fresher cases. For policy, forensic laboratories and accrediting bodies should consider developing standardized sampling, sequencing, and statistical-threshold protocols before microbial vitality evidence is introduced into casework or testimony, given the current reliance on a limited evidence base. For future research, priority should be given to: (i) replicating the antemortem/postmortem divergence findings in human cadaveric models rather than animal proxies alone; (ii) defining the earliest detectable time-point of divergence under controlled conditions; (iii) explicitly modelling and correcting for postmortem bacterial translocation as a confound; and (iv) harmonizing sequencing and statistical methodology across forensic-microbiology groups to enable future quantitative meta-analysis.

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