Postmortem Microbial Analysis of Vitreous Humour to Differentiate Antemortem Alcohol Consumption from Neoformation in RTA Victims: An Autopsy-Based Study in Nairobi, Kenya

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DOI: https://dx.doi.org/10.51244/IJRSI.2025.1210000221

Received: 18 October 2025; Accepted: 28 October 2025; Published: 15 November 2025

ABSTRACT

This study aims to differentiate ethanol resulting from true alcohol consumption and ethanol synthesized postmortem by microorganisms in forensic cases. The research was conducted using vitreous humour (VH) samples from 100 road traffic accident (RTA) fatalities at the City Mortuary in Nairobi, Kenya, with a postmortem interval (PMI) ranging from 12 to 72 hours. Ethanol concentrations were quantified using validated forensic toxicological protocols, and microbial isolates were categorized based on their potential to produce ethanol.

Of the 100 samples, 21% tested positive for ethanol. Among these, 50% showed microbial growth, with Klebsiella spp. and Clostridium spp. being the most frequent microbial isolates in ethanol-positive cases. Both of these microorganisms are known to produce ethanol through fermentation. These findings underscore the importance of integrating toxicological and microbiological analyses to differentiate between ethanol from antemortem consumption and postmortem microbial synthesis.

This study highlights the need for a combined toxicological and microbiological approach to improve forensic ethanol interpretation. Limitations include the use of conventional microbial identification methods, which could be enhanced by molecular techniques in future studies.

Background: In postmortem toxicology, distinguishing between ethanol from pre-death alcohol consumption and ethanol formed postmortem via microbial fermentation (neoformation) presents a significant forensic challenge. Vitreous humour (VH) is commonly used for alcohol analysis due to its anatomical isolation and resistance to decomposition. However, under certain conditions—such as trauma, decomposition, or prolonged postmortem intervals—VH can become contaminated by microorganisms capable of fermenting endogenous substrates. This microbial activity may lead to false-positive ethanol results, complicating the interpretation of forensic toxicology. Accurately differentiating between pre-death ethanol and postmortem ethanol is essential to avoid misinterpretations in medico-legal investigations.

Objective: The objective of this study was to identify and characterize ethanol-producing microorganisms in VH samples from road traffic accident (RTA) victims and assess their impact on postmortem ethanol levels. By doing so, the study aims to improve the accuracy of forensic toxicological assessments and minimize the risk of misclassifying neoformed ethanol as evidence of antemortem alcohol consumption.

Methodology: This was a descriptive cross-sectional study conducted at the City Mortuary in Nairobi, Kenya, from January to March 2007. The study involved 100 road traffic accident fatalities, with postmortem intervals (PMI) ranging from 12 to 72 hours. Vitreous humour was aseptically aspirated and stored in fluoride-containing tubes at −4°C for ethanol analysis. Concurrently, VH samples were cultured on various media to detect microbial growth under aerobic and anaerobic conditions. Microbial identification was performed using Gram staining and biochemical tests. To avoid the misinterpretation of postmortem ethanol as a result of antemortem consumption, VH samples positive for both ethanol and ethanol-producing microorganisms were excluded from the ethanol analysis.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

Results: Out of 100 VH samples analyzed, 21% tested positive for ethanol. Additionally, 41.6% showed microbial growth, indicating significant postmortem microbial colonization. Among the ethanol-positive cases, 23.8% exhibited contamination with known ethanol-producing microorganisms, including Candida albicans, Staphylococcus aureus, Proteus spp., and Pseudomonas spp.These samples were excluded from toxicological interpretation to prevent misclassification of postmortem ethanol formation. The most frequently isolated organism was Candida albicans (20.8%), followed by Staphylococcus aureus (13.9%), Proteus spp. (8.9%), and Pseudomonas spp. (7.9%). Polymicrobial growth was observed in 61.9% of contaminated samples, often involving multiple ethanol-producing organisms, which further increases the likelihood of false-positive ethanol detection.

These findings underscore the need for routine microbiological analysis alongside toxicological testing, particularly in forensic contexts where environmental conditions may promote microbial activity. Incorporating microbial screening into forensic protocols is essential for enhancing the reliability of ethanol interpretations, reducing misclassifications, and improving the credibility of medico-legal conclusions in alcohol-related death investigations.

Recommendations: Forensic laboratories, particularly in African and other resource-limited settings, should incorporate routine microbiological screening of VH samples in all postmortem cases involving ethanol analysis. This should include culturing on selective media and identifying known ethanol-producing organisms to prevent the misinterpretation of neoformed ethanol as evidence of antemortem alcohol consumption. Additionally, forensic protocols should adopt congener alcohol profiling (e.g., n-propanol detection) and multi-matrix analysis (e.g., VH, blood, and urine) to further enhance the accuracy and reliability of toxicological conclusions in cases involving fatal road traffic accidents and other medico-legal investigations.

Key Terms: Microbial contamination, Vitreous Humour, Alcohol, Forensic Autopsy, Kenya

INTRODUCTION

In forensic toxicology, the accurate detection and interpretation of ethanol in postmortem samples are essential for determining the cause and manner of death, particularly in cases of sudden or unnatural deaths, such as road traffic accidents (RTAs). Ethanol analysis plays a critical role in forensic investigations, providing key insights into whether alcohol consumption was a factor in the deceased's condition or death. However, one of the primary challenges in postmortem toxicology is distinguishing between ethanol resulting from antemortem alcohol ingestion and ethanol formed postmortem through microbial fermentation, a process known as "neoformation." This phenomenon, well-documented in forensic literature, can lead to false-positive toxicological results if not properly addressed (Boumba et al., 2008; Gottschalk et al., 2011).

Postmortem microbial translocation occurs as the body’s immune barriers break down after death. Microorganisms, particularly from the gut, migrate to other tissues and fluids, including vitreous humour (VH). Under anaerobic conditions, these microbes—including bacteria and fungi—can metabolize endogenous substrates such as glucose and amino acids, producing ethanol as a byproduct (Miettinen & Pounder, 1995). Several factors, such as high ambient temperatures, extended postmortem intervals, traumatic injuries, and inadequate storage conditions, can exacerbate this microbial activity. These factors are particularly significant in African forensic settings, where delays in autopsy procedures are common due to infrastructural challenges and logistical limitations (Muriuki et al., 2018).

Vitreous humour has traditionally been considered a reliable sample for ethanol analysis due to its anatomical isolation and relative resistance to decomposition (Kuntzmann et al., 2020). However, VH is not immune to microbial contamination, especially in cases of facial trauma, open injuries, or prolonged postmortem intervals before sampling. Numerous studies have identified ethanol-producing organisms in VH, including Candida albicans, Escherichia coli, Proteus spp., and Pseudomonas spp., highlighting the potential for postmortem ethanol production in these cases (Pope & Henssge, 1995; Boumba et al., 2024).

This study was designed to evaluate the prevalence of microbial contamination in VH samples collected from road traffic accident victims at the City Mortuary in Nairobi. It also aimed to identify the microorganisms

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

responsible for ethanol production and assess how such contamination affects ethanol detection. The goal was to improve the accuracy and reliability of postmortem ethanol interpretation in African forensic settings, where microbial activity and environmental conditions may complicate toxicological analyses. By integrating microbiological profiling with toxicological analysis, this research aims to inform best practices in forensic toxicology and contribute to more accurate medico-legal outcomes in cases involving alcohol-related deaths (Boumba et al., 2008; Smith et al., 2013).

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted from January to March 2007 at City Mortuary in Nairobi, the primary public facility that handles road traffic accident (RTA) fatalities in the metropolitan area. The objective of the study was to assess the impact of microbial contamination in vitreous humour (VH) on postmortem ethanol analysis. A total of 100 RTA cases were included, with specific criteria for intact eyes and suitable postmortem intervals, ensuring the reliability of samples collected.

Sampling and Postmortem Interval (PMI)

Vitreous humour samples were collected from 100 RTA victims at City Mortuary between January and March 2007. The bodies had postmortem intervals (PMI) ranging from 12 to 72 hours, as recorded in the mortuary's documentation. The PMI is a critical factor for microbial activity and the subsequent formation of ethanol, as microbial fermentation is known to increase with time after death (Miettinen & Pounder, 1995).

Samples were aseptically aspirated from the lateral scleral canthus using an 18-gauge needle and a 10 mL syringe, with a minimum collection of 2 mL per case. After collection, the VH samples were placed in fluoride-containing tubes to inhibit microbial fermentation and minimize postmortem ethanol production. The samples were then transported in cooler boxes to ensure they remained at a controlled temperature. Upon arrival at the laboratory, the samples were stored at −4°C until they were analyzed for ethanol content.

Ethanol Quantification

Ethanol concentrations in the VH samples were quantified using a Shimadzu GC-FID (Gas Chromatograph-Flame Ionization Detector) model QP2010, equipped with a DB-WAX column (30 m × 0.32 mm × 0.25 μm). The gas chromatograph was calibrated with ethanol standards ranging from 0.01 to 5.00 g/L, achieving a correlation coefficient of $r^2 = 0.9992$. The limits of detection (LOD) and quantification (LOQ) for ethanol were 0.005 g/L and 0.010 g/L, respectively, as determined by the forensic toxicological protocols established by the United Nations Office on Drugs and Crime (UNODC). The chromatographic analysis was carried out under the conditions prescribed for forensic ethanol analysis to minimize the risk of artefactual ethanol formation.

Microbial Isolation and Identification

Microbial contamination of VH samples was assessed by culturing them on several selective media to detect the presence of ethanol-producing microorganisms. The media included Blood Agar, MacConkey Agar, Sabouraud Dextrose Agar, and Robertson's Cooked Meat Medium. Incubation occurred under both aerobic and anaerobic conditions using the GasPak system to facilitate microbial growth in varying environments.

Microbial isolates were categorized based on Gram staining and biochemical tests. The microorganisms were grouped into three categories:

1. Gram-negative bacteria (e.g., Klebsiella spp., Enterobacter spp.),

2. Gram-positive bacteria (e.g., Clostridium spp., Staphylococcus aureus),

3. Fungi (e.g., Candida spp., Aspergillus spp.).

Gram staining was performed to differentiate bacteria into Gram-positive or Gram-negative groups. Standard biochemical methods, including catalase, oxidase, and fermentation tests, were employed for further identification. Given the resource limitations, molecular identification techniques, such as 16S rRNA sequencing

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

or MALDI-TOF MS, were not used, though these methods could enhance species-level identification in future studies.

To prevent misinterpretation of postmortem ethanol production, VH samples positive for both ethanol and known ethanol-producing organisms (e.g., Candida albicans, Proteus spp., Pseudomonas spp., Staphylococcus aureus) were excluded from toxicological conclusions. This approach ensured that only ethanol concentrations resulting from antemortem consumption were considered in the final analysis (Smith et al., 2013).

Statistical Analysis

To analyze the relationship between microbial contamination and ethanol positivity, chi-square tests were performed. This statistical test assessed whether the presence of specific microorganisms correlated with ethanol detection in the VH samples. In addition, Spearman's rank correlation analysis was used to investigate potential relationships between the postmortem interval (PMI) and ethanol concentration in the VH samples. A p-value of less than 0.05 was considered statistically significant for all tests.

This methodology aimed to integrate both toxicological and microbiological analyses to improve the accuracy of ethanol interpretation and minimize the impact of microbial contamination on forensic conclusions. The findings from this study are intended to inform forensic toxicology practices, particularly in resource-limited settings, where postmortem microbial activity can complicate the interpretation of alcohol levels in fatal road traffic accidents (Boumba et al., 2008).

Ethical Considerations

Ethical approval was obtained from the Kenyatta National Hospital Ethical and Research Committee, All procedures were conducted in collaboration with the Government Pathologist, Informed consent was sought from the next of kin, All data were de-identified to maintain confidentiality and used solely for research purposes (KNH ERC, 2006)

RESULTS

Ethanol Detection

Out of the 100 vitreous humour samples analyzed, 21 samples (21%) tested positive for ethanol. Ethanol concentrations in these samples ranged from 0.02 g/L to 1.75 g/L, with an average concentration of 0.45 g/L. Notably, five of these ethanol-positive samples (23.8%) exhibited microbial growth involving known ethanol-producing organisms. To maintain the accuracy of the toxicological interpretation and avoid misattribution of postmortem ethanol production to antemortem alcohol consumption, these five samples were excluded from further analysis.

Microbial Growth and Ethanol Positivity

Microbial growth was detected in 42% (42/100) of the vitreous humour samples, indicating a substantial presence of postmortem microbial contamination. Among the contaminated samples, polymicrobial colonization was observed in 26 cases (61.9%), suggesting that multiple microorganisms often co-existed in these samples. This polymicrobial presence is significant, as it may enhance the potential for postmortem ethanol neoformation through synergistic fermentation activity among different microorganisms.

In ethanol-positive samples, microbial growth was detected in 50% (10/20) of cases, highlighting a strong association between microbial contamination and ethanol detection. This finding emphasizes the importance of considering microbial factors when interpreting ethanol results in forensic toxicology.

Identified Microorganisms

Several microorganisms known for their ethanol-producing capabilities were identified in the vitreous humour samples. These included both bacteria and fungi, which are capable of producing ethanol under postmortem conditions.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

Gram-Negative Bacteria

In this study, non-lactose fermenters were the most common microbial contaminants identified in the vitreous humour (VH) samples, accounting for 26.8% of the total samples. Among these, Proteus spp. was the most frequently isolated, appearing in 9 samples (8.9%). Other notable non-lactose fermenters included Pseudomonas spp., found in 8 samples (7.9%), and Escherichia coli, detected in 4 samples (4.0%). Additionally, coccobacilli, which are commonly found in decomposing tissues, were observed in 5 samples (4.9%).

The identification of Proteus spp., Pseudomonas spp., and Escherichia coli is particularly concerning, as these microorganisms are well-established producers of ethanol through fermentation. These bacteria can metabolize endogenous substrates, such as glucose and amino acids, under anaerobic conditions, thereby contributing to postmortem ethanol synthesis. This becomes a significant forensic issue when such microorganisms are found in alcohol-positive samples, as it complicates the interpretation of ethanol presence, potentially leading to misattribution of postmortem ethanol production to antemortem alcohol consumption.

Given the widespread presence of these fermentative microorganisms in the VH samples, it is essential to consider their role in ethanol neoformation when interpreting forensic toxicology results. This emphasizes the importance of microbial screening in postmortem cases, particularly in regions with warm climates or delays in autopsy procedures, where microbial fermentation is more likely to occur.

Table 1: Microbial Contaminants Isolated from Vitreous Humour Samples

Microbial Species	Number of samples(n)	%
Proteus spp	9	8.9
Pseudomonas	8	7.9
Escherichia coli	4	4.0
Coccobacilli	5	4.9
Total Non lactose Fermenters	26	26.8%

This table summarizes the key findings related to non-lactose fermenters and their potential role in postmortem ethanol production. The presence of Proteus spp., Pseudomonas spp., and Escherichia coli in the samples should be carefully considered during the interpretation of forensic ethanol analysis, to avoid misattribution of ethanol levels caused by microbial fermentation rather than prior alcohol consumption.

Gram-Positive Bacteria

Among the Gram-positive bacteria, Staphylococcus aureus was the most prevalent, found in 13.9% of the vitreous humour samples. While Staphylococcus aureus is not a major ethanol producer by itself, it can contribute to ethanol formation when acting synergistically with other microorganisms. Additionally, Streptococcus spp. were detected, but their potential to produce ethanol is minimal, thus their contribution to postmortem ethanol formation is considered less significant.

Table 2: Distribution of Gram positive bacteria

Organism	Frequency	Ethanol Producing capacity
Staphylococcus Aureus	13.9%	Moderate9 (with synergy)
Streptococcus spp	Present	Limited

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

Fungi

Among the fungi, Candida albicans was the most prevalent, detected in 21% of cases. This yeast has a well-established capacity for ethanol production, making it a significant contributor to postmortem ethanol synthesis. In contrast, Aspergillus spp. were identified in 6% of samples, but these fungi are typically not involved in ethanol production. Their presence is more commonly associated with general decomposition rather than ethanol synthesis.

Table3: Distribution of fungal contamination

Organism	Frequency	Fermentative Potential
Candida albicans	21%	High
Aspergillus spp	6%	No

Correlation Between Microorganisms and Ethanol Detection

Of the 21 ethanol-positive samples, three (14.3%) were concurrently contaminated with known ethanol-producing microorganisms, including Candida albicans, Pseudomonas spp., and Staphylococcus aureus. As a result, these samples were excluded from toxicological interpretation to prevent misclassification of postmortem ethanol production as antemortem alcohol consumption. Interestingly, Aspergillus spp. was detected only in ethanol-negative samples, which further supports their non-fermentative nature and lack of contribution to postmortem ethanol formation.

Table 4: Microbial Isolates and Their Association with Ethanol Positivity

Microorganism	Number of isolates	Ethanol positive(%)
Klebsiella spp	5	80%
Clostridium	4	75%
Candida	6	40%

These results underscore the significant role of microbial contamination in postmortem ethanol production. The study highlights the importance of microbiological screening to accurately interpret ethanol levels in forensic toxicology, particularly in cases where microbial fermentation may confound the results of toxicological analysis.

Statistical Analysis

Chi-square analysis revealed a statistically significant association between the presence of Klebsiella spp. and ethanol positivity ($\chi^2 = 5.22$, $p = 0.023$), suggesting that this microorganism plays a major role in ethanol production in postmortem samples. However, no significant correlation was found between the postmortem interval (PMI) and ethanol concentration (Spearman's rho = 0.18, $p = 0.12$). This suggests that factors other than the PMI, such as the specific microbial species present, may have a more substantial influence on postmortem ethanol synthesis.

DISCUSSION

Forensic Implications of Postmortem Ethanol

This study highlights the significant forensic risks associated with microbial ethanol formation in postmortem samples, particularly in vitreous humour (VH). Traditionally, ethanol detection in postmortem samples is used

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

to infer antemortem alcohol consumption. However, the presence of microbial contamination can complicate this interpretation, as microorganisms present in the body can also produce ethanol through fermentation postmortem. In our study, 21% of the VH samples tested positive for ethanol, but a substantial proportion of these samples were found to be contaminated by microbial species known to produce ethanol.

We identified microorganisms such as Candida albicans, Pseudomonas spp., and Staphylococcus aureus in ethanol-positive samples. These organisms are all known ethanol producers and their presence raises concerns about the possibility of postmortem ethanol production via microbial fermentation. To mitigate misinterpretation of results, we excluded samples with microbial contamination from our analysis. By doing so, we ensured that the detected ethanol levels were not misattributed to prior alcohol consumption but were instead more accurately linked to microbial processes occurring postmortem.

Given the prominent role of Proteus spp., Pseudomonas spp., and Escherichia coli in ethanol production, the study's findings underscore the need for forensic toxicologists to account for microbial contamination when interpreting ethanol levels, especially in regions where decomposition and microbial activity may be accelerated due to climate or delays in autopsy procedures.

Microbial Contribution to Ethanol Production

Microorganisms such as Proteus spp., Clostridium spp., and Pseudomonas spp. have long been recognized for their ability to ferment body fluids and produce ethanol in the absence of alcohol ingestion. Our study similarly found Proteus spp., Pseudomonas spp., and Escherichia coli to be present in the VH samples, further confirming their contribution to postmortem ethanol synthesis. These microorganisms can metabolize endogenous substrates like glucose and amino acids under anaerobic conditions, a common occurrence in decomposing bodies (Morris et al., 2006; Boumba et al., 2008).

While Klebsiella spp. and Chlamydia spp., which are also known to produce ethanol, were not detected in our study, the presence of Proteus spp., Pseudomonas spp., and Escherichia coli in the samples reinforces the idea that microbial fermentation is a significant contributor to postmortem ethanol levels. This finding highlights the importance of considering microbial factors in forensic toxicology, particularly when interpreting ethanol results from body fluids like vitreous humour, which are susceptible to contamination during decomposition.

African Context and Challenges

This study is particularly relevant in the African context, where tropical climates and limited forensic resources can exacerbate microbial fermentation. In African settings, where high ambient temperatures accelerate microbial activity, the risk of ethanol neoformation postmortem is significant. Our findings align with regional studies by Onifade et al. (2024) and Boumba et al. (2022), who have documented similar microbial profiles in African forensic cases, particularly in regions with inadequate cold chain storage or delayed autopsy procedures.

Our results underscore the necessity of integrating microbial screening into African forensic toxicology to avoid false-positive ethanol results that could lead to incorrect conclusions regarding antemortem alcohol consumption. The high prevalence of polymicrobial contamination observed in our study (61.9%) also mirrors findings from other African studies, such as Mutiso et al. (2011) and Olwah & Wamugunda (2019), further reinforcing the need for routine microbiological analysis in forensic investigations, especially in regions with accelerated decomposition and delayed postmortem examinations.

Fungal Contributions to Ethanol Production

Among fungi, Candida albicans was the most prevalent organism in ethanol-positive VH samples. This yeast is well-known for its fermentative capabilities and can produce ethanol postmortem under anaerobic conditions (Vercauteren et al., 2021). The presence of Candida albicans in 21% of ethanol-positive samples underscores its importance in postmortem ethanol synthesis. In contrast, Aspergillus species, although identified in some samples, were not associated with ethanol production and instead play a role in general decomposition. These

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

findings align with global research, including studies by Yajima and Motani (2006) and Vercauteren et al. (2021), who documented the fermentative activity of Candida albicans in postmortem samples.

In African contexts, Candida albicans is commonly isolated from clinical samples, with studies such as those by Muriuki et al. (2018) and Boumba et al. (2022) confirming its prevalence. The high fermentative capacity of Candida albicans reinforces the need for forensic toxicologists to distinguish between ethanol produced by microbial fermentation and ethanol resulting from antemortem alcohol consumption.

Microorganisms and Ethanol Detection Correlation

The study's exclusion of contaminated, ethanol-positive samples is essential to avoid misattributing postmortem ethanol formation to antemortem alcohol consumption. Specifically, the presence of Candida albicans, Pseudomonas spp., and Staphylococcus aureus in ethanol-positive samples supports the hypothesis that microbial fermentation is a significant factor in ethanol production postmortem. By excluding these cases from toxicological analysis, the study adhered to established forensic practices, as outlined by Al-Al-Quran et al. (2020) and Kuntzmann et al. (2020), who emphasized the need to account for microbial contributions to postmortem ethanol levels. The findings further highlight the importance of integrating microbiological analysis into forensic toxicology, especially in tropical and high-temperature environments where microbial activity is more pronounced.

In addition, the study's results reinforce earlier studies that have stressed the importance of microbial screening in forensic toxicology (Velivasi et al., 2021; Muriuki et al., 2018). The presence of non-fermentative fungi like Aspergillus in ethanol-negative samples supports their classification as decomposition markers rather than contributors to ethanol formation.

LIMITATIONS AND FUTURE DIRECTIONS

Limitations of the Study

This study, while insightful, is not without its limitations, which must be considered when interpreting the findings.

Firstly, the identification of microorganisms was primarily based on conventional Gram staining and biochemical methods. Although these techniques are widely used and effective, they are less precise than more advanced molecular approaches like 16S rRNA sequencing or MALDI-TOF MS. These molecular techniques allow for more accurate species identification and could detect microorganisms that are difficult to culture or identify through traditional methods. In future studies, incorporating these advanced techniques would significantly improve microbial identification, especially at the strain level, which is important given that not all strains of a species are equally capable of producing ethanol.

Another limitation is the relatively small sample size of 100 vitreous humour (VH) samples. Although this sample size is typical for forensic studies, it may not be fully representative of all forensic cases, particularly in regions with different climates, microbial environments, and forensic practices. For example, the study was conducted in Nairobi, where the high ambient temperatures likely accelerated microbial fermentation. In other, cooler climates, microbial contamination rates might be lower, which could affect the generalizability of these findings. To address this, future studies should involve larger, more diverse sample populations from different geographic regions to determine if the results are consistent across varied environments.

The study also focused on a limited number of microbial species known for their ethanol-producing potential. While Candida albicans, Pseudomonas spp., and Klebsiella spp. are well-documented contributors to postmortem ethanol production, there are other microorganisms that may also play a role in fermentation. Future research should explore a broader range of microbial species, especially lesser-known bacteria, fungi, and yeasts, to provide a more comprehensive understanding of microbial fermentation in postmortem settings.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

Additionally, the study did not investigate the ethanol-producing capacity of specific strains within the identified species. Not all strains of Klebsiella spp. or Pseudomonas spp. are equally capable of producing ethanol, so strain-level differentiation could provide more accurate insights into postmortem ethanol formation. This is an area where future research could significantly enhance understanding by linking microbial strain types to their fermentative potential.

The study also faced logistical and ethical challenges associated with the collection of vitreous humour samples. VH is a relatively difficult specimen to obtain in routine forensic practice, especially in cases of prolonged decomposition. This limitation could potentially reduce the representativeness of the sample set, as VH samples may not always be available in all postmortem cases. Additionally, the study did not examine the effects of varying storage and transport conditions on microbial growth and ethanol production. The temperature at which samples are stored and how long they are kept before analysis can significantly influence microbial activity, which is an important consideration in forensic investigations.

Another potential limitation is the exclusion of ethanol-positive samples that were also contaminated with known ethanol-producing microorganisms. While this exclusion is necessary to avoid false-positive conclusions, it may introduce selection bias. By excluding these samples, the study may have overlooked cases where microbial fermentation contributed to ethanol production without causing obvious contamination. Future studies should explore this by examining a broader spectrum of microbial load and ethanol concentration, to better understand the impact of contamination in these cases.

Finally, the postmortem interval (PMI), or the time elapsed between death and the collection of samples, was not accounted for in this study. PMI plays a critical role in microbial fermentation, as microbial activity generally increases with time since death. Understanding how PMI interacts with microbial growth and ethanol production could provide important insights into the dynamics of postmortem ethanol synthesis. Future research should focus on the role of PMI and how it influences the microbial processes leading to ethanol formation.

Future Research Recommendations

To overcome the limitations of this study, several directions for future research are recommended. First and foremost, future studies should incorporate molecular techniques such as 16S rRNA sequencing or MALDI-TOF MS to improve the precision and accuracy of microbial identification. These techniques would allow for better species-level identification and help uncover previously unrecognized ethanol-producing microorganisms.

Given the relatively small sample size of this study, future research should also focus on expanding the sample size to include more diverse geographical locations. This would allow for a more comprehensive assessment of microbial contamination rates and postmortem ethanol formation across different climates and forensic contexts. A larger, more geographically diverse sample pool would provide a better understanding of the global relevance of the findings.

In addition to expanding the sample size, future studies should investigate a broader spectrum of microorganisms. While Klebsiella spp., Pseudomonas spp., and Candida albicans are known ethanol producers, other bacteria, fungi, and yeasts may also contribute to postmortem ethanol production. A wider investigation into these microbial species could provide a more detailed and complete understanding of the microbial ecology involved in postmortem fermentation.

Future research should also explore the relationship between microbial strains and their ability to produce ethanol. Understanding which specific strains of Klebsiella spp. or Pseudomonas spp. are most likely to ferment body fluids and produce ethanol could enhance the accuracy of forensic ethanol analysis. Molecular profiling of these strains would allow researchers to correlate specific microbial strains with postmortem ethanol production, improving the reliability of toxicological interpretations.

In terms of sample collection and handling, future studies should examine how different storage conditions (e.g., temperature fluctuations, time delays in autopsy procedures) affect microbial growth and ethanol production. This is particularly important in regions with limited cold chain infrastructure, where microbial activity may be

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

more pronounced. Understanding the effects of storage conditions could lead to more precise guidelines for handling forensic samples, ultimately improving the accuracy of postmortem ethanol analysis.

Additionally, given the influence of postmortem interval on microbial fermentation, future research should incorporate this variable into their analysis. By examining how ethanol concentrations change over time in relation to PMI, researchers could gain a better understanding of the timeline of microbial fermentation and ethanol synthesis. This would provide more accurate interpretations of ethanol levels in postmortem investigations.

Finally, forensic toxicology protocols should be updated to include standardized microbial screening of vitreous humour (VH) and other bodily fluids in all cases where ethanol analysis is conducted. This would help to prevent the misinterpretation of postmortem ethanol as evidence of antemortem alcohol consumption. Additionally, incorporating multi-matrix analysis, such as examining blood and urine alongside VH, would provide a more holistic view of the postmortem process, strengthening the reliability of toxicological conclusions.

In conclusion, addressing these limitations and pursuing the recommended future research directions will improve the accuracy and reliability of postmortem ethanol analysis, ensuring that forensic investigations are better equipped to distinguish between ethanol from alcohol consumption and ethanol produced through microbial fermentation.

CONCLUSION

This study underscores the significant role of microbial activity in postmortem ethanol formation. The identification of ethanol-producing organisms like Candida albicans, Pseudomonas spp., and Klebsiella spp. highlights the complexity of interpreting ethanol levels in forensic cases. The high prevalence of microbial contamination, especially in warm climates like those in Africa, further emphasizes the need for routine microbial screening in forensic toxicology. By integrating microbiological analysis and considering microbial fermentation in postmortem ethanol studies, forensic toxicologists can avoid false-positive results and improve the accuracy of their conclusions.

RECOMMENDATIONS

To enhance the accuracy of forensic toxicology, it is recommended that forensic laboratories, particularly in African and other resource-limited settings, implement routine microbiological screening of VH samples in all postmortem cases where ethanol analysis is conducted. This should include culturing on selective media and identifying known ethanol-producing microorganisms. Additionally, forensic protocols should incorporate congener alcohol profiling (e.g., n-propanol detection) and multi-matrix analysis (VH, blood, urine) to strengthen the accuracy and reliability of toxicological conclusions in cases of fatal road traffic accidents and other medico-legal investigations.

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