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ORIGINAL ARTICLE

Comparative Studies on Degradation of Forensic Biological Fluids Recovered from Crime Scene

[1]Aswathi Anurudhan, [2]Rajshree Borah, [3]Anshu Nanda, [4]Karan Singh, [5]Ekampreet Kaur, [6]Jaskaran Singh

ABSTRACT

CONTEXT: Evidence plays crucial role in forensic investigation in solving criminal cases. These evidences should be properly collected and preserved to avoid degradation and loss of evidential value. Hence, this paper describes the experimental study of collected and degraded biological fluids which can be recovered from the crime scene.

AIMS: The study aims to assess the microbial growth on different biological fluids recovered from crime scene in percolation with varied temperature durations. **SETTINGS AND DESIGN:** An experimental setup was designed to study the different biological fluids and their variation of impact on different conditions like type of sample, temperature differences and time of exposure. **METHOD AND MATERIALS:** Biological fluids such as saliva, urine, semen and vomit were collected and used for the study. The identification procedure of microorganisms and extent of degradation was studied by means of physical analysis, bacterial culture, fungal culture, staining for bacteria and fungus, and biochemical testing. Followed by assessment of collected samples inoculated with cotton cloth piece with specified time interval and temperatures. **RESULTS:** Candida albicans and Escherichia coli show maximum profuse growth in inoculated urine samples exposed to temperature 37°C with the time interval of 7 to 27 hours and 20-25°C with time interval of 48 hours. Likewise, Pseudomonas aeruginosa shows maximum growth in inoculated saliva samples exposed to temperature ranging from 20-37°C with time interval of 40 hours. Furthermore, the micrococcus mucillogens, proteus vulgaris and streptococcus pneumoniae show maximum growth in inoculated vomit samples exposed to temperature ranging from 20-37°C with time interval of 7-27 hours. Lastly, Micrococcus mucillogens shows maximum growth in inoculated semen sample exposed to temperature ranging 20-37°C with time interval of 48 hrs. **CONCLUSION:** Forensic biological samples are more susceptible to the contamination by the growth of microorganism because of the nutritive substances present in each fluid. Since compositions of each biological fluid are different, therefore types of microbes growing and their effect on samples will also be different leading to destruction of forensic biological fluid samples. This study reveals the determination of microbes such as a Candida albicans, Escherichia Coli, Pseudomonas aeruginosa, micrococcus mucillogens, proteus vulgaris and streptococcus pneumoniae in urine, saliva, vomit and semen samples. Under ambient-conditions of high temperature and with specific time durations, the growth of microbes was found to be rapid. It is also to be concluded that temperature plays a major role in the preserving the integrity of samples, at high temperatures for more time, the samples will get dry and minimal amount of microorganism will grow. **KEY MESSAGES:** Detection and determination of varied microbial growth on different biological fluids of forensic importance provide prudent information to forensic experts for combating the loss of evidential value for trials and for maintaining proper chain of custody. Hence, the study aids forensic experts to collect and preserve biological fluids with in specific time duration and temperature conditions.

KEYWORDS | forensic microbiology, biological fluids, bacterial culture, biochemical tests

Author's Credentials:
[1]*M.Sc. Nursing Student, Deparment of Forensic Science, Lovely Professional University, Phagwara, Punjab 144001, India.*

[2]*Assistant Professor, Department of Dentistry, Assam Medical and Hospital, Dibrugarh 86005, Assam, India.*

[4]*Assistant Professor, Deparment of Forensic Science, Punjabi University, Patiala-147002, Punjab, India.*

[5]*Intern, Toxicology division, Regional Testing Forensic Science Laboratory, Ludhiana 141008, Punjab, India.*

[3,6]*Assistant Professor, School of Allied Health Sciences, Sharda University, Greater Noida 244001, Uttar pradesh, India.*

Corresponding Author:
Jaskaran Singh, Assistant Professor, School of Allied Health Sciences, Sharda University, Greater Noida 244001, Uttar pradesh, India.

Email:
jaskaran. singh@sharda.ac.in

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INTRODUCTION

In the field forensic analysis, one of the main drawbacks has been the quantity of samples collected and their purity. Biological samples are more susceptible to contamination.[1] Biological fluid evidence is easily tampered evidence.[2] Evidences collected from the crime scene plays a vital role in solving criminal case.[3] Especially in the case of biological evidence, they are more susceptible to the growth of microorganisms. For this reason, many times the required results are not given by samples for DNA test and non-DNA tests.[4,5] The growth of microorganism in the biological sample will lead to the loss of integrity of the sample.[6] Microbial growth will lead to the destruction of cells, proteins, metabolic products, drugs and other materials present in it, making the analysis a difficult task.[7,8] This is one of the main drawbacks of forensic biological samples. Usually, the samples are collected long after the occurrence of crime.[9] Because of this, the chances for degradation of fluid evidence gets worse. Microbial degradation can be reduced by collecting samples as soon as possible. The compositions of different biological fluids are different, so the effect of microbial growth also will be different.[10]

The growth of microorganisms in different biological samples makes its detection and analysis a very difficult task. One of the most important sample analysis report that is admissible in court is the DNA analysis report. The permissibility of the DNA evidence before the court of law always depends on its accurate and proper collection, preservation and documentation which can reassure the court that the evidence which has been put in front is reliable. In studies it is shown that microbial genome will interfere with the sample genome and makes the DNA typing method difficult. Sometimes the microbial growth will lead to poor PCR amplification or no PCR amplification.[11] For the detection of body fluids, RNAs of degraded samples are taken and it is being detected by transcriptomic analysis. By massively parallel sequencing technology the sample is detected with least possible sample.[12,13] In sexual assault cases biological fluid evidences have great importance, so the samples should not be degraded. In some countries there are certain protocols and guidelines for evidence management but sometimes it will vary from one region to another in the same country. One of the most common sample collected from the crime scene is blood. Because of improper storage, collection and packaging, many of the tests are not giving appropriate results. Studies done on blood samples shown that with the changing temperature, packaging type and environmental conditions will affect the sample very badly leading to the growth of different types of microbes on it. In this study, it is dealing with the analysis of types of microorganisms growing on different biological samples, the effect of outer environment on sample, the effect of temperature and exposure of time is studied.[12] The study reveals that, according to the changing temperature, time and samples, the microbial growth on samples will differ. Samples which are exposed to high temperatures will suffer less degradation in as it gets dried so fast but samples at room temperature for long duration will suffer more microbial degradation. This is due to less temperature condition will take more time for drying the samples.[15] This study helps us to understand how much the samples are degraded, is the sample suitable for analysis and what time is suitable for sample collection.

MATERIALS AND METHOD

Biological fluids used for this study are saliva, urine, semen and vomit. Methods used for the identification of microorganisms and extent of degradation is done mainly by four methods: physical analysis, bacterial culture, fungal culture, staining for bacteria and fungus, and biochemical testing.[9] After collection of samples, physical analysis of color, texture, coagulation status and smell are done. Five samples are collected with specified time interval and temperatures (e.g. 20-25°C and 37°C). Each

SAMPLE	INDOOR	TEMPERATURE (°C)
Urine	Cloth	20-25 and 37
Semen	Cloth	20-25 and 37
Saliva	Cloth	20-25 and 37
Vomit	Cloth	20-25 and 37

Table 1: Temperature and inoculation surface of samples

biological fluid is inoculated into the cotton cloth piece. The cloth piece is exposed to the environment with different time intervals at different temperatures.

So the sample of inoculated cloth is exposed to the environment for 3 hours (as referred to in the literature), after that the first sample is collected and marked as S1, after 9 hours, S2 is collected, after 15 hours S3 is collected, after 21 hours, S4 is collected, after 27 hours S5 is collected. This procedure is carried out twice for each sample, under two different temperatures (20-25°C and 37°C). For each biological fluid, 40 samples are analyzed, so a sum total of 160 samples were analyzed for this entire study.

When each sample is collected, it is inoculated into the culture media like nutrient agar, blood agar, macconkey agar, peptone water (bacterial culture) and sabourdes dextrose agar (fungal culture) for isolation of microorganism.[13,15] Staining for bacteria and fungus is done by staining methods like gram's staining, lacto phenol cotton blue (LPCB), India ink, and motility test. For identification of organism biochemical testing is done by indole test, methyl red test, voges-proskauer test, citrate test, urease test, nitrate reductase test, catalase, coagulase, triplesugar iron agar test, oxidase test as mentioned in Table 1 above.

The minimum average time for collection of sample after the occurrence of crime is 3 hours.[7] After that first sample is collected and marked as S1, after 9 hours, S2 is collected, after 15hours, S3 is collected after 21 hrs, S4 is collected after 27 hrs S5 is collected. This procedure is done twice for each sample, under two different temperatures 20-25°C and 37°C. For each biological fluid, 40 samples are analyzed, so a total of 160 samples are analyzed for this entire study as mentioned in Figures 1-10.

RESULT

Urine Sample (at 37°C)

1. Sample collected after 6 hours of inoculation is not showing any microbial growth, which indicates that up to6thhour after occurrence of crime the sample is intact.

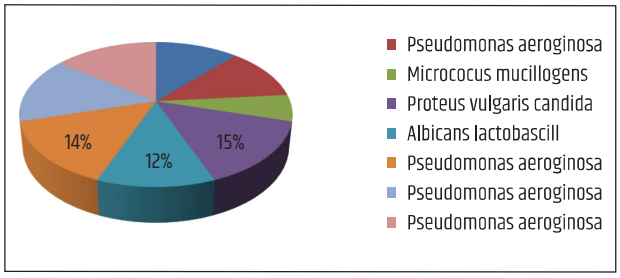

Figure 1: Urine sample (at 37oC)

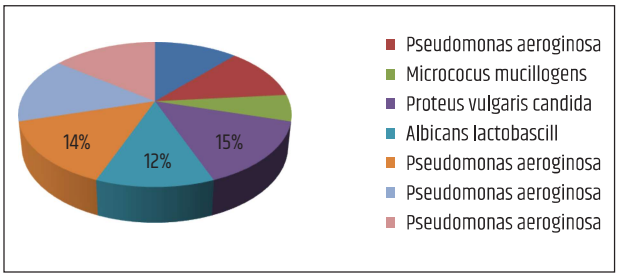

Figure 2: Urine sample (at 20-25oC)

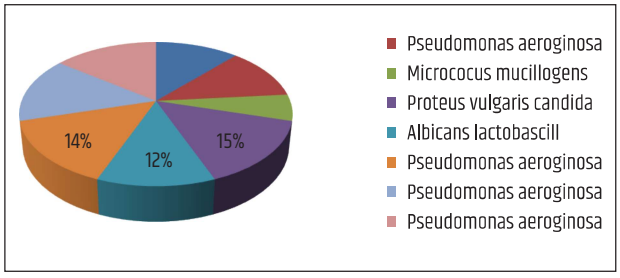

Figure 3: Saliva sample (at 37oC)

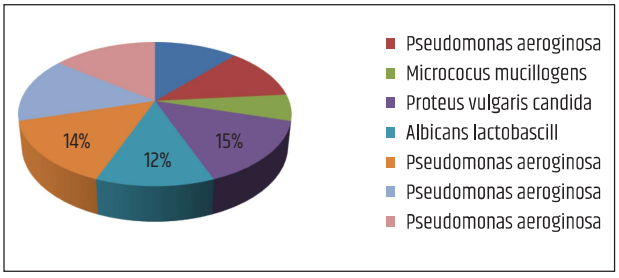

Figure 4: Saliva sample (at 20-25oC)

2. Samples collected in the time interval of 7th to 27th hour, profuse growth of micro-organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation.
3. Sample collected after 28th hour is not showing any microbial growth, which indicates two reasons, either the sample is completely dry or the sample is completely degraded.

Urine Sample (at 20-25°C)

1. Samples collected after 3 hours of inoculation is showing microbial growth.
2. Samples collected within 48 hours are showing microbial growth. This indicates that from the time of inoculation till about 48 hours, microorganisms are growing continuously as it takes more time for the samples to dry.
3. Samples collected after 49th hours onwards showing no trace of microbial growth.

Saliva Sample (at 37°C)

1. Sample collected after 6hours of inoculation is not showing any microbial growth, which indicates that up to 6th hour after occurrence of crime the sample is intact.
2. Samples collected in the time interval of 7th to27th hour, profuse growth of micro-organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation
3. Samples collected after 28th hour is still showing growth, and after 40th hour there is no growth of micro-organisms in the sample due to drying.

Saliva Sample (at 20-25°C)

1. Sample collected after 6 hours of inoculation was not showing any microbial growth, which indicates that up to 6th hour after the occurrence of crime, the sample is intact.
2. Samples collected in the time interval of 7th to 27th hour, profuse growth of micro-organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation.
3. Samples collected after 28th hour is still showing growth, and after 40th hour there is no growth of micro-organisms in the sample due to drying.

Vomit Sample (at 37°C)

1. Sample collected after 6hours of inoculation is not showing any microbial growth, which indicates that up to 6th hour after occurrence of crime the sample is intact.
2. Samples collected in the time interval of 7th to 27th hour, profuse growth of micro-

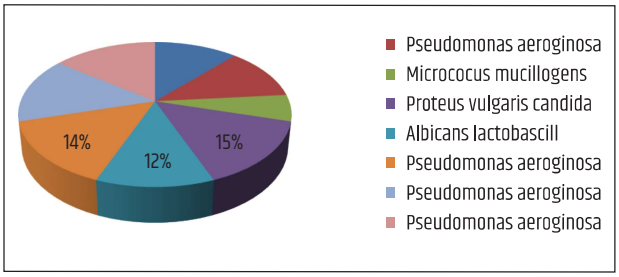

Figure 5: Vomit Sample (at 37oC)

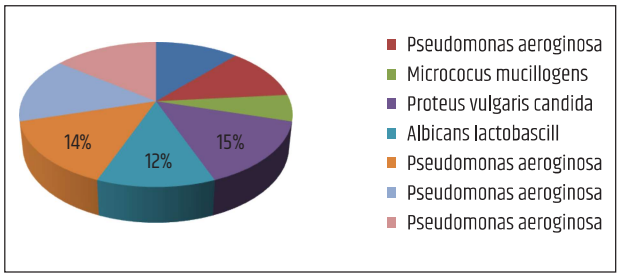

Figure 6: Vomit sample (at 20-25oC)

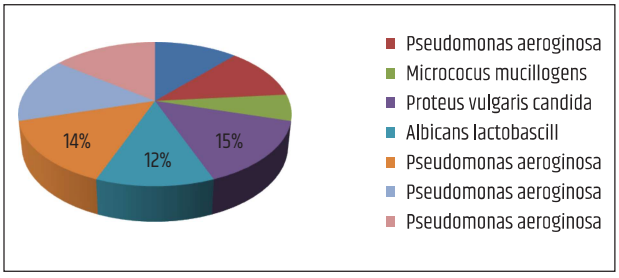

Figure 7: Semen Sample (at 37oC)

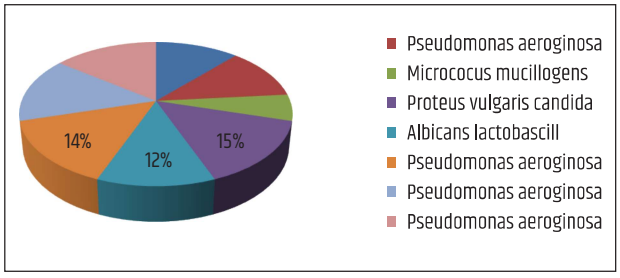

Figure 8: Semen Sample (20-25oC)

organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation.

3. Sample collected after 28th hour is not showing any microbial growth, which indicates two reasons, either the sample is completely dry or the sample is completely degraded.

Vomit Sample (at 20-25°C)

1. Sample collected after 6 hours of inoculation is not showing any microbial growth, which indicates that up to 6th hour after the roccurrence of crime the sample is intact.
2. Samples collected in the time interval of 7th to 27th hour, profuse growth of micro-organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation.
3. Samples collected after 28th hour is still showing growth, and after 40th hour there is no growth of micro-organisms in the sample due to drying.

Semen Sample (at 37°C)

1. Samples collected after 3 hours of inoculation is showing microbial growth.
2. Samples collected within 48 hours are showing microbial growth. This indicates that from the time of inoculation till about 48 hours micro-organisms are growing continuously as it takes more time to dry the sample.
3. Samples collected after 49th hours onwards showing no trace of microbial growth.

Semen Sample (at 20-25°C)

1. Samples collected after 3hours of inoculation is showing microbial growth in the sample.
2. Samples collected within 48 hours are showing microbial growth. Which indicates that from the time of inoculation till about 48 hours micro-organisms are growing continuously as it takes more time to dry the samples.
3. Samples collected after 49th hours onwards showing no trace of microbial growth.

GRAM STAINING OF MICROORGANISMS IDENTIFED

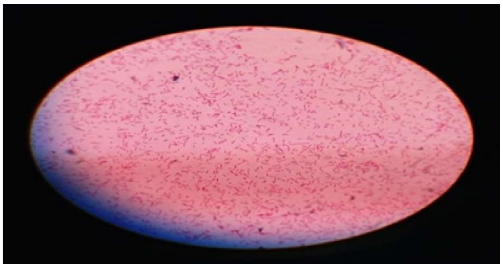

Figure 1: Pseudomonas aeroginosa

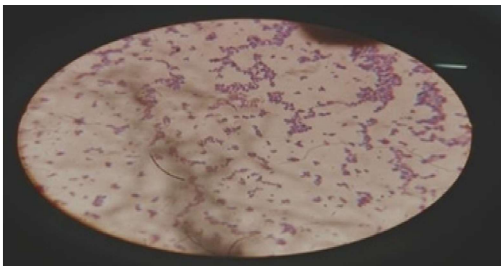

Figure 2: Micrococcus mucillogens

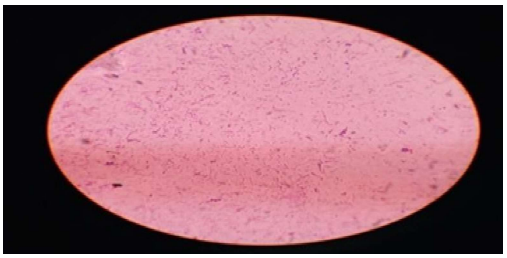

Figure 3: Clostridium tetani

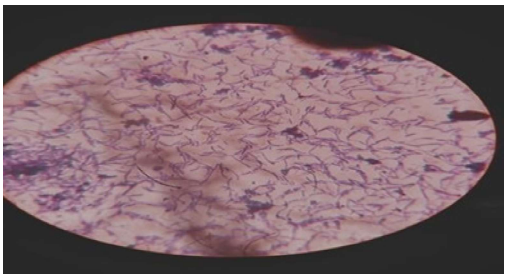

Figure 4: Lactobacillus lactai

Figure 5: Streptococcus pneumoniae

GRAM STAINING OF MICROORGANISMS IDENTIFED

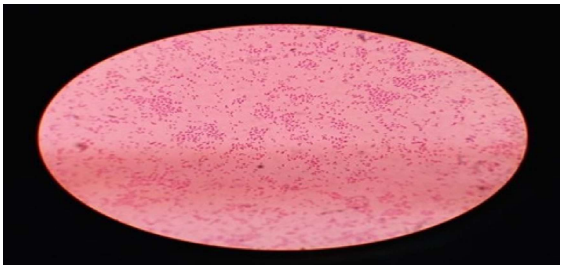

Figure 6: Klebsilella pneumonia

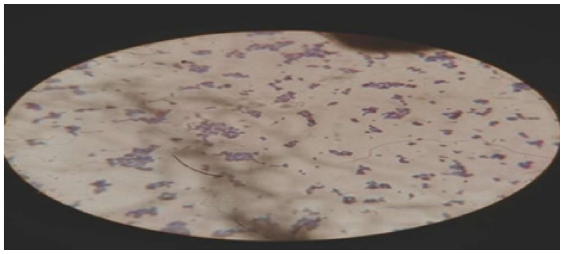

Figure 7: Staphylococcus aureus

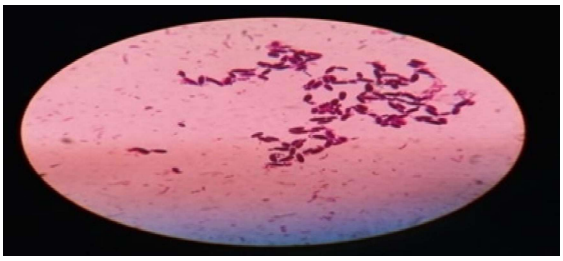

Figure 8: Candida albicans

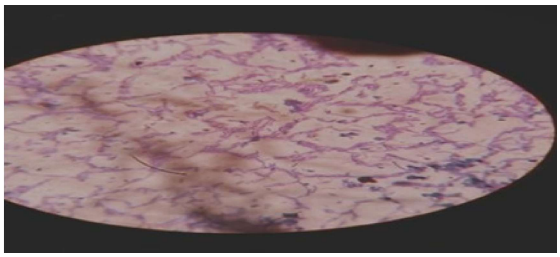

Figure 9: Escherichia coli

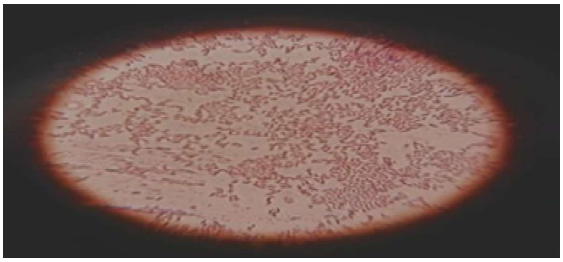

Figure 10: Proteus vulgaris

DISCUSSION

Sample gets degraded at 37°C temperatures for 9-21 hours. Compared 37°C less degradation is seen at room temperature. If the sample is collected before 4 hour from inoculation, the degradation will be less, and more accurate results will result in both temperatures. More contamination is observed in the sample collected from both indoor and outdoor region at the time interval from 9th hour to an average of 30th hour at 37°C 6th to 40th hour at room temperature (20°C-25°C) and showing relevant growth that leads to the loss of integrity of sample. Samples collected after the 31st hour at 37°C, gradually the growth is reducing, which implies that, with the time samples get dry and degradation decreases. Samples collected after 31st hour will be dry but more chances of getting false positive or negative result due to microbial degradation. It is clear that by collecting samples at early stage of crime occurrence the microbial degradation can be reduced so that the integrity of the samples can be maintained. This detailed study of degradation of samples by micro organisms helps in reducing false positive and false negative result to occurrence.

CONCLUSION

In a nutshell, it may be concluded that under ambient-conditions of high temperature and in specific time duration, the growth of microbes were found to be rapid. It is clear that temperature plays a major role in the preserving the integrity of samples. The samples will get dry at high temperature and minimal amount of microorganism will grow. Additionally, within 3 hours of exposure to different temperature and time interval, the microbial grow this minimal.

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