

## Oral Microbiome in Forensic Odontology to Identify Bioterrorism Attack

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### Abstract

Throughout human civilization, various biological agents have been used as deadly weapons and terrorist media for decimating or wiping out a population or even a country from the map. Such bioterrorism may aim to achieve instability of political, economic, social and/or cultural condition of its target. Forensic odontology or forensic dentistry in the process of identification, prevention and handling of bioterrorism can offer robust biological markers of the threat and crucial evidence from the oral cavity in the form of oral microbiome.

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### Introduction

Natural disasters, accidents, and crime, including bioterrorism, could happen anytime and anywhere. After such tragedy, it is hard to identify the victims, especially if their bodies are no longer intact.<sup>1</sup> Terror attacks with biological agents could happen without warning and causing long-term damaging effects.<sup>2</sup> For example, the bioterrorism attacks by mail containing *Bacillus anthracis* spores in the United States during black September infected 22 people who opened the letter, five of whom died.<sup>3</sup> Bacteria can evade the human defences by producing effective virulence factors and then spreading to important organs. The toxins produced by the bacteria can interact with the host cells through receptors, resulting in irregular immune responses.<sup>4</sup> Endotoxins that enter the submucosal layer will stimulate chemotaxis activity in polymorphonuclear leukocytes (PMNs) to secrete proinflammatory cytokines.<sup>5</sup> On the other hand, this creates the opportunity for collecting biological markers from individuals to identify whether they are exposed to an external substance that affects the body before death.<sup>6</sup>

### Materials and methods

This review was initiated to provide knowledge on bioterrorism agents that could endanger the community, and on means to prevent and analyze the occurrence of biological agents attacks from the point of view of forensic odontology with the use of oral microbiome as a biomarker. All relevant information from electronic sources were systematically reviewed to identify papers that published between 2008-2018. The data collection was done by studying and analyzing reputable international journals as well as current literature related to oral microbiome, bioterrorism and forensic odontology. RCTs (randomized control trials) and other study designs such as non-randomized trials, cohort studies, and case series were considered in order to give relevant information.

### Forensic Dentistry and Bioterrorism

Forensic odontology is a branch of dentistry that focuses on the law regarding the examination or discovery and identification of evidence relating to dentition.<sup>7</sup> The use of a dental identification card can assist in the gathering of data for epidemiological purposes that gives data about condition of the mouth. Such data can be used as a basis for policy-making step to prevention, including prevention to bioterrorism area.<sup>1</sup>

Bioterrorism is an act of terror by using biological agents to cause unrest in society resulting in economic, social and political instability. Various chemical and infectious

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agents have been used as a tool to inflict terror since more than 1 century ago.<sup>2</sup>

### Anthrax and Smallpox

Anthrax and Smallpox are agents that commonly used for bioterrorism activities. Anthrax can now be detected by PCR-based rapid tests. Smallpox cases happen again after routine immunization stopped since the 1970s in America so that today many citizens are vulnerable to smallpox attacks. The deaths from smallpox in Asia reached 30% during the epidemic. In the past, the Soviet Union Biological Warfare Program produced smallpox agents, anthrax and other pathogens that can now be attained by terrorists. The various steps used as a way to carry out bioterrorism is contaminating the food and water sources that people consume, administering infectious agents to animals and insects, or dispersing aerosols containing biological agents in the air so as to infect many people.<sup>8</sup> Oral manifestations from bioterrorism agents can become consideration for forensic odontologists in conducting analysis and identification. Anthrax as bioterrorism agent causes hoarseness, macula and oral ulcer.<sup>9</sup> *Bacillus anthracis* can be isolated from cutaneous lesions, respiratory tract, gastrointestinal tract, cerebrospinal fluid and blood cultures.<sup>10</sup>

### Categories of Biological Agents

The US Centers for Disease Control and Prevention (CDS) divides the biological agents that can be used for bioterrorism activities into three categories:

1. Category A: biological agents that easily spread between persons, causing high mortality rates and endangering the public interest, causing public panic and social damage and requiring accurate government handling
2. Category B: biological agents that are fairly easy to control, cause low mortality rates and still require special attention from the government
3. Category C: Biological agents that can still be further developed to become future biological weapons and potential cause high mortality rates and create great social impacts in the future.<sup>11</sup>

### Results

From this systematic review, with the keywords, oral microbiota, bioterrorism and forensic, 130 potentially relevant studies from 2000 until 2017 were identified using Pubmed-NCBI, and 17 studies fulfilled the inclusion criteria for this review. The research studies were English peer-reviewed research report and the abstract was used for data extraction. The major biological agents that are possible to be used as bioweapons according to CDC 2013 were shown in table 1.

Groups	Disease	Agents	
A	Anthrax	<i>Bacillus anthracis</i>	
	Botulism	<i>Clostridium botulinum</i> toxin	
	Plague	<i>Yersinia pestis</i>	
	Smallpox	<i>Variola major</i>	
	Viral hemorrhagic fevers	<i>Viroviruses and Arenaviruses</i>	
	Tularemia	<i>Francisella tularensis</i>	
	B	Brucellosis	<i>Brucella spp.</i>
		Epsilon toxin	<i>Clostridium perfringens</i>
		Food safety threats	<i>Salmonella spp, E.coli O157;H7, Shigella</i>
		Glanders	<i>Burkholderia mallei</i>
Melioidosis		<i>Burkholderia pseudomallei</i>	
Psittacosis		<i>Chlamydia psittaci</i>	
Q Fever		<i>Coxiella burnetti</i>	
Ricin toxin		<i>Ricinus communis</i>	
Staphylococcal enterotoxin B		<i>Staphylococcus spp.</i>	
Typhus Fever		<i>Rickettsia prowazekii</i>	
C	Viral encephalitis	<i>Alphaviruses</i>	
	Water safety threats	<i>Vibrio cholerae, Cryptosporidium parvum</i>	
	Emerging infectious diseases	<i>Nipahvirus and Hantavirus</i>	

**Table 1.** The major biological agents that are possible to be used as bioweapons according to CDC 2013.

## Discussion

The existence of oral microbiome was first reported by Antony Van Leeuwenhoek at the end of 1670 to the British Royal Society. The report states that on the surface of a person's teeth, there are a variety of different microbes in each individual. The microbes are known to be associated with the diseases found in the oral cavity.<sup>12</sup> There are three types of toxins produced by bacteria. Type I toxin is a superantigen (SAGs) that produced by streptococcus aureus and streptococcus pyogenes. Type II toxins such as hemolysin and phospholipase could destroy host cell membranes and interfere with host defence processes within cells. Type III toxin, also known as A/B toxin due to its binary structure, disrupts the host cell's defences to allow dissemination to distant organs. B Component of this toxin binds to the host cell surface, while A component has an enzymatic activity to damage the cell. Some deadly toxins include shiga toxin, cholera toxin, and deadly anthrax toxin are including in the Type III family of toxins.<sup>13</sup> The presence of gingival lesions also alters the balance of normal flora so the immune cells from capillary blood vessels interact with existing microorganisms.<sup>14</sup> Besides in the oral cavity, the presence of microbiota can also be found in saliva. Saliva is a completion fluid that contains various components of the mucosal surface, crevicular gingival and tooth surfaces. Saliva also contains microorganisms that colonize within the mouth and contain exogenous substances. Saliva serves to maintain oral hygiene, helps the process of dental mineralization, helps the process of wound healing, neutralize harmful food components, affect the microbiota in the oral cavity, maintain the balance of microbiota in the oral cavity as well as useful for the lubrication and hydration of the oral mucosa.<sup>15</sup>

Various salivary components are useful for examination, including forensic examination. Proteins derived from the blood can enter into the saliva in relatively small amounts, passing through the narrow gap between the epithelial cells by diffusion. As a result, the mean serum IgG concentration of 11 mg/mL is also present in saliva with a concentration of 0.016 mg/mL. The least amount of IgG present in the saliva is effective enough to be used to detect the presence of a virus.<sup>16</sup> DNA extraction of the tooth can be done by opening the access to the pulp

by perforation in the occlusal, cervical perforation and cervical area cutting. Tooth pulp contains DNA because there are fibroblasts, macrophages, and lymphocytes. The pulp areas used as sources of DNA extraction can be useful for obtaining mitochondrial DNA (MtDNA).<sup>17</sup>

## Conclusions

In conclusion, the review showed that the diverse biomarkers originating from the oral cavity are useful for the purposes of examination by the expert in the field of forensic odontology. Bioterrorism requires proper treatment because the incidence is unpredictable. Understanding the nature of catastrophic consequences of bioterrorism is required by experts in the field of odontology forensics to perform its functions in helping to identify and to analyze cases caused by exposure to biological agents.

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## Declaration of Interest

The authors report no conflict of interest.

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