Contamination of honey products by *Clostridium botulinum* spores and fungi along with their effects on human health

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ABSTRACT

Bee products, such as honey, are widely consumed as food and medicine. Because of its sticky nature, honey does absorb bacterial spores from dust or bee activity, and their contamination may carry serious health hazards. Databases searched to obtain articles included “Google Scholar”, “SID”, “Scopus”, “PubMed”, “Science Direct”, and “ISI”. Keywords used in this study included *Clostridium botulinum*, *Honey*, *Bees*, and *Fungi* in their titles. This study focused on published articles from 2016 to 2022. Result showed that this product may contain a great variety of bacteria and particularly, fungi that eventually entered the food chain at an early stage (e.g., via pollen). The ranges of samples with *C. botulinum*, yeast and mould infections were 0.5% - 68%, 15.78% - 100% and 17.22 - 100%, respectively. Overall, the amount of honey contaminated with *Cl. botulinum* spores, yeast, and molds in some of the samples that were evaluated was nil, however certain samples from the Lithuanian Kazakh, Turkey, and Brazil regions exhibited varying levels of contamination.

Keywords: *Clostridium botulinum*, *Honey*, *Bees*, *Fungi*.

Article type: Review Article.

INTRODUCTION

Honey is a natural sweet substance produced by bees from nectar, blossoms or from the secretion of parts of plants or their excretions. Primarily, honey consists of sugar and water, with sugar making up 95–99% of the dry matter (Manouchehri et al. 2021). Due to its consumption qualities, this product is very well liked by people, including kids. In addition, honey's anti-inflammatory and antibacterial properties and its usefulness in boosting immunity, wound healing, gastrointestinal disorders, skin illnesses, and even cancer are well-known (Pasias et al. 2018). A
total of 1,540,242 tons of honey was produced in 2010, according to FAOSTAT. China (398,000 t), Turkey (81,115 t), the United States (79,789 t), Ukraine (70,900 t), and Argentina were the top five producers (59,000 t). However, Europe produces the most honey (31.4% of global production). Asia, the Americas, Africa, and Oceania each produced 29.3, 26.0, 10.66, and 2.7%, respectively (Grabowski et al. 2017). Foods can be contaminated with chemical, microbial and harmful factors for various reasons (Janbakhsh et al. 2018; Heidari et al. 2018; Pirhadi et al. 2021; Ragimov et al. 2021; Soltanbeigi et al. 2021; Grenda et al. 2009). Since the infant's intestinal microflora is still developing, after the baby consumes contaminated honey, the health benefits of honey have been studied in recent years. Though, the harmful effects of undesirable C. botulinum and Bacillus (Finola et al. 2007). C. botulinum is a gram-positive, anaerobic bacterium, existing naturally in soil, dust, and agricultural products in the form of spores (Wojtacka et al. 2016). Several studies have been conducted on the abundance of C. botulinum spores in honey produced in different countries of the world such as Iran (Vahdani et al. 2009), Brazil (Rall et al. 2003), and Japan (Ebrahimi et al. 2019). In Europe, honey consumption was linked to 59.2% of infant botulism cases, according to Aureli et al. (2009). Since the infant's intestinal microflora is still developing, after the baby consumes contaminated honey, C. botulinum spores can grow and produce BoNTs. The symptoms can range widely. There are often digestive disorders present. Multiple attacks are possible, and the neurological manifestation of the attacks is a descending flaccid paralysis (Aureli et al. 2009). Fungal contamination of honey is another significant pollutant, and Iran's national rules have virtually set limits for this contamination. When honey's moisture content rises above 171 or 200 g kg$^{-1}$, the fermentation process begins. Osmophilic yeasts may develop in honey if the relative humidity exceeds this threshold (Tosi et al. 2004). These moulds may be pathogenic and present a health risk to those who are vulnerable. However, the primary threat stems from the creation of mycotoxins, particularly aflatoxins, which exhibit a number of negative effects on human health, including the development of cancer, mutagenesis, and malformations (Hasanvand et al. 2019). Therefore, this study aims to review the contamination of honey with C. botulinum spores and fungi and also its harmful effects on human health.

MATERIALS AND METHODS
Databases searched to obtain articles included “Google Scholar”, “SID”, “Scopus”, “PubMed”, “Science Direct”, and “ISI”. Keywords used in this study included Clostridium botulinum, Honey, Bees and fungi in their titles. This study focused on published articles from 2016 through 2022.

RESULTS AND DISCUSSION
The health benefits of honey have been studied in recent years. Though, the harmful effects of undesirable C. botulinum spores from the consumption of honey on the health of humans is a question that cannot be ignored considering its therapeutic benefits. The present study focused on published articles from 2016 through 2022 (Table 1). The sample size in reviewed studies ranged from 1 to 300. The levels of honey contamination by C. botulinum in Polish, Kazakh, Brazil, Serbia and Turkey were relatively low. However, the occurrence in samples from Lithuania (60%) was estimated to be significantly higher. The levels of mold contamination in Brazil and Serbia were 34 and 15.78 respectively.

Honey samples from the Lithuanian, Kazakh, Turkey, Brazil, Polish, Argentina, Italy, Iran, and Nigeria displayed varying levels of contamination by yeast. Honey samples from Tuzla Canton and Africa were safe from C. botulinum spores, yeast, and molds. The presence of C. botulinum spores in honey from various parts of the world has been the subject of numerous studies. C. botulinum spores are widely dispersed in the gastrointestinal tracts of people, animals, and soil. Bees carrying the C. botulinum spores from soil into hive may cause beeswax to accumulate or honey to become directly infected during extraction (Nevas et al. 2006).

Honey consumption has been linked to cases of botulism in Brazil (Pinheiro et al. 2018), Lithuania (Wojtacka et al. 2017), Poland (Wojtacka et al. 2016), and Polish (Grenda et al. 2018), and honey is a source of the C. botulinum spores type A and B.
<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Number of samples</th>
<th>Infection rate (%) to Clostridium botulinum</th>
<th>Infection rate (%) to a type of toxin</th>
<th>Infection rate (%) to yeast</th>
<th>Infection rate (%) to moulds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serbia</td>
<td>2019</td>
<td>61</td>
<td>1.63</td>
<td></td>
<td></td>
<td></td>
<td>(Matović et al. 2019)</td>
</tr>
<tr>
<td>Poland</td>
<td>2016</td>
<td>102</td>
<td>21.56</td>
<td>A: 15.68</td>
<td>B: 2.94</td>
<td>E: 2.94</td>
<td>(Wojtacka et al. 2016)</td>
</tr>
<tr>
<td>Serbia</td>
<td>2018</td>
<td>19</td>
<td>0</td>
<td></td>
<td></td>
<td>E: 5.26</td>
<td>(Matović et al. 2018)</td>
</tr>
<tr>
<td>Turkey</td>
<td>2020</td>
<td>300</td>
<td>3.33</td>
<td></td>
<td></td>
<td></td>
<td>(Bayrakal et al. 2020)</td>
</tr>
<tr>
<td>Iran</td>
<td>2017</td>
<td>130</td>
<td></td>
<td></td>
<td>26.15</td>
<td></td>
<td>(Hajimohammadi et al. 2017)</td>
</tr>
<tr>
<td>Brazil</td>
<td>2020</td>
<td>67</td>
<td>34</td>
<td></td>
<td>83</td>
<td></td>
<td>(Galhardo et al. 2020)</td>
</tr>
<tr>
<td>Maldives</td>
<td>2022</td>
<td>34</td>
<td></td>
<td></td>
<td>35.5</td>
<td></td>
<td>(Naila et al. 2022)</td>
</tr>
<tr>
<td>Bosnia and Herzegovina</td>
<td>2022</td>
<td>33</td>
<td></td>
<td></td>
<td>24.24</td>
<td></td>
<td>(Landeka et al. 2022)</td>
</tr>
<tr>
<td>Tuzla Canton</td>
<td>2021</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Brčina et al. 2022)</td>
</tr>
<tr>
<td>Africa</td>
<td>2021</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Vosloo et al. 2021)</td>
</tr>
<tr>
<td>Polish</td>
<td>2018</td>
<td>34</td>
<td></td>
<td></td>
<td>34.38</td>
<td></td>
<td>(Kiš et al. 2018)</td>
</tr>
<tr>
<td>Argentina</td>
<td>2017</td>
<td>163</td>
<td></td>
<td></td>
<td>43.55</td>
<td></td>
<td>(Fernández et al. 2017)</td>
</tr>
<tr>
<td>Brazil</td>
<td>2018</td>
<td>35</td>
<td>2.85</td>
<td>C</td>
<td>25.71</td>
<td></td>
<td>(Pinheiro et al. 2018)</td>
</tr>
<tr>
<td>Lithuania</td>
<td>2017</td>
<td>50</td>
<td>60</td>
<td>A: 40</td>
<td>B: 14</td>
<td>E: 8</td>
<td>(Wojtacka et al. 2017)</td>
</tr>
<tr>
<td>Kazakh</td>
<td>2019</td>
<td>197</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td>(Maikanov et al. 2019)</td>
</tr>
<tr>
<td>Polish</td>
<td>2018</td>
<td>240</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>(Grenda et al. 2018)</td>
</tr>
<tr>
<td>Italy</td>
<td>2016</td>
<td>40</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td>(Nardoni et al. 2016)</td>
</tr>
<tr>
<td>Iran</td>
<td>2021</td>
<td>43</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td>(Kazeminia et al. 2021)</td>
</tr>
<tr>
<td>Niger</td>
<td>2018</td>
<td>5</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td>(Hocine et al. 2018)</td>
</tr>
<tr>
<td>Iran</td>
<td>2018</td>
<td>180</td>
<td></td>
<td></td>
<td>17.22</td>
<td></td>
<td>(Namini et al. 2108)</td>
</tr>
</tbody>
</table>

Pinheiro et al. (2018) reported the honey samples contamination (2.85%) with the *C. botulinum* spores type C in Brazil. Positive samples of *C. botulinum* were calculated in a study by Wojtacka et al. (2017) who reported *C. botulinum* type A, B, E, and F contamination in Lithuania honey samples at the levels of 40, 14, 8, and 6% respectively. None of the Iranian honey samples were contaminated with *C. botulinum* (Namini et al. 2018). Nevas et al. (2005) reported the average contamination of 26%, 10%, and 2% in honey samples from Denmark, Sweden,
and Norway. Group B was the most often identified serotype in this investigation. According to several studies, the \textit{C. botulinum} low incidence in European honey may be due to the bacterium’s strain. The type of strain of this bacterium, which destroys many spores after heating (30 min at 65 °C or 25 min at 80 °C; Nevas et al. 2005). Infant botulism was first identified in the 1970s and usually occurs in infants under one year of age when the \textit{C. botulinum} spores enter their digestive tract. A number of sources, including honey, have been reported for this bacterium. The results show that due to their exposure to dust, children who live in rural areas are more likely to be infected. It seems that due to their immaturity and inadequate digestive tract microbial ecology, newborns are more likely to develop \textit{C. botulinum} colonization in their intestines (Ebrahimi et al. 2019). One of the contaminants in honey is fungus. Bees can contaminate the environment inside the hive as they transport pollen or nectar and airflow (Tosi et al. 2004). The sheet and wax pumice, especially those left over from past years, is another route of introducing spores into the beehive (Tosi et al. 2004). There are reports of different yeasts, \textit{Penicillium}, and \textit{Aspergillus flavus}. These molds may be pathogenic and offer a health concern to those who are susceptible (Hasanvand et al. 2019). \textit{Alternaria tenuissima} and \textit{Cladosporium cladosporioides} are frequently found in the intestines of honeybees, while the genus \textit{Paecilomyces} is an entomopathogenic fungus isolated from insects (Tosi et al. 2004; Hasanvand et al. 2019). Using medicinal plants as a rich source of antioxidants (Abbasi et al. 2020; Bahmani et al. 2020; Solati et al. 2021; Zarei et al. 2017) is recommended to reduce the load of honey contamination. \textit{Acremonium} and \textit{Aureobasidium} are saprophytes isolated from plants, soil, wood, and indoor air environments. The genera \textit{Botrytis} and \textit{Alternaria} are prevalent plant causing diseases on agricultural and forest plants (Tosi et al. 2004). If the environmental parameters, such as those related to temperature, humidity, etc., are good, these spores develop and multiply (Tosi et al. 2004).

CONCLUSION
Numerous studies demonstrate that honey produced in many nations across the world frequently contains \textit{C. botulinum} spores, yeast, and mould. Honey absorbs bacterial spores from dust or bee activity, and retains them for a long time as a result of its sticky nature and chemical composition. The hives should not be exposed to dust as much as possible because dirt is where \textit{Clostridium} microbes originated. In addition, honey should be packed in 100% sanitary containers.

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