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

Yahya Ebrahimi[1], Andrés Alexis Ramírez-Coronel[2], Aiman Mohammed Baqir Al-Dhalimy[3], Raed H. C. Alfilm[4], Mohammed Al-Hassan[5], Rasha Fadhel Obaid[6], Ameer A. Alameri[7], Fatemeh Rastiani[8], Yousef Khaledian[9], Samira Shokri[10]*

1. Department of Cardiology, School of Medicine, Shahid Madani Hospital, Lorestan University of Medical Sciences, Khorramabad, Iran
2. Catholic University of Cuenca, Azogues campus, Ecuador, University of Palermo, Buenos Aires, Argentina & National University of Education, Azogues, Ecuador; CES University, Colombia
3. Department of Nursing, Altoosi University College, Najaf, Iraq
4. Refrigeration & Air-conditioning Technical Engineering Department, College of Technical Engineering, The Islamic University, Najaf, Iraq
5. Experiential Learning Center Lead, Department of Nursing, University of Calgary in Qatar, Doha, Qatar
6. Department of Biomedical Engineering, Al-Mustaqbal University College, Babylon, Iraq
7. Department of Chemistry, College of Science, University of Babylon, Babylon, Iraq
8. Department of Food Hygiene and Safety, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
9. Department of Food Hygiene and Quality Control, Faculty of Veterinary, Shahrekord University, Shahrekord, Iran
10. Department of Environmental Health, Food Safety Division, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran

** Corresponding author's Email: shokrisamira22@yahoo.com*

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, 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

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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*. 2022). One of these foods is honey. The microorganisms in honey are those that can endure a high sugar concentration, acidity, and the presence of antimicrobial compounds. Pollen, honeybee digestive tracts, dirt, dust, air, and flowers are considered to be the main causes of microbial contamination. Humans, tools, containers, the wind, dust, etc. are secondary causes of microbial contamination in honey (Finola *et al*. 2007). A high concentration of sugars, low water activity, high osmotic pressure, low pH, and formation of hydrogen peroxide are not favourable conditions for survival and growth of vegetative microflora (Ebrahimi *et al*. 2019). However, it is still feasible for anaerobic spore-forming bacteria to exist under such conditions, including the spores of fungi and the spore form of bacteria such as *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 1. The proportion of honey samples from various regions contaminated with yeast, *Clostridium botulinum*, and moulds

Country	Year	Number of samples	Infection rate (%) to *Clostridium botulinum*	Infection rate (%) to a type of toxin	Infection rate (%) to yeast	Infection rate (%) to moulds	References
	2020	19		0			(Koike *et al*. 2020)
Serbia	2019	61	1.63				(Matović *et al*. 2019)
Poland	2016	102	21.56	A -15.68 B- 2.94 E- 2.94			(Wojtacka *et al*. 2016)
Serbia	2018	19		E- 5.26	15.78		(Matović *et al*. 2018)
Turkey	2020	300	3.33				(Bayrakal *et al*. 2020)
Iran	2017	130				26.15	(Hajimohammadi *et al*. 2017)
Brazil	2020	67			34	83	(Galhardo *et al*. 2020)
Maldives	2022	34				35.5	(Naila *et al*. 2022)
Bosnia and Herzegovina	2022	33				24.24	(Landeka *et al*. 2022)
Tuzla Canton	2021	6					(Brčina *et al*. 2022)
Africa	2021	1					(Vosloo *et al*. 2021)
Polish	2018	34				34.38	(Kiš *et al*. 2018)
Argentina	2017	163				43.55	(Fernández *et al*. 2017)
Brazil	2018	35	2.85	C		25.71	(Pinheiro *et al*. 2018)
Lithuania	2017	50	60	A -40 B -14 E- 8 F- 6			(Wojtacka *et al*. 2017)
Kazakh	2019	197	0.5				(Maikanov *et al*. 2019)
Polish	2018	240	2				(Grenda *et al*. 2018)
Italy	2016	40				100	(Nardoni *et al*. 2016)
Iran	2021	43				100	(Kazeminia *et al*. 2021)
Niger	2018	5				100	(Hocine *et al*. 2018)
Iran	2018	180				17.22	(Namini *et al*. 2108)

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 *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 ºC or 25 min at 80 ºC; Nevas *et al*. 2005). Infant botulism was first identified in the 1970s and usually occurs in infants under one year of age when the *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 *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, *Penicilliums*, and *Aspergillus flavus*. These molds may be pathogenic and offer a health concern to those who are susceptible (Hasanvand *et al.* 2019). *Alternaria tenuissima* and *Cladosporium cladosporioides* are frequently found in the intestines of honeybees, while the genus *Paecylomyces* 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. *Acremonium* and *Aureobasidium* are saprophytes isolated from plants, soil, wood, and indoor air environments. The genera *Botrytis* and *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 *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 *Clostridium* microbes originated. In addition, honey should be packed in 100% sanitary containers.

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