

Foodborne botulism: A brief review of cases transmitted by cheese products (Review)

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Abstract. Food safety constitutes a basic priority for public health. Foodborne botulism occurs worldwide; it is an acute paralytic disease caused by the consumption of food containing the botulinum toxin. Growing consumer demand for cheese products could result in increased exposure of the population to this toxin, and thus the risk of foodborne botulism. The majority of cases of botulism caused by dairy products are related to cheese products specifically. Epidemic outbreaks and isolated cases have been reported over time. Domestically canned foods are still among the primary causes of the disease. Cheese products are not regularly involved in botulism incidents; it is however, necessary to take control measures for manufacturing and domestic preparation due to the high risk of occurrence of this particular disease. The aim of this review is to discuss foodborne botulism caused by cheese products, providing a brief epidemiological history, and to examine certain control measures that should be taken throughout the production process to better protect public health.

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1. Introduction

Food safety constitutes a key priority for public health. Unsafe foodstuffs containing harmful micro-organisms (bacteria, viruses, parasites etc.) or chemical compounds, may cause various illnesses and even death (1). Unsafe food has always been considered a major issue for public health throughout history. It is estimated, that the majority of foodborne diseases that cost the lives of ~1.8 million individuals each year occur as a result of contaminated water or food (2).

The diseases caused by bacterial pathogens can be classified into two types: Foodborne infection and foodborne intoxication (3). Foodborne infections result from the development of bacteria in the human body (4). Bacteria are transmitted through contaminated food, multiply within the intestines, and excrete toxins that affect the proper function of the tissues or other organs. Foodborne intoxication is an illness caused by the ingestion of food contaminated by microbial toxins even though the microorganisms responsible for the production of toxins may have already been destroyed (5). Therefore, intoxication is caused by toxins produced by bacteria in the food (3,4,6). The World Health Organization defines foodborne intoxication as an ‘*illness caused by the ingestion of toxins produced by bacteria in the food as a natural by-product of their metabolic processes*’ (7). Related terms regarding toxins include bacterial toxins, phytotoxins produced by algae, mycotoxins produced by fungi, and poisons produced by animals (8).

Clostridium botulinum (*Cl. botulinum*) is a bacterium that produces a dangerous toxin that has been reported to be one of the most lethal substances known to man (9-11). Foodborne botulism constitutes an acute paralytic disease caused by a toxin produced by the bacterium. The disease may be caused by ingesting food that contains this toxin or due to the development of spores within the intestines of young

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children (3,12,13). *Clostridium* took the name *botulinum* from the Latin '*botulus*' which means lunch meat, salami, sausage, etc. The corresponding ancient Greek word is '*Ἀλλᾶς*' from which the word allantiasis comes (14).

Growing consumer demand for traditional food products, such as cheese products, poses significant challenges for the food industry (15). Greece has a long tradition in the production and consumption of cheese products. It is ranked among the countries with the highest consumption of cheese worldwide (16). Cheeses are widely consumed all over the world and they have often been involved in outbreaks of foodborne illnesses (17). Consumers consider local dairy products, such as cheese, as a cost-effective solution, which may occasionally expose the population to the risk of foodborne botulism (15).

The risk of *Cl. botulinum* in cheese products is still observed today. Recently, a well-known food company in the United Kingdom and Ireland recalled its products (cheese spread in tubes) due to possible infection with *Cl. botulinum*. Company officials report that they were in contact with local public health inspectors and the UK Food Standard Agency (FSA) (18). The UK FSA issued a press release on 16th of June 2020 informing consumers about the recall of products and the risk of consuming related products (19). However, the risk of foodborne botulism associated with the consumption of dairy products has not been discussed in detail (20). The aim of this review is to highlight the occurrence of foodborne botulism caused by cheese products and their significance to public health.

2. Foodborne botulism: An overview

Human botulism can occur in six different forms: Foodborne botulism caused by the ingestion of food contaminated with bacterial toxins, wound botulism caused by *Cl. botulinum* colonization of a wound and the production of toxins in the wound, infant botulism caused by *Cl. botulinum* intestinal colonization and the production of toxins, and unclassified botulism that is even rarer and is characterized by intestinal colonization and the production of toxins in the intestine of adults (21). Iatrogenic botulism due to improper neurotoxin administration in the systemic circulation instead of the predefined therapeutic goal and inhalation botulism caused by toxin inhalation in the form of aerosol, have also been observed (22).

Foodborne botulism is an acute and potentially fatal disease (11,23). Exotoxins of *Cl. botulinum* are proteins, and they are very toxic to the host (10). They are large polypeptides of similar structure and their effects manifest as neuromuscular blockade that usually leads to flaccid paralysis (24). Botulinum toxin is one of the most potent neurotoxins. It is a 150 kDa polypeptide [consisting of a 100-kDa heavy chain joined to a 50-kDa light chain via a disulfide bond] with three separate domains: N, middle and C. The C domain binds to the pre-synaptic membrane, the N domain is a specific polypeptidase and the middle domain facilitates the light chain into the cytosol. The most remarkable aspect is the affinity of this substance for the most active synapses (25,26).

The mechanism of action relies on blocking acetylcholine at the neuromuscular junction, which blocks the transmission of the nervous impulse resulting in secondary flaccid paralysis (25,27). The average lethal dose (LD₅₀) is 1 ng of

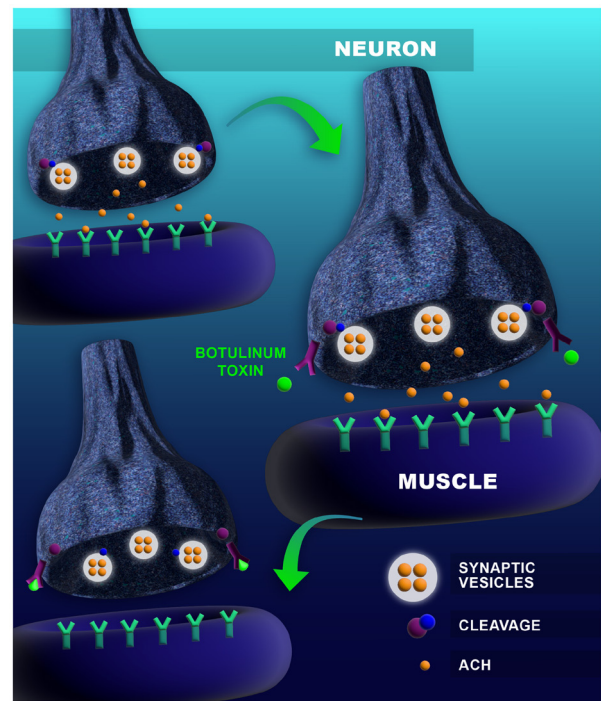


Figure 1. Representation of the mechanism of action of botulin toxin. Botulin toxin binds to the presynaptic membrane and cleaves the proteins blocking the synaptic vesicle at the plasma membrane preventing Ach release into the synaptic cleft. Adapted from Proverbio *et al* (69). Ach, acetylcholine.

toxin per a Kg of body weight (22); it is, however, relatively rare (11,13,28). This intoxication is caused by the ingestion of *Cl. botulinum* neurotoxins that have already been formed in the contaminated food (9). Foodborne botulism is not spread from one individual to another (11). The mechanism of action of the toxin is represented in Fig. 1.

Visual difficulty (blurred or double vision), dysphagia and dry mouth are usually the first symptoms. These symptoms can also lead to symmetrical flaccid paralysis (3,23). The original symptoms may also involve nausea, vomiting, abdominal pain and diarrhea (7). Fever is not observed unless infectious complications occur (3,7). After the onset of neurological symptoms, constipation is common. Symptoms usually occur within 12-36 h after exposure to toxins (from 6 h to 8 days) (5,7,23). Extensive paralysis of respiratory muscles may lead to respiratory insufficiency and death within 3 days if no supportive therapy is received (7,23). Death may occur within 20 h to 1 week following the consumption of suspicious food (12). The removal of contaminated food from the intestine either by subclasses or by inducing vomiting is considered critical. Ventilatory support may be deemed necessary for 2-8 weeks, although cases of ventilatory support for a longer period have been reported (22). The most typical signs and symptoms of foodborne botulism are described in Table I.

Treatment in addition to supportive care, intubation and mechanical ventilatory support when deemed necessary may include the administration of a botulinum antitoxin. Early administration of antitoxin may mitigate the extent and severity of paralysis, and in some cases, may prevent respiratory paralysis and in other cases, reduce the duration of mechanical support and hospitalization in intensive care (29,30). The administration of antibiotics is not indicated

Table I. Typical signs and symptoms of foodborne botulism with toxin production^a.

Medical history	Negative for infectious diseases
First clinical evaluation	Normal mental status, afebrile Gastrointestinal manifestations Xerostomia/sore throat and/or dysphagia Weakness Normal cardiac activity (rarely bradycardia) Anxiety
Physical examination	Symmetric descending neurological alterations Symmetric oculobulbar signs

^aAdapted from Lonati *et al* (70).

in foodborne botulism (7,22). Vaccination with a toxoid form of botulinum toxin applies to all types of botulism (29,31).

Recognizing an agent that causes the disease is the necessary condition for the choice of appropriate therapy. PCR is the primary method for the confirmation of *Cl. botulinum* presence or absence (32,33). Another opportunity for detecting botulism is to detect the residual levels of the toxin in the blood plasma or blood. Though direct assay of botulinum toxin appears to be an ideal means of diagnosis of botulism, it is not simple to achieve in reality. Any method relying on direct detection of botulinum toxin has very low limits of detection. A number of immunochemical tests such as ELISA (34), chromatography, mass spectrometry (35) and single-step test strips (lateral flow immunochromatographic assays) (36) are suitable analytical methods for botulinum toxin detection.

3. *Clostridium botulinum* as a pathogen

Clostridium botulinum is an anaerobic Gram-positive spore-forming bacillus (10). Bacterial spores are heat-resistant and can survive in foods even after intense heat-treatment. According to the antigenic specificity of the toxin produced by each bacterial strain seven types (A-G) have been identified (*Clostridium Argentinense*) (37). Types A, B, E and F are involved in human botulism. Types C and D are involved in the majority of botulism events in animals (29,38). Some associated *Clostridium* species, such as *Clostridium baratii* and *Clostridium butyricum*, can also produce botulinum toxins (3,23,39). All toxins cause a clinically similar syndrome (3).

The strains that produce the toxins can be divided into proteolytic and nonproteolytic strains (40). The optimum temperature for toxin development and production by proteolytic strains is 35°C, and for nonproteolytic it is 26-28°C. It is worth noting that nonproteolytic strains B, E and F can produce toxins at cooler temperatures (3-4°C) (40,41). Its bacterial spores are widely spread within the environment and can be traced in the dust, on the ground, in the water that has not undergone treatment and in the peptic system of animals and fishes (5,37,42). They can be found in cultivated and forest

lands, river, lake and coastal sediments, as well as in gills and the internal organs of crabs and other shellfishes (9).

Usually, the spores survive cooking and food preparation. The combination of environmental conditions however, that allow for spore formation (anaerobic environment, non-acidic pH and low salt and sugar concentration) are rarely observed in food, which explains the low number of foodborne botulism cases (29,39). The marginal conditions for bacterial growth are indicated in Table II.

4. *Clostridium botulinum* in foods

Any food that facilitates spore germination and botulinum toxin production allows spores to survive through food preparation, if not subjected to heat treatment before consumption (43). Almost every type of food lacking sufficient acidity (pH >4.6) can lead to the development and production of bacterial toxins. The toxins are typically sensitive and can be destroyed by heating to 85°C for 5 min (5). Spores are destroyed in conditions of wet sterilization at 120°C for 5 min (9). Spore germination and toxin production are achieved when foods are exposed to conditions of an anaerobic environment, pH >4.6, low salt and sugar concentration and temperatures from 4-12°C (21).

Most cases of foodborne botulism are related to the consumption of canned foods (such as tuna fish), homemade foods or containerized food available on the market (such as canned vegetables) (15).

5. Epidemiological data on foodborne botulism

Foodborne botulism occurs worldwide (37). It is a disease with a high mortality rate. Before 1950, 60% of botulinum cases in America resulted in death. Later the mortality rate was reduced to <10%, due to the production of antitoxins and the provision of intensive supportive care (44). More recent data in the United States (2016) refer to 205 confirmed cases of botulinum intoxication, 14% of which were associated with foodborne botulism (45). The majority of botulinum cases that have been reported in the United States are related to insufficiently processed canned foods, home-canned foods and occasionally products that are available on the market (37).

In Europe, the botulinum rate is generally low with ~200 cases per year (0.03 cases per 100,000 persons). The highest rate of cases over the last 10 years has been reported in Poland and Lithuania (13). Recent cases of foodborne botulism have been reported in 2015 in France and Slovakia. In France 3 botulinum cases were reported, involving the consumption of Bolognese sauce at a restaurant and one case involved *Cl. baratii* in minced meat. In Slovakia botulinum toxin (type A) was detected in three hummus products (46). In Greece botulism constitutes one of the rarest diseases for which declaration is mandatory, and the statistical data from the National Agency for Public Health (EOΔΥ) did not reveal any cases of food-borne botulism in the recent years (47).

6. Cheese making data

Codex Alimentarius defines cheese as the fresh or mature product obtained by draining following coagulation of the

Table II. Marginal growth conditions for *Cl. botulinum*^a.

Growth conditions	Proteolytic strains	Nonproteolytic strains
Minimum water activity value (AW)	0.935	0.970
Minimum pH value	4.6	5.0
Maximum pH value	9.0	9.0
Maximum NaCl concentration	10%	5%
Minimum temperature value	10-12°C	3.3°C
Maximum temperature value	50°C	45°C

^aAdapted from Poggas (40).

whole, partly skimmed or nonfat milk or buttermilk, or a mixture of some or all of these products (48). The Greek Food and Drinks Code defines ripened cheeses that are produced from milk, as products obtained from curd-ripening (maturation) free of whey-to the desired degree each time-that have been prepared by using rennet (rennet effect) or other enzymes that function accordingly in milk, partly skimmed milk or a mixture of those, and a mixture of those with milk cream (49,50).

Cheese products may contain several types of microorganisms at all stages from manufacturing to the consumption thereof. These may be lactic-acid bacteria (*Lactococcus*, *Streptococcus*, *Lactobacillus*) developed during the production of cheese and used at the stages of fermentation and ripening (maturation), microorganisms that may enter milk from environmental contamination (*Micrococcus*, *Streptococcus*, *Proteus*, *Pseudomonas*, *Bacillus* etc.), yeasts and moulds. The microbial population may undergo constant qualitative and quantitative changes depending on several factors, such as water activity, microbial competition, degree of acidity, temperature and salt concentration. Under normal conditions of maturation, the initial microbial load steadily decreases. If the milk used for cheese-making is not pasteurized, the rich microbial flora can be transmitted to the product. If the changes in the microbial flora at the ripening stage for cheeses that are ripened are not constant and they do not ensure cheese sanitation, then the consumption of this cheese may prove dangerous to the consumer (50). Only high-temperature pasteurization can neutralize types a and b botulinum toxin (51).

A low number of botulism cases related to dairy products, produced domestically or at food-businesses, have been reported. Milk contamination may originate from the manufacturing environment or while adding ingredients that may transmit *Cl. botulinum* spores. The maintenance conditions of dairy products also constitutes a key factor involved in the possible development of vegetative forms (of microorganisms) and the production of toxins (51). Cheese and other dairy products are the cause of botulism, (<1% in the United States) (21,52). The rare presence of botulinum spores in dairy products has been observed. In a study relating to the presence of *Cl. botulinum* spores, involving a limited number of cheese samples, such as Edam and Cheddar, as well as spreadable cheese (40 and 10 samples, respectively) the results were all negative (53,54).

7. A brief history of foodborne botulism outbreaks transmitted by cheese products

The cases of botulism that have been reported (Table III) occurred in France and Switzerland following the consumption of ripened cheese, such as Brie, contaminated with *Cl. botulinum* toxin type B. The epidemiological investigation of these cases demonstrated the involvement of the straw on which the products were ripened (54). Cheese contamination from straw was investigated experimentally by Billon *et al* (55) in a research paper published in 1980. The storage of soft cheese on inoculated straw (1,000 spores' type B/cm²) resulted in the production of the toxin on cheese surfaces, but not in the cheese body. The toxin was found to be unstable and disappeared in the subsequent ripening stages.

An outbreak of botulism from toxin type A, involving a commercially available, spreadable onion cheese, was reported in Buenos Aires. Specific intrinsic parameters, such as water activity (aw=0.97) and pH value (5.6-6.1) of this product facilitated germination and production of *Cl. botulinum* toxins type A (54). An experimental study by Briozzo *et al* (56) indicated that substrates with similar pH values to those of spreadable cheeses facilitate the development of *Cl. botulinum* and the production of the toxin at a lower water activity value of water activity (aw)=0.965 and not at 0.949.

A rather rare outbreak of foodborne botulism cases was observed in 1993 in Georgia, United States, resulting from the consumption of cheese sauce at a restaurant, contaminated during handling. After the laboratory investigation of the suspicious food, it was found that the contaminated food was a canned cheese sauce. A laboratory investigation of two samples of containerized cheese from the same batch showed negative results. *Cl. botulinum* type A was detected in cultures obtained from the suspected can. Experimental inoculation studies for this particular cheese sauce demonstrated the development of botulinum toxin at an ambient temperature (22°C) but not at refrigeration temperatures (5°C) (57).

In 1996, eight cases of botulism from *Cl. botulinum* type A involving an industrially produced Mascarpone cheese (spreadable cheese) were reported in South Italy (Campania and Calabria). The patients consumed the suspicious cheese itself or as an additive to a tiramisu dessert, which had not been subjected to heat-treatment. The toxin was detected in samples from the remains of tiramisu that had been consumed by the patients and in mascarpone cheese samples that were

Table III. *Botulinum* cases from the consumption of cheese products between 1912-2019.

First author, year	Year	Type of <i>Cl. botulinum</i>	Product involved	Area	Outbreaks	Cases	Deaths	(Refs.)
Collins-Thompson and Wood, 1992	1912	-	Cottage cheese	California, USA	1	7	2	(54)
	1914	-	Neufchatel	California, USA	1	2	2	
	1914	B	Cottage cheese	New York, USA	1	3	3	
	1935	-	Curd cheese	California, USA	1	3	0	
	1939	A	Cottage	New York, USA	1	3	0	
	1951	B	Liederkrantz	California, USA	0	1	1	
	1973	B	Brie	Marseilles, France	1	22	-	
	1973	B	Brie	Lausanne, Switzerland	1	43	-	
	1974	A	Cheese spread	Buenos Aires, Argentina	1	6	3	
Townes <i>et al</i> , 1996	1993	A	Cheese sauce	Georgia, USA	1	8	1	(57)
Aureli <i>et al</i> , 1996	1996	A	Mascarpone	Calabria and Campania, Italy	4	8	1	(58)
Pourshafie <i>et al</i> , 1998	1997	A	Intangible	Northern Province, Iran	1	27	1	(60)
Rosen <i>et al</i> , 2018	2018	-	Nacho cheese sauce	California, USA	1	10	1	(71)
Kamaloddini and Kheradmand, 2021	2019	-	Local dairy cheese	Iran	0	1	-	(15)
Total					15	144	15	

collected from the points-of sale that supplied the cheese to the other patients (58). Experimental analysis on inoculation of bacterial spores in Mascarpone cheese demonstrated that non-compliance with temperature regulations throughout maintenance favors the production of this toxin (59).

Studies on botulism from *Cl. botulinum* type A caused by the consumption of cheese were also conducted in Iran. Toxin type A was detected in cheese and 37% of serum and stool samples from the patients and the bacterium were isolated from cultures for clinical samples and cheese (60). Furthermore a case of foodborne botulism was recently observed in Iran caused by the consumption of a local product (15).

8. Control measures

Control measures for the manufacturing of cheese products. The majority of botulism cases that have been observed in dairy products are related to cheese or cheese products (20). Cheese products are not regularly involved in botulism incidents; it is, however, necessary to take into consideration some inhibitory factors throughout the production process. European regulation allows for the production of some cheese products from unpasteurized milk (61). The intensity of heat-treatment throughout the production and cheese maintenance temperature constitute basic inhibitory factors (52). An apparent cause of botulism cases that has been observed is inadequate cooling. Unlike milk, which can be easily spoiled without cooling, cheese products can be spoiled without obvious macroscopic features and maintaining the refrigeration chain may not be deemed necessary by consumers or sales persons in order to ensure safety and quality of products (20). Water activity and salinity factors also inhibit microorganisms. The development of proteolytic strains of *Cl. botulinum* is inhibited at aw values <0.935, while for non-proteolytic strains of *Cl. botulinum* the corresponding minimum growth rate is 0.937. The salinity levels for the

production of cheese may also have a negative influence on the production of toxins, but it does not constitute a sufficient means for the prevention of toxin production alone (20,52). Various additives are extensively used in foods to control the risk of botulism. The main additives used are nitrates and nitrites (52). More specifically, it has been observed that the addition of nitrites to curd prevents the risk of botulinum toxin growth, therefore the Food and Agriculture Organization/World Health Organization allows the addition of nitrates (≤ 200 ppm) in the production of several types of cheese (50). European legislation allows the use of nitrites and nitrates in some dairy products (i.e., matured/ripened cheese and whey, as well as cheese products, so that maximum permitted levels are defined as amounts added during manufacture and not as amount of residue in the final product (62). The Greek Food Code limits the use of potassium nitrate [E251] and sodium nitrate in hard, semi-hard and semi-soft cheeses, as well as in dairy-based cheese analogues, and sets the maximum amount that may be added during manufacture to 150 mg/kg (49). Despite the advantages from their addition to products, their use has raised concerns since the 1970's due to the fact that nitrates/nitrites react with secondary amines resulting to the formation of N-nitrosamines, substances that exhibit carcinogenic activity (63,64).

Control measures regarding home production of food. Due to the emergence of several epidemic outbreaks, originating primarily from the consumption of home-made canned food (65), it is recommended to follow the directives below: Canned foods, including those produced for private domestic consumption must be subjected to inspection before use. Moreover, container swelling/bulging, damaged containers and foods with an abnormal flavor should not be consumed as they are indicative of bacterial growth that produces gases inside the package.

If they are intended for consumption, canned foods of low acidity must be heated to at least 80°C for 30 min. The products that

contain oils with garlic or herbs must be properly cooled during storage. For the safe preparation of canned products, and in order to ensure that all appropriate requirements of time, temperature and pressure are met to prevent the development of vegetative forms and spores of the microorganism; thus it is necessary to use pressure-cooking equipment. Spores can be inactivated by heat at extremely high temperatures of 116–121°C (66).

Although foodborne botulism is not regularly associated with canned products available at the market, their content must not be consumed if expired or the packaging is damaged. If the canned foods are produced for private domestic consumption, it is suggested to use nitrites in brine solution to reduce the growth of *Cl. botulinum*. During food storage the appropriate temperatures must be met. Cooked/baked foods must be kept warm (>57°C) and cooled (<5°C) in order to prevent germination of spores and the consecutive development of toxins (67). Conditions of personal and environmental hygiene must be preserved (68). For the best implementation of preventative measures, hygiene training of the food handlers regarding the home preparation of food and especially canned food is required to understand the conditions of destruction of the spores of the bacterium (14).

9. Conclusions

Foodborne botulism remains an important public health concern. Although rarely observed, it remains important for environmental health. Domestically canned foods are still among the primary causes of the manifestation of the disease. It is of prime importance that suspected foodborne botulism cases are immediately reported to public health services.

Cheese products are not regularly involved in the occurrence of botulism, due to high risk of the disease; however, it is necessary to take under consideration some bacterial growth inhibitors throughout the production process. The intensity of heat treatment during production, the regulation of acidity and the maintenance of cheese products' temperatures are considered key inhibitory factors. Water activity and salinity factors are inhibitory to the development of *Cl. botulinum*, but salinity is not a sufficient means for the prevention of toxin production alone. Moreover, the use of nitrates and nitrites prevents the risk of botulinum toxin formation throughout the preparation of several cheese products. Therefore, further research is required to evaluate the development of spore-forming bacteria in different temperatures regarding their composition, packaging and storage, to improve the safety of cheese products.

Finally, given the severity of cases of botulism, even one incident is considered as an emergency for public health as it may signal the beginning of an outbreak. Public health authorities at the national and international level must be informed in the case of suspected foodborne botulism to investigate possible causes, and to examine the need for further research and possible precautionary measures.

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EC conducted the review, conceived the subject of review based on the literature, collected the relevant data and wrote the manuscript. PP and DK drafted and reviewed the manuscript. IM and AM edited and reviewed the manuscript. ACL and NK provided scientific input. Data authentication is not applicable. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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