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Food microbial contamination - the main danger in the catering type food industry in Romania

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Abstract

An epidemiological study on food-borne pathogens, bacterial infection aetiology in Romanian catering and food industry was performed in the period 2003-2008. The scope of this study was the identification and evaluation of food-borne bacterial agents present in several catering products, involved in human infections and intoxications (poisonings). In our study most cases of human food-borne infections were caused by Staphylococcus aureus, Clostridium perfringens, Clostridium botulinum, and Salmonella spp. Disease outbreaks caused by Staphylococcus aureus were usually due to the enterotoxin A (SEA). The main serotype found in diseases caused by Clostridium perfringens was serotype A. The maximum incidence of C. botulinum infections was reported in 2007, when 110 cases were identified (5 deaths). The main serotypes of Salmonella spp. isolated were: S. Montevideo *(9150 cases), S.* München *(100 cases), S.* Enteridis *(75 cases), S.* Newport *(82 cases), S.* Oranienburg *(18 cases) and S.* Stanley *(50 cases).*

Keywords: food-borne infection, bacteriology, epidemiology, food industry, microbiology

Introduction

The patients with food-borne diseases exhibit a clinical symptomatology with explosive syndromes at digestive tract level. The disease has a short incubation period, produced by microbial agents or its metabolites, represented by microbial toxins [1].

More than 250 food-borne diseases have been described, and these had mainly bacterial or viral etiology. These diseases display many and different symptoms, so no single "syndrome" can describe the food-borne illnesses [2].

A food-borne illness is presumed if some of the following signs, in association with several epidemiological data, are reported [3]:

a) one or more persons are affected who eat the same food;
b) it has an acute onset in the initially healthy subject;
c) it has a superacute or acute course either to a rapid recovery or death;
d) the disease is not transmissible;

e) the symptoms are very well defined, being specific for the course of food-borne infections and are represented by:

- a superior digestive syndromes (nausea, vomiting);
- an inferior digestive syndromes (gastroenteritis, colon enteritis);
- a neurological syndromes (paralysis, neuromotor-disturbances).

According to a statistical analysis of epidemiological data, in food-borne infections the commonly recognized are bacteria are *Campylobacter*, *Salmonella*, and *E. coli* O157:H7 as well as a group of viruses like calicivirus, also known as the Norwalk or Norwalk-like viruses [2]. Although in food industry or catering the mainly identified organisms that are incriminated in food-borne pathology are: *Staphylococcus aureus, Clostridium perfringens, Clostridium botulinum* and *Salmonella spp*. [3].

Materials and Methods

The samples were received from different Romanian catering units from January 2003 to May 2008.

Isolation and identification methods were previously described [1, 4], and were in concordance with international regulations [5]. In diagnostic protocol both presumptive diagnostic tests and confirmation laboratory analyses were involved.

The epidemiological study consists in the evaluation of the outbreaks occurring during the whole study period for several catering food products and in the assessment of the *C. perfringens* serotype.

The statistical evaluation of food-borne outbreaks, disease cases and deaths was performed, for the period 2003-2008.

In most cases the bacteria contained in food are in a latent state. Their activation is more difficult in solid media, and therefore the product making the object of research was first inoculated in liquid media, in which not only the revitalization of germs, but also their multiplication was achieved. 1 ml of homogenized product and each dilution was inoculated in a test tube with tomato sauce, with liver or tomato sauce, with thioglycolate or tomato sauce – glucose – amidone – cisteline. The rapport of inoculums / culture-medium was 1/5-1/10. On blood solid medium *C. perfringens* created big colonies: shining, curved, and opaque colonies, sometimes with green pigments. The colonies surrounded by a beta-haemolysis area and a bigger area of incomplete haemolysis. On Willis-Hobbs agar red colonies were created (lactose fermentation) having around an opaque halo (lecithinase activity).

Botulinum toxin was detected by testing the toxicity on mice (the mouse neutralization test), ELISA or electrochemiluminescent (ECL) tests [7]. The toxicity testing was performed by intraperitoneally injection with 0.5 ml of undiluted and untreated liquid and 0.5 ml from each untreated sample of separate pairs of mice. Usually, in food-borne botulism the extracted food samples were prepared and tested for botulinum toxin. Growth on egg yolk agar produces colonies that exhibit a surface iridescence when they are examined under oblique light. This is due to lipase activity. *C. botulinum* is a spore-forming Gram-positive rod (13).

Isolation and identification of *Salmonella spp.* were performed according to ISO 6579/1995 standard. The isolation was made on selective agar (BS, HE, and XDL). Biochemical identification was performed with API 20E system. The confirmation was realized on TSI and LI agar. For confirmation, somatic serological test (O) and Spicer-Edwards flagella test were used. *Salmonella* was confirmed by commercial kits as being presumptive, when it was positive for somatic test (O) and Spicer-Edwards flagella test (9, 10).

Results and Discussions

According to the statistical analysis of the epidemiological reported in the food industry in Romania, most of the food-borne cases were caused by: 1. *Staphylococcus aureus*; 2. *Clostridium perfringens*; 3. *Clostridium botulinum*; 4. *Salmonella spp*.

The sources of food-born disease outbreaks were various, being represented by:

- foodstuffs favouring anaerobe conditions and moderate temperatures (room temperature) (*C. perfringens*);
- home made tins and semi-tins of meat, liver pate, fish, vegetables, without thermal treatment for toxins inactivation (*C. botulinum*);
- food prepared and rapidly cooled, but left at room temperature; then warmed inadequately (*S. aureus*);
- consumption of food contaminated by animal or bird feces, containing *Salmonella spp.* The contaminated foods were: beef, poultry, milk, eggs, and vegetables.

C. perfringens was identified as a Gram-positive bacillus, producing black colonies on TCS agar, reducing nitrates to nitrites, fermenting the lactose, releasing acid and gas, and liquefying gelatine after 48 hours [3].

The symptoms of *Clostridium perfringens* as alimentary intoxication (food poisoning) appear when the contaminated food contains 10^8 CFU/g bacteria. The symptoms consist in diarrhoea and abdominal pains, appearing in 8-16 hours after ingestion of the incriminated food, sometimes with fever and vomiting [1]. Recovery occurs usually without treatment within 1-4 days. The pathogenesis mechanism is hyper secretion at gut level caused by *C. perfringens* enterotoxin [5].

The contamination sources were the foods in anaerobe conditions and medium temperatures (room temperature). Food-borne diseases caused by *C. perfringens* were described mainly as restaurant problems, when the heat treatment (cooking) was not sufficient to destroy all endospores; or when food was cooled or warmed up again and the spores were germinated into vegetative forms [3].

C. perfringens has 5 serotypes; in our study, the most frequent was serotype A (Chart 1).

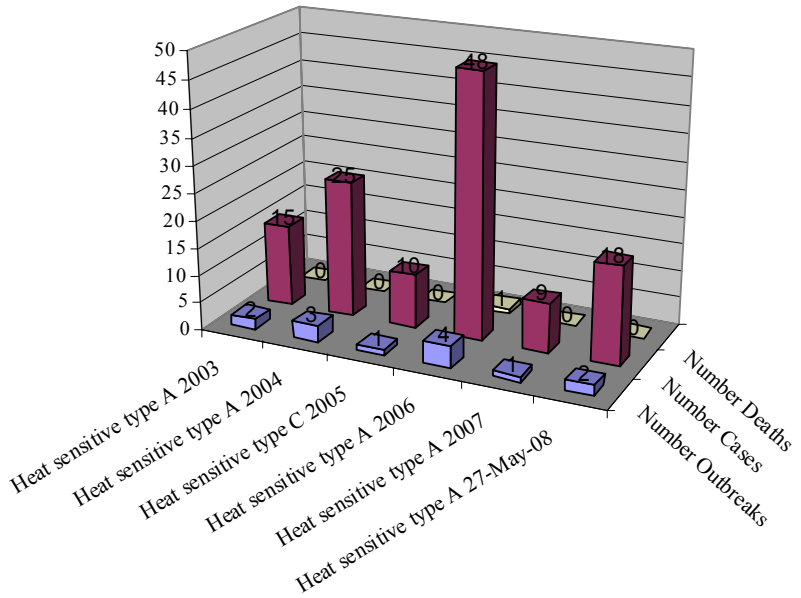

Chart no. 1. *C. perfringens* prevalence (absolute values) in Romania, during the period 2003 to 2008. (Source: *National Institute of Research and Development for Microbiology and Immunology "I. Cantacuzino"*)

In case of botulinum intoxications evaluated in this study the symptoms appeared usually in 12-72 hours after ingestion and in 3 cases after 6-10 days. Clinical features were: nausea, vomiting, tiredness, headaches, dry skin, mouth and trachea, constipation, no fever, paralysis of muscles, double vision or unclear vision, unclear speaking, and respiratory stop; and in 5 cases, death. The contaminated food products were: tins and semi-tins of meat, liver pate, fish, and vegetables. Outbreaks of *C. botulinum* toxicosis in Romania between the years 2003-2008 are detailed in chart 2.

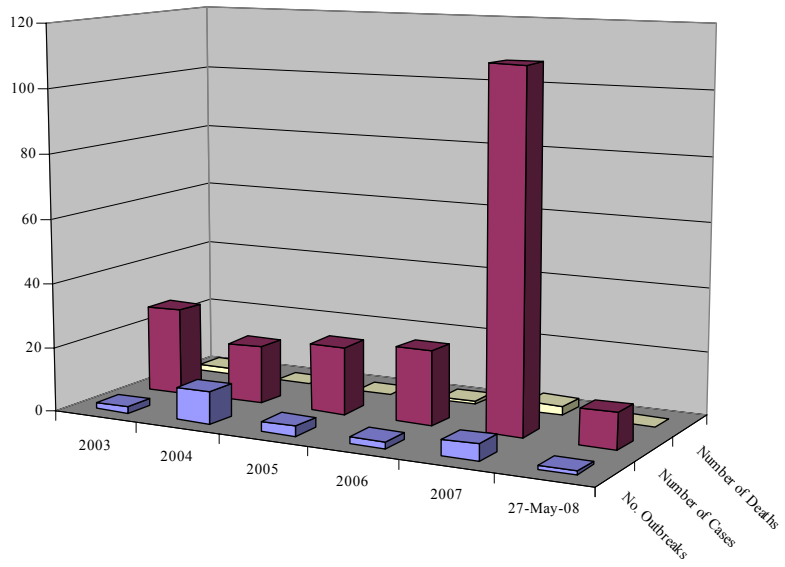

Chart no. 2. Botulism prevalence (absolute values) in Romania, from 2003 to 2008
(Source: *National Institute of Research and Development for Microbiology and Immunology "I. Cantacuzino"*)

Staphylococcal food poisoning is one of the major food-borne diseases reported in USA [8]. Nevertheless, the first contamination sources in human outbreaks are the staff, alimentary products being only on the second position. Staphylococcal intoxication is caused by *Staphylococcus aureus*. The bacteria can grow on hams, processed meats, chicken salad, pastries, ice cream, and cheese [6].

Severe nausea and vomiting usually are registered at 2 - 8 hours after the consumption of the contaminated food. Those may be associated with abdominal cramping, diarrhea, and sometimes headache and fever. Staphylococcal food poisoning symptoms usually last less than 12 hours [9]. In our study the symptoms appeared in about 4 hours after the ingestion of the food contaminated with staphylococcal enterotoxin. The symptoms were as follows: nausea, vomiting, serious abdominal pains, diarrhea, transpiration, headaches, prostration, and lowering of body temperature.

The relative incidence of five staphylococcal enterotoxins (SE) is shown in table 1. SE A was recovered from food-poisoning outbreaks more often than any of the others; SE D was the second most frequent serotype. The fewest numbers of outbreaks were associated with SE E type.

Table 1. Relative incidence of staphylococcal enterotoxins in some food products in Romania, from 2003 to 2008

Source	No. of cultural colonies	Enterotoxins A	B	C	D	E
Raw milk	246	1.8	0.8	1.3	6.3	0
Hams	180	40.2	4.8	13.0	16.1	7.8
Chicken salad	50	1.5	0.0	0.9	30.2	0
Beef	220	7.3	3.0	3.4	7.5	10.7
Icecream	350	7.2	15.5	6.3	6.9	0.8
Poultry	150	1.4	5.0	1.6	3.9	0
Cheese	270	7.9	15.3	6.3	5.9	0.8

Table 2. The effect of pH and NaCl on the production of SE C in an inoculum of 10 CFU/ml of *S. aureus* in a protein hydrolyzed medium incubated for 8 days at 37ºC

	pH 3.0-9.5	4.2-9.2	4.3-8.3	5.5-7.2	4.4-8.6
NaCl concentration	0	4	8	9	11
Enterotoxin production	+	+	+	+	-

Sources: *Faculty of Veterinary Medicine Bucharest, Institute of Diagnosis and Animal Health*

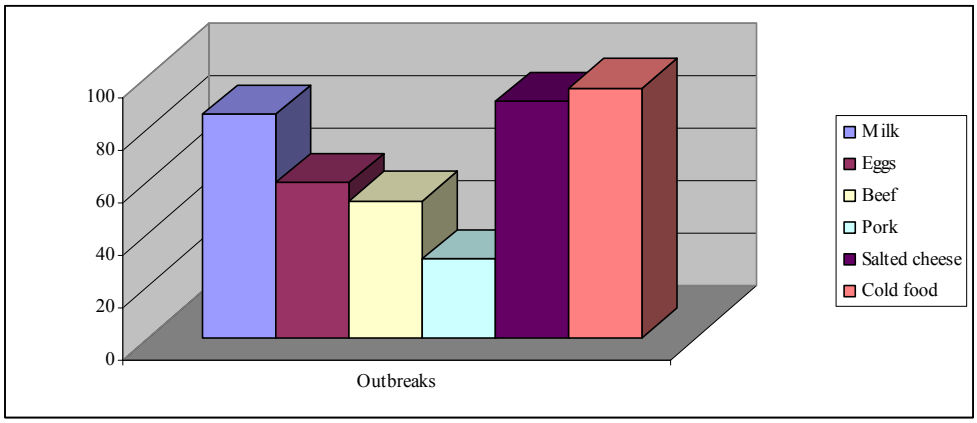

Chart no 3. Food as sources in staphylococcal gastroenteritis outbreaks in Romania, from 2003 to 2008 years *(Source: Center of Study and Research for Biodiversity from Animal Genetic Resources Academy David Davidescu)*

Foods identified in staphylococcal food poisoning in Romania were: milk, eggs, beef, pork, salted cheese, and cold/refrigerated food (chart no. 3). The conclusion of epidemiological analysis of all staphylococcal food poisoning outbreaks described in this study is that there are some main causes (chart no. 4); and a good management of all the risk factors are the bases of the prevention.

Using a protein hydrolyzed medium incubated at 37ºC for 8 days, without NaCl, the production of enterotoxin C occurred at the pH range 3.0-9.2. With 4% NaCl, the pH range

was restricted at 4.2-9.2 (table 2). In the medium with 9% NaCl, enterotoxin was produced at pH 5.5-7.2. SE was not produced in the medium containing 11% NaCl.

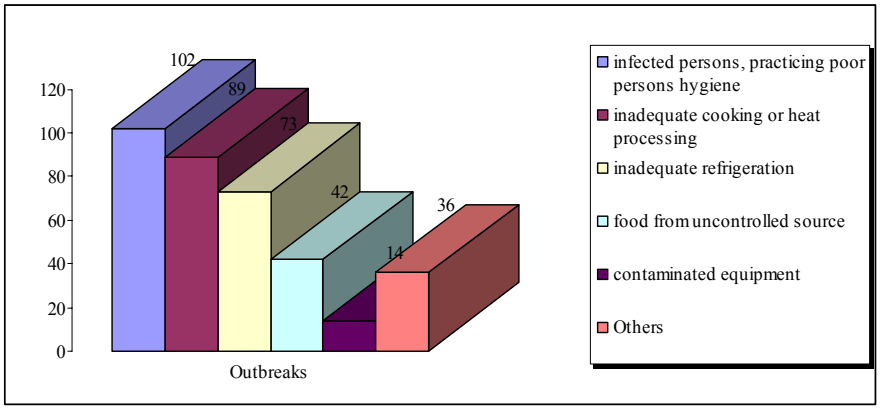

Chart no. 4. Main factors inducing outbreaks of staphylococcal food-borne gastroenteritis in Romania, from 2003 to 2008.
(Source: *Center of Study and Research for Biodiversity from Animal Genetic Resources Academy David Davidescu*)

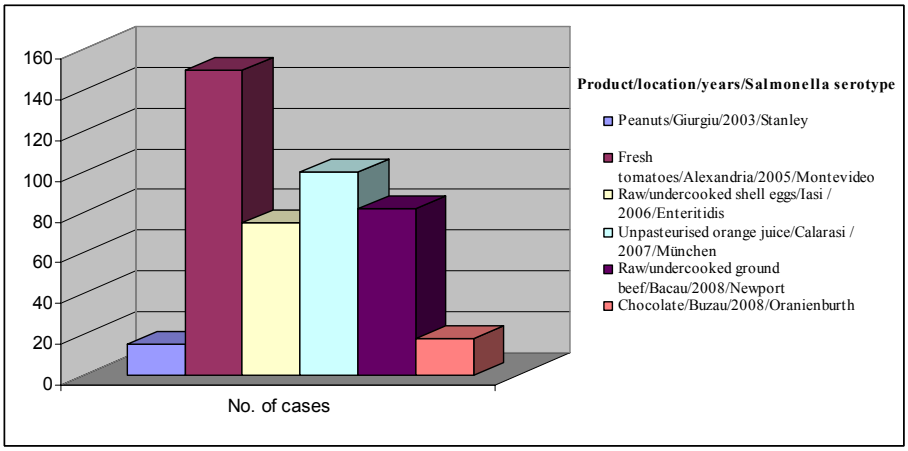

Chart no. 5. Synopses of some food-borne outbreaks of non-typhoidal salmonellosis in Romania, from 2003 to 2008 years (*Source: Institute for Diagnosis and Animal Health, Bucharest, Romania*)

Salmonella spp. is transmitted to humans by the consumption of food contaminated with faces from different sources. Salmonella can be found in: beef, meat, poultry, milk, eggs and even vegetables. It is important to notice, that old persons and children without a strong immunity system have a bigger risk to salmonellosis [3]. Usually, it is difficult to predict the association of *Salmonella spp.* with specific food products. But in some situations, like the *S.* Enteritidis, can be associated with poultry and eggs or egg–products; this association is frequently suspected in human salmonellosis outbreaks. In our study several serotypes were identified from salmonella food-borne diseases, in various towns (chart no. 5). The initial source of the bacteria was the intestinal tract of birds or other animals [6]. In salmonella outbreaks presented in this paper some alimentary products were also involved (11, 12).

Conclusions

1. Most Romanian cases of food-borne diseases caused by *C. perfringens* were reported in 2006 (48 cases). The main *C. perfringens* serotype isolated in Romanian was type A.

2. The annual incidence of food-borne disease cases caused by *C. botulinum* was variable, showing an ascendant curve with a maximum value in 2007 (52%). It was no correlation between annual numbers of outbreaks and numbers of cases registered. This situation was a consequence of some collective food-borne disease outbreaks.
3. Staphylococcal enterotoxin A (SE A) was recovered from food poisoning outbreaks more often than any other serotypes. SE D was the second most frequent. The fewest number of outbreaks were associated with SE E. Growth and enterotoxin C production occurred on the pH range 3.00-9.20 without NaCl. Using 4% NaCl, the pH range was restricted to 4.20-9.20. In the presence of 9% NaCl, the toxin was produced within a pH range of 5.50-7.20, but it was not produced in the presence of 11% NaCl. The main vehicle foods products in staphylococcal poisoning in Romania were: milk, eggs, beef, pork, salted cheese and refrigerated fish.
4. The main *Salmonella* serotypes isolated in food-borne diseases were Montevideo (150 cases), München (100 cases), Enteritidis (75 cases), Newport (82 cases); Oranienburg (18), and Stanley (15 cases).

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