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INCIDENCE OF FOOD CONTAMINATION WITH *STAPHYLOCOCCUS AUREUS* IN SUCEAVA COUNTY, ROMANIA

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Abstract: *Staphilococcus aureus is one of the most wide spread bacterial pathogens initiating food-borne disease worldwide. An investigation was conducted, between 2002 and 2011, in order to evaluate S. aureus contamination in various types of animal origin food, commonly consumed in Suceava County. A total of 781 samples were examined and 15.6% were found contaminated with S. aureus. Prevalence rates varied, recording the highest rate on 2006 when over 50% among the investigated food samples and over 20% among the food handlers (nasal cavity and hands) samples were contaminated. Of a particular case from 76 samples analyzed on 2002 and 2006 at Suceava-Bucsoaia students' camp 38.1% were confirmed positive in both food samples and food handlers samples. None of the samples analyzed on 2011 were contaminated. Our findings indicate a substantial improvement of GMP in food processing units and catering divisions as well as the benefit of food safety systems implemented during this period in Suceava County.*

Keywords: *Staphylococcus aureus, food contamination, food-borne illness*

1. Introduction

Contaminated food consumption frequently results in the illness, which is called food-borne illness of food poisoning. *S. aureus* contamination is recognized as a major cause of food-borne illness worldwide, being indicated as one of the most prevalent among the bacterial pathogen agents in both community-acquired as well as nosocomial infections [1]; [2]. It has been suggested that over 30%-50% of the population represent the numbers of carriers [3]. Kluytmans and Werheim [4] reported that in fact only 20% of people almost never carry *S. aureus*. Foods storage at improper temperature, inadequate handling as well as the capacity of microorganism to develop in a wide range of pH conditions and salt concentrations indicate that a wide range of food products are the main epidemiological features to provide appropriate conditions for an epidemic of *S. aureus* food poisoning. Meat and milk-based products were found likely to be the most frequently involved matrices of food poisoning during the investigations done after an outbreak [5]; [6]. Work surfaces and equipment used to prepare foods are an important source of indirect contamination. A study carried on 2003

[3], reveled that 25% of swabs taken from work surfaces were contaminated with *S. aureus* and 71.7% of ready-to-eat products handled after heat treatment were contaminated. Some *S. aureus* strains are capable to produce one or more enterotoxins this consist in pathogenity of this microorganism. It is estimated that the enterotoxigenic strains account for about 25% of all isolated strains [5].

Staphylococci are catalase - positive, oxidase-negative, facultative anaerobes [7]. Enterotoxin production is adversely affected by anaerobic condition far more than growth [8]. Staphylococcal food poisoning is caused by the; ingestion of foods containing enterotoxins produced by some species of staphylococci [9]. The disease is described by sudden start of symptoms, including nausea, vomiting, abdominal cramps, and diarrhea within few hours after ingestion of toxin-contaminated foods. Staphylococcal food poisoning is generally considered a mild, self-limited illness with low mortality rate lasts only a few hours with no consequence. However, the hospitalization rate has been reported to be as high as 10% in United States of America [10]. The diagnosis of this food-borne illness is based primarily on recovering enterotoxigenic staphylococci and enterotoxins from leftover food.

Staphylococci are common in nature can be found in the air, in dust, in water, and on humans and animals. The main human reservoirs of these organisms are the skin, nasal cavities and throat [11]. About 40 to 44% of healthy humans carry staphylococci in the nose [12]. Strains present in the nose often contaminate the back of hands, fingers and face; nasal carriers can easily become skin carriers. Although it is difficult to determine the origin of the strains involved in staphylococcal food poisoning outbreaks, food handlers are usually regarded as one of the primary source of these organisms [13]; [14]. It has been reported that, one of the important pathogens often transmitted via food contaminated by infected food handlers is *S. aureus* [15]. Health risks are also linked with subsequent contaminations by the workers during handling. Staphylococci are included in the bacterial group that contaminates food products in this way [16]. For many years, *S. aureus* was the only staphylococcal species known to produce enterotoxins [9]. An important characteristic that differentiates *S. aureus* from most staphylococcal species is its ability to produce coagulase, an enzyme that clots blood plasma. Staphylococcal food poisoning is widespread and quite frequent. The incidence of staphyloenterotoxicosis cases is probably underestimated considerably, and there may be a lot of reasons for that: not calling medical services by many ill people due to the short duration of the disease or mild symptoms, improper both sample collection and laboratory examination [17]. The aim of the present study was to investigate microbiological quality and to detect the presence of the pathogenic of *S. aureus* in various kinds of animal origin food, as well as in food handlers, occurred in Suceava Country during 2002 - 2011. Although, food safety is one of the most important issues for maintaining human health therefore, to prevent *S. aureus* contamination becomes an important apprehension.

2. Materials and methods

According to the method of Lancette and Tatini [18], 25 g of each implicated food were homogenized with in 225 mL of 0.1% buffered peptone water. A 0.1 mL aliquot of the suspension was then spread on the surface of a Baird-Parker agar plate. Additional plates were prepared with successive decimal dilutions. The plates were incubated for 48 hours at 37ºC, and the suspected colonies were counted. Ten

typical colonies (jet black to dark grey, smooth, convex, well-defined contours, off-white edge, presenting an opaque zone and/or a clear halo beyond the opaque zone) and 10 colonies classified as atypical (gray and mucoid showing one halo) were transferred to tubes containing nutrient agar (stock culture) for further testing. For detecting small numbers of *S. aureus* in raw food ingredients and non-processed foods expected to contain large numbers of competing organisms, incubation is in trypticase soy broth containing 10 % NaCl and 1 % sodium pyruvate before transferring to Baird-Parker agar plates. For detecting relatively large numbers of staphylococci, the food extract is plated directly on Baird-Parker agar. Typical colonies of *S. aureus* on Baird-Parker agar are circular, smooth, convex, moist, cca. 1.5 mm in diameter, gray-black to jet-black, off-white edges and may show an opac zone with a clear halo extending beyond it.

Isolation and identification of *S. aureus*: For the bacterial colony isolation serial dilutions of the samples were made and diluted sample (100µl) was transferred on Baird-Parker agar supplemented with egg yolk tellurite enrichment suspension (Oxoid-England) and incubated at 37°C for 48 hours as previously described elsewhere [19]. The physical identification characteristics of the bacterial colonies such as black, smooth, convex to uniform outline with one or two halos were recorded [20]. *S. aureus* was confirmed by colonial morphology, Gram staining, catalase activity, and coagulation of citrated rabbit plasma (Sigma – Aldrich Group)

Isolation of staphylococci from food handlers

Each swab collected from the nasal cavity, throat and from under the fingernails was introduced in tubes containing 5 mL of Triptic Soy Broth (TSB) with 10% of NaCl. The tubes were incubated for 24 hours at 37ºC. The cultures were streaked on Baird-Parker plates and incubated for 48 hours at 37ºC. Ten typical and 10 atypical colonies were selected for further testing as described above.

3. Results and discussion

A total of 781 samples of animal origin, from Suceava County were collected and examined between 2002 and 2011, of which 15.6% were found contaminated with *S. aureus*. The highest peak was recorded on 2006 when over 50% among the investigated food samples and over 20% among the food handlers sample were contaminated with *S. aureus* as can be seen in the Figure 1. Also, a slight increase of the incidence rate can be observed during monitoring; however on 2011 there was no contaminated samples recorded. In 2004, the incidence of food handlers samples riches the highest incidence over 37% among the analyzed samples were positive. The *Staphylococci sp*. is omnipresent microorganisms that cannot be eliminated completely from our environment. As it was mentioned by Di Ginatnatale [3], at least 30-50% of individuals are carriers of these types of microorganisms in their nasal cavity or throats, or on their hands. The possibility that the food to be contaminated with *S. aureus*, is strongly correlated with the expose to the human handling therefore, the risk of contamination with Staphylococci that can produce enterotoxins can rich at least 30% - 50%. The contamination incidence of food sample and food handler samples by year are summarized in the Table 1.

The highest incidence was recorded during 2004 - 2006, and the incidence decreased considerably from 2007. Recently, Oliveira [21], show that in Korea on 2007 the incidence of raw milk samples contaminated with *S. aureus* was 0.34% among the investigated samples, in Norway in 2005, 11 samples were found positive, in 2010 in United States, 29% of

samples were positive for *S. aureus* in cow's milk.

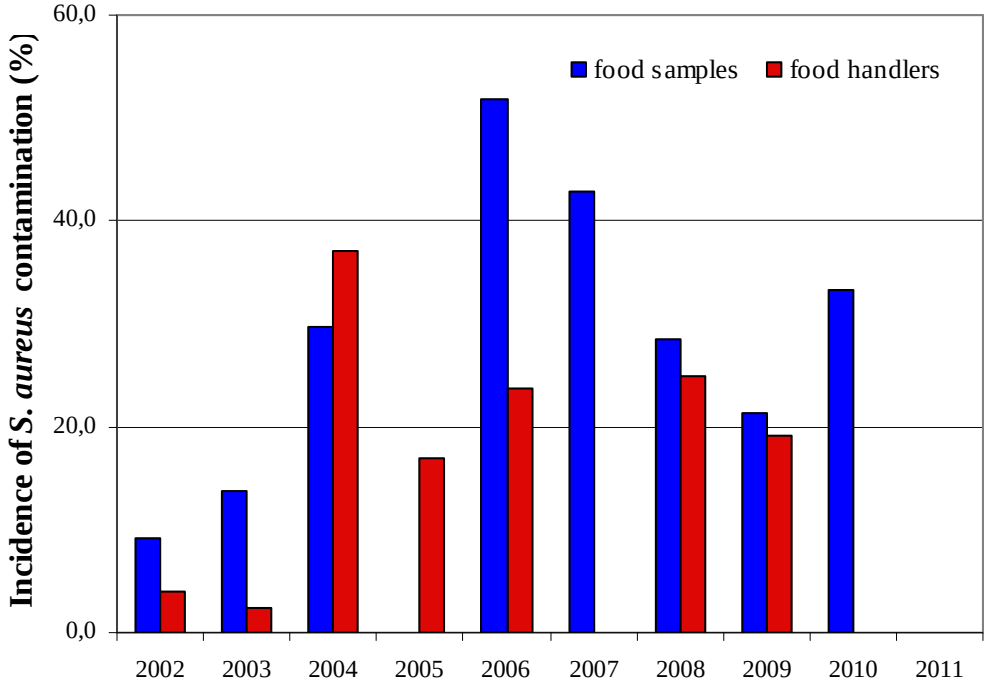

Figure1. The incidence of *S. aureus* contamination (foods and food handlers) occurred in Suceava County between 2002 - 2011

Table 1.
Summary of the contamination incidence with S. aureus in food samples and food handlers occurred in Suceava County between 2002 – 2011

year	Samples	No. of examined samples	No. of sample positive for S. aur	% of isolat
2002	food samples	66	6	9.09
	food handlers	82	4	4.87
2003	food samples	51	7	13.72
	food handlers	85	2	2.35
2004	food samples	27	8	29.62
	food handlers	27	10	37.04
2005	food samples	56	0	0.00
	food handlers	59	10	16.95
2006	food samples	54	28	51.85
	food handlers	105	25	23.81
2007	food samples	7	3	42.86
	food handlers	12	0	0.0
2008	food samples	14	4	28.57
	food handlers	12	3	25.0
2009	food samples	14	3	21.43
	food handlers	21	4	19.05
2010	food samples	15	5	33.33
	food handlers	9	0	0.0
2011	food samples	35	0	0.0
	food handlers	30	0	0.0

The same study show that in Turkey in 2007, 18.18% had, in Morocco in 2004, registred 40% and in India in 2008, with a 61.7% infestation respectively from raw milk samples were found contaminated with *S. aureus.*

Table 2.
Summary of the contamination incidence with S. aureus in food samples and food handlers occurred in Suceava Bucsoaia student's camp on 2002 and 2006

year	Samples	No. of examined samples	No. of sample positive for S. aureus	% of isolation
2002	food samples	35	14	40.0
	food handlers	16	0	0.0
2006	food samples	20	10	50.0
	food handlers	5	5	100.0

In Romania according to Ivana 2010 [22], the situation is worrying and the *S. aureus* contamination incidence rises to over 50%. On the other hand, of a particular case from 76 samples analyzed on 2002 and 2006 at Suceava-Bucsoaia students' camp 38.1% were confirmed positive in both food samples and food handlers samples, the results are summarized in the Table 2. Bucsoaia students' camp is organized usually during summer. The warmer summertime temperatures may complicate the situation resulting in increasing the contamination incidence. Another factor as a particular summer problem is that if the food products are not stored under adequate refrigeration until consumption may also increase the risk.

4. Conclusion

In conclusion, this study provides a summary of the evaluation regarding prevalence of *S. aureus* contamination occurred in various food of animal origin, in Suceava County during the past decade. A decrease of contamination incidence with *S. aureus* can be observed during 2002 - 2011. These results show a low incidence of food contamination in Suceava County as compared with the average of contamination incidence in Romania, in this case the level rises 50% according to literature.
These results, in our opinion, suggest that the improvement in applied HACCP and GMP in food processing units in Suceava County were implemented successfully.

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