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INCIDENCE OF ENTEROTOXIGENIC STAPHYLOCOCCUS AUREUS AND ITS ENTEROTOXINS IN MILK AND MEAT PRODUCTS

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SUMMARY: Coagulase positive *Staphylococcus aureus* isolated from 44 out of 94 samples (46.8%) of dairy products in counts that varied from 3.0×10^2 to 1.0×10^7 and from 20 out of 50 samples (40 %) of meat products in counts ranging from 1.0×10^2 to 1.6×10^4. Enterotoxins type A, D, AB, AD, AE, ABC and ACD were detected in 40.9 % of the isolates recovered from dairy products in concentrations ranging from 0.5 to 6 ug per ml culture supernatant fluid. 45 % of the meat products isolates yielded enterotoxins type A, D, E and AD in concentrations varying from 0.5 to 2.0 ug per ml culture supernatant fluid. Of 4 *Staph.aureus* isolates recovered from 4 samples of various foods incriminated in cases of food poisoning, 3 of them were found to produce enterotoxins A, D and AD.

INTRODUCTION

The incidence of enterotoxigenic *Staphylococcus aureus* varies according to the type of food. In foods associated with food poisoning the incidence is high. Casman et al. (1967) found that 96.2 % of such strains were enterotoxigenic. Simkovicova and Gilbert (1971) obtained a comparable value of 92 %. On the other hand, Toshach and Thorsteinson (1972) reported an incidence of only 44 %.

In foods not associated with food poisoning the percentage of enterotoxigenic *Staphylococcus aureus* was always lower. Casman et al. (1967) found that 10 % of 236 strains from raw milk

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and 30 % of 260 strains from frozen foods produced enterotoxins. Simkovicova and Gilbert (1971) found that 1/3 of the strains recovered from various foods produced enterotoxins. Wieneke (1974) reported that 21-26 % of 112 strains isolated from raw meat, sausages and poultry and 32-36 % of 183 stains isolated from cooked foods were enterotoxigenic. Sedik (1982) tested 100 strains isolated from frozen beef and could detect enterotoxins in 36 %.

The present work is dealing with the incidence of enterotoxigenic *Staphylococcus aureus* in foods associated or not associated with food poisoning.

MATERIAL AND METHODS

A total of 144 samples of dairy and meat products collected from markets and shops in Giza and 4 samples of foods associated with food poisoning were examined for enterotoxigenic Staphylococcus aureus.

25 g of each sample were homogenized in 225 ml buffered peptone water and 10-folds dilutions were prepared. From each dilution 0.1 ml was plated on duplicate plates of Staphylococcus medium No. 110 containing 0.75 mu Sodium azide (Smuckler and Appleman, 1964 and Refai,1979). After 48 hours incubation at 35°C the plates were counted and pure colonies were subcultured on sheep blood agar and Difco brain-heart infusion agar. Each strain was tested for production of coagulase (using fresh citrated rabbit-plasma), DNA hydrolysis, heat-stable nuclease and enterotoxins (Sac-culture method and optimal sensitivity plate method) according to the methods described by Donnelly et al. (1967), Barry et al. (1973) and Robbins et al. (1974).

Reference enterotoxins (A-E) and antisera were kindly supplied by Prof. Dr. M. Bergdoll, Food Research Institute Wisconsin, U.S.A.

RESULTS

Incidence of Staphylococcus aureus

Dairy products

Coagulase positive *Staphylococcus aureus* were recovered from 44 out of 94 samples (46.8 %)of dairy products. The highest incidence was recorded in mish, processed and kariesh cheese (60, 56 and 54 % respectively). None of the 10 Youghurt samples yielded *Staphylococcus aureus* All positive samples showed colony counts more than 100 bacteria per gram. The lowest average count (9.0×10^2) was recorded in mish cheese and the highest (5.0×10^6) in white cheese (**Table 1**).

Meat products

As shown in **Table 2**, 20 out of 50 samples examined (40 %) yielded coagulase positive *Staphylococcus aureus* in counts that varied from 1.0×10^2 to 1.6×10^4. The highest average count (1.2×10^4) was reported in frozen raw minced meat and the lowest (1.4×10^2) in frozen raw chicken skin.

Types and concentrations of enterotoxins

Dairy products

18 of 44 isolates of *Staphylococcus aureus* (40.9 %) recovered from dairy products were found to be enterotoxigenic. As shown in **Table 3** a wide range of enterotoxins were detected in Kariesh cheese. The concentrations of enterooxins

Table 1: Incidence of *Staphylococcus aureus* in dairy products

Type of food	No. of samples examined	No. of isolates	%	Colony counts		
				Minimum	Maximum	Average
Kariesh cheese	50	27	54	3.0×10^2	1.5×10^4	7.7×10^3
Processed cheese	16	9	56	1.5×10^3	1.8×10^6	9.0×10^5
Mish cheese	10	6	60	3.0×10^2	1.5×10^3	9.0×10^2
White cheese	8	2	25	5.0×10^2	1.0×10^7	5.0×10^6
Youghurt	10	0	0	--	--	--
Total	94	44	46.8			

Table 2: Incidence of *Staphylococcus aureus* in meat products

Type of food	No. of samples examined	No. of isolates	Colony counts		
			Minimum	Maximum	Average
Frozen raw minced meat	14	6	9.0×10^3	1.6×10^4	1.2×10^4
Frozen raw sausage	5	4	1.0×10^2	3.6×10^3	1.9×10^3
Frozen raw liver	5	4	1.0×10^2	4.5×10^3	2.3×10^3
Frozen raw beef meat	5	1	2.0×10^2	5.0×10^2	3.5×10^2
Frozen raw chicken (skin)	8	2	1.2×10^2	1.6×10^2	1.4×10^2
(muscle)	3	0	-	-	-
Cooked sausages	5	3	4.3×10^2	1.5×10^3	9.6×10^2
Total	50	20 (40%)			

Table 3: Staphylococcus aureus enterotoxins detected in dairy and meat products

Type of food	No. of isolates tested	No. of toxigenic isolates	Types and concentration of enterotoxins in ug
Kariesh cheese	27	10	2A/1.0, 1D/6.0, 2AD/0.75, 0.5, 2AE/0.5,0.5 1ABC/4.0,0.5,0.5,2ACD/0.5,0.5,2.0.
Processed cheese	9	4	2D/1.0, 2AB/2.0,0.5
Mish cheese	6	2	1AB/4.0,1.0, 1AD/0.5, 2.0
White cheese	2	2	2AE/0.5,0.5
Frozen raw minced meat	6	3	2A/2.0, 1AD/0.5,2.0
Frozen raw sausages	4	1	1D/1.0
Frozen raw liver	4	2	1E.0.5, 1AD/1.0,1.0
Frozen raw beef	1	1	1D/0.5
Frozen chicken (skin)	2	1	1D/0.5
Cooked sausages	3	1	1AD/0.5,1.0,1.6
Total	64	27	

* 2 A/1.0 = type/concentration (ug) of enterotoxins

per ml of culture supernatant fluid varied from 0.5 to 6.0 ug. In processed and mish cheese only two types were detected. On the whole, the enterotoxin AE was the most frequent, followed by D, AB and AD then A, ACD and ABC. Enterotoxins B, C and E were not detected in cheese.

Meat products

9 out of 20 *Staphylococcus aureus* isolates recovered from meat products were toxigenic (45 %). The most common enterotoxins were types D and AD (each in 3 isolates). Type A was detected in 2 isolates and type E in one isolate. The concentration of toxins varied from 0.5 to 2.0 ug per ml of culture supernatant fluid.

Cases of food poisoning

3 cases of food poisoning were reported to the laboratory. In the first case 80 students were involved. In the second case 2 persons in a family and in the third case 5 persons in another family were affected. The incriminated food is presented in **Table 4**. From the samples of dairy and meat products examined 4 *Staphylococcus aureus* were recovered in counts that ranged from 3.6×10^2 to 2.2×10^6 per gram of food. 3 isolates were found to be enterotoxigenic, of which one yielded enterotoxin A (processed cheese), one D (luncheon) and one AD (cooked liver). The concentration of enterotoxins varied from 0.5 to 2.0 ug per ml culture supernatant fluid.

Table 4: Enterotoxigenic *Staphylococcus aureus* and enterotoxins in food incriminated in food poisoning

Cases of food poisoning (No. of individuals involved).	Food incriminated	Colony count/g	Types of enterotoxins/concentrations in ug/ml.
80 students	Processed cheese white cheese	2.2×10^6 4.7×10^5	A (2.0 ug/ml) ----
2 persons in family A	Luncheon	3.6×10^2	D (1.0 ug/ml)
5 persons in family B	Cooked liver	1.6×10^3	AD (0.5-1.0 ug/ml)

DISCUSSION

The incidence of coagulase positive *Staphylococcus aureus* in Karish cheese (54 %) obtained in this study is almost similar to that recorded by El-Bassiony and Ahmed (1979) who isolated the organism from 16 out of 31 samples (51 %). A much higher incidence (86.7 %) was found by Sallam et al. (1985). The latter authors reported a higher incidence also in white cheese (93.3 %) in comparison to the result obtained in the present work (25 %). The mean colony counts in our samples varied between 10^2 and 10^6/g. Donnelly et al. (1964) reported that coagulase positive *Staphylococcus aureus* was recovered from market cheese in counts ranging from less than 50 to more than 200000 per g. Sallam et al. (1985) reported a mean count of 3.3×10^4 - 1.7×10^5 per g in different types of cheese.

In meat products the incidence of coagulase positive *Staphylococcus aureus* (40 %) was much lower than that reported by other authors. Sedik(1982) detected *Staphylococcus aureus* in 71.23 %, 3.33% and 90 % of examined beefn, chicken and pork products respectively. El-Hashimy et al. (1982) found *Staphylococcus aureus* in 50 % and 70 % in cooked and raw liver samples respectively and represented an incidence of 80 % in raw and cooked brain. The mean colony count reported by these authors is almost in agreement with that obtained in the present study.

It is worthy to note that all samples of dairy products examined in the present work showed colony counts higher than 10^2 per g, i.e. the products are considered to be of potential hazard according to the limits proposed by ICMSF (Refai, 1979). On the other hand, the limits of *Staphylococcus aureus* in meat proposed by Mohs (1972) is 10^4/g. Accordingly only the positive cases of frozen minced meat examined in this work are to be considered hazardous.

The low count recommended in cheese is based on the fact that cheese will not be subjected to heat and there is a possibility that the number of organisms increases further in as much as the *Staphylococcus aureus* is a halotolerant organism, i.e. it will not be suppressed by the salt present in the cheese. The limit recommended by Mohs (1972) for meat is in our opinion high, particularly if the food is cooked and will not be subjected to further heating. The counts in luncheon and cooked liver associated with food poisoning were 3.6×10^2 and 1.6×10^3 respectively, i.e. below the limits recommended by Mohs (1972). On the other hand, the processed cheese involved in the massive food poisoning among students showed a high

count (2.2×10^6). Although the white cheese consumed by the students showed also a count of 4.7×10^5/g, yet the recovered *Staphylococcus aureus* was not enterotoxigenic. This demonstrats the importance of enterotoxin detection and the critical evaluation of the colony count of *Staphylococcus aureus* in foods.

27 out of 64 *Staphylococcus aureus* isolates tested (42.2 %) were found to be enterotoxigenic. The incidence of enterotoxigenicity was higher in meat products isolates (45 %) than that of dairy products isolates (40.9 %). Such incidence was in general high if compared with results of Casman et al. (1967) who found that 10% of strains isolated from foods produced enterotoxins. Also Simkovicova and Gilbert (1971) reported an incidence of 33.3 %. Of 100 isolates recovered from frozen meat and chicken products, Sedik (1982) detected enterotoxin in 36 isolates. On the other hand, Payne and Wood (1974) mentioned that 62.5 % of strains isolated from meat and dairy products were enterotoxigenic. Comparable value (44 %) to that obtained in the present work was however reported by Toshach and Thorsteinson (1972).

Over 90 % of *Staphylococcus aureus* isolates recovered from foods associated with food poisoning were found to be enterotoxigenic (Casman et al., 1967 & Simkovicova and Gilbert, 1971). Such high percentage was not found in the present work as only 3 out of the 4 isolates (75%) recovered from foods incriminated in food poisoning produced enterotoxins.

There is no agreement among the various authors with regard to the types of enterotoxins in foods. This may be due to the pattern of enterotoxin antisera and the sensitivity of the technique used. Donnelly et al. (1967) detected only

enterotoxin A in cheese. Casman et al. (1967) and Wieneke (1974) mentioned that most of the isolates recovered from cheese produced enterotoxin D and E.

In the present study 7 types of enterotoxins could be detected in the supernatant fluid of sac culture of 18 isolates from dairy products namely type AE (4 isolats), D, AB and AD (3 each), A and ACD (2 each) and ABC (once). This wide range of types was met with in Kariesh cheese; a result which is not surprising as the way this cheese is produced subjects it to the various sources of contamination.

Only 4 types of enterotoxins were detected in strains isolated from meat products in the present study, namely types A, D, E, and AD. Wieneke (1974) reported that *Staphylococcus aureus* isolated from raw meat, sausage and poultry produced enterotoxins A, B, C, D or E. Ostovar and Bremier (1975) reported that most isolates of coagulase positive staphylococci recoved from frozen foods produced type A or B. Sedik (1982) detected enterotoxins A, B, C, D, AB and AD in culture supernatant of isolates recovered from frozen meat and chicken products.

Our study showed that enterotoxins A, D, and AD were produced by coagulase positive *Staphlococcus aureus* isolated from processed cheese lunchoen and cooked liver respectively and implicated in cases of food poisoning. These findings are consistent with various reports of Donnelly et al. (1967), Casman et al. (1967), Bergdoll (1970), Simkovicova and Gilbert (1971) and Wieneke (1974) who found that *Staphylococcus aureus* incriminated in food poisoning is most likely to produce enterotoxins A,B,C,D or AD. Furthermore, Donnelly et al (1967) reported that most *Staphylococcus aureus* isolated from

cheese food poisoning produced enterotoxin A. Moreover the determined concentration of enterotoxins per ml of culture supernatant fluid (0.5-2.0 ug) of *Staphylococcus aureus* recovered from incriminated food was found to be within the limits (0,5 ug or more Bergdoll, 1973) that can cause food poisoning outbreaks.

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