BACTERIOPHAGE TYPING OF STAPHYLOCOCCI ISOLATED FROM FOODSTUFFS AND FOOD HANDLERS IN TEHRAN

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Abstract

Studies were done to assess contamination of 3247 samples of foods and food handlers with coagulase-positive staphylococci. Food specimens were collected from retail shops and a Tehran milk pasteurizing plant; in addition 510 food handlers of different professional groups were examined. Three swabs for bacteriological examination were randomly collected from nose, hands, and throat. Of samples tested for coagulase-positive staphylococci, 26.02% were positive and almost half of them were obtained from foodstuffs. Of 546 isolates that were phage typed, we found that group III predominated in the nose and cream cake, while group II was more prevalent in raw cream, throat, and hands. Although group IV was not prominent, nose and hands yielded 3 and 1 positives, respectively. Untypable isolates comprised about 47.2% with most from hands and nose, 61.7 and 60.5%, respectively, and the least from raw cream, 33.3%.

Wide-spread adoption of phage typing for grouping coagulase-positive staphylococci makes this technique very useful for comparing the results reported by different laboratories *(2)*. Phage typing of staphylococci isolated from milk and cheese *(24)*; raw milk *(8)*; certified milk *(16)*; Cheddar cheese *(4, 5)*; skin of pig and chickens *(22)*; whipping cream, half cream, and butter milk *(10)*; fish *(21)*; market meats and non-frozen meats *(14, 15)*; minced kebab *(15)*; and some other foods and food handlers *(4, 7, 19)* has been reported from different countries in recent years, but in Iran no report is available concerning the phage typing of staphylococci of food origin.

Food poisoning, although common in Tehran, particularly during the warmer months, is not reportable, therefore there are no data available regarding the extent of food poisoning and the role that different organisms play in these episodes *(6, 11)*. It is hoped that further studies towards isolation and phage typing of staphylococci of food origin and comparison of the results with similar studies elsewhere may help towards the understanding the epidemiology of these bacteria in food poisoning.

Materials and Methods

Food specimens collected from 717 retail shops and a Tehran milk pasteurizing plant were prepared and cultured on plates containing Baird-Parker medium *(1)* for isolation of coagulase-positive staphylococci.

Sampling food handlers

Five hundred and ten food handlers from different professional groups were examined. Three swabs for bacteriological examination were randomly collected from nose, hands, and throat of each person.

Before collection swabs were soaked in trypticase soy broth (TSB) and the specimens were then kept in TSB medium for 24 hr at 37 C. A loopful of each incubated sample was inoculated on Baird-Parker medium, and plates were incubated at 37 C for 48 hr. Typical colonies were transfered to 10 ml of broth and after sufficient growth, the coagulase test was done using 1:3 dilution of fresh rabbit plasma.

Phage typing of staphylococci

Bacteriophage typing was done according to the standard method *(4, 27)*, using the following phages: Group I: 29, 52, 52A, 79, 80; Group II: 3A, 3B, 3C, 55, 71; Group III: 67, 42E, 47, 53, 54, 75, 77; Group IV: 42D; and Miscellaneous Group: 81, 187, 83A, 77ad, D, B5.

Results

Table 1 demonstrates that from 3247 samples of foods and food handlers that were tested for coagulase-positive staphylococci, 846 were positive and of these 367 were collected from food handlers and 479 were obtained from foodstuffs. In this table, we also have shown the percentage of coagulase-positive staphylococci.

The phage type strains from each source are presented separately in Tables 2-8. Group III staphylococci prevailed in the nose and cream cake, while Group II was more prevalent in raw cream, throat and hands. There were 3 and 1 group IV staphylococci recovered from nose and hands, respectively. About 47.2% of all samples were untypable, with most from hands and nose, 61.7 and 60.5%, respectively, and the lowest from raw cream, 33.3%.

Discussion

It was found that contamination of minced kebab in Tehran with coagulase-positive staphylococci was much greater than that of other foodstuffs tested in

TABLE 1. NUMBER AND PERCENTAGE OF COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM FOODSTUFFS AND FOOD HANDLERS

Source	Cream cake	Raw milk	Minced kebab	Raw cream	Food handlers			Total
					Nose	Hands	Throat	
No. samples	250	1000	350	117	510	510	510	47
No. of staph. coa-pos.	19	147	307	6	190	141	36	846
% Pos.	7.6	14.7	87.7	5.1	37.2	27.6	7.1	26.0
No. of strains typed	19	95	63	6	190	141	36	550

our study. More than 87% of the samples contained the bacteria. This high percentage can be attributed to the nature and composition of the minced kebab which contains about 90% meat.

It has been reported that meat marketed in developing countries is so contaminated that one has to consider it as poor quality meat *(25)*. Kebab is excessively manipulated during processing and before roasting, and this can also account for such a high level of staphylococcal contamination. Although the percentage of staphylococcal contamination is too high, there is no report of a high incidence of food poisoning produced by kebab in Tehran areas. The number of staphylococci in the kebab specimens examined were < 400,000/g, and only four specimens exceeded such a value *(15)*. Hobbs *(12)* has shown that the enterotoxin produced by < 500,000 staphylococci/g of food is not sufficient to induce poisoning. It is necessary to mention that all kebab, milk, and cream specimens examined were in the raw condition.

TABLE 2. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM CREAM CAKE

Group	No. of strains	Percentage
Group I:		
80	1	5.3
Group II:		
3A	1	5.3
71	2	10.5
Total Group II	3	15.8
Group III:		
6/42E	1	5.3
6	1	5.3
Total Group III	2	10.5
Group IV	0	0
Mixed Group		
80/71	1	5.3
42E/53/81	1	5.3
52/79/80/6/47/54/83A/81	2	10.5
Total Group	4	21.0
Non typable	9	47.3
Total Strains	19	

Since staphylococci are usually killed in roasted kebab and pasteurized milk and cream, the enterotoxin produced by these microorganisms persist and may cause food poisoning which can not be traced to staphylococci *(12)*.

Table 1 indicates that the number of coagulase-positive staphylococci isolated from raw milk, raw cream, and cream cake is very low in comparison with kebab specimens. It is suggested that such a discrepancy in milk and cream is due to a high level of contamination of these materials with other microorganisms, particularly lactic bacteria. The latter act to inhibit staphylococcal growth *(6)*. It was observed that, in typable strains, the nose of food handlers examined was more afflicted with strains of group III-IV, and hands and throat with group II staphylococci.

Strains of group I were most frequently observed in minced kebab and raw milk. Petersen *(18)* reported that in Great Britain the yearly incidence of food-borne intoxication, from 1950 to 1962, was due to strains of staphylococci in phage group III, ranging from 64.5 to 96.7%. Another investigation has also revealed that strains more commonly implicated in food poisoning are members of phage groups III and IV *(20)*. In our experiments a high proportion (about 47.2%) of the strains isolated from food handlers and foodstuffs were untypable, but these also may produce enterotoxins *(3, 24)*. Hence, staphylococcal food poisoning in Tehran is associated with group III and untypable strains of staphylococci.

Tables 6 and 7 show that the percentages of untypable strains of staphylococci isolated from the nose

TABLE 3. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM RAW MILK

Group	No. of strains	Percentage
Group I:		
79/52A	1	1.06
29/52/52A/79/80	5	5.31
52/52A/79	1	1.06
Total Group I	7	7.43
Group II:		
3C/55/71	4	4.2
3A/3C/55/71	1	1.06
3A	1	1.06
Total Group II	6	6.32
Group III:		
75	2	2.1
53/54	2	2.1
42E/53	2	2.1
Total Group III	6	6.3
Group IV	0	0
Mixed group		
81/6/7/42E/54/75/83A	2	2.1
52/80/6/42E/81	1	1.06
52A/80/81	1	1.06
52/79/80/3A/3C/47	9	9.5
83A	5	5.3
80/6/42E/75/81	6	6.3
52A/42E/75	1	1.06
53/81/80/3A/3C/6	7	7.4
Total Group	32	33.78
Non typable	44	46.7
Total Strains	95	

TABLE 4. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM MINCED KEBAB

Group	No. of strains	Percentage
Group I:		
79/80	5	7.9
52/79	2	3.1
52A/79/80	2	3.1
Total Group I:	9	14.1
Group II:		
3A	2	3.1
55	1	1.5
71	1	1.5
Total Group II:	4	6.1
Group III:		
54	1	1.5
53/6/7/42E/47	2	3.1
6/75/54	3	4.7
6/7/47/53/54/75	1	1.5
Total Group III	7	10.8
Group IV	0	0
Mixed Group		
42E/81/52/7/54	1	1.5
54/53/79/6/7/42E/83A	10	15.8
42E/81/7/52	5	7.9
52/53	1	1.5
79/7/53	1	1.5
Total Group	18	28.2
Non typable	25	39.6
Total Strains	63	

TABLE 5. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM RAW CREAM

Group	No. of strains	Percentage
Group I:		
79	1	16.6
Group II:		
3A/3C	2	33.3
Group III:		
54	1	16.6
Group IV	0	0
Non typable	2	33.3
Total Strains	6	

and hands of food handlers are very similar, namely 60.4 and 61.7% respectively.

This is compatible with findings of other investigators who reported that 15 to 20% of the people who carry staphylococci in their nose, also carry the organisms on their hands *(7, 13)*. In addition, phage type 42D, which is the only group IV staphylococcus, was isolated from the nose and hands at a frequency of 1.5 and 0.7%, respectively. Tables 6 and 7 also reveal that the incidence of the following phage types was similar in both nose and hands: 29, 52, 52A, 80, 3A, 3C, 55, 71, 7, 42E, 187, 83A. It is therefore suggested that a significant relationship exists between contamination of nose and hands with coagulase-positive staphylococci.

ACKNOWLEDGMENTS

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TABLE 6. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM THE NOSE

Group	No. of strains	Percentage
Group I:		
29/52/52A/79	10	5.2
80	6	3.1
22A/79	2	1.05
Total Group I	18	9.35
Group II:		
55/71	14	7.3
3A	1	0.5
3C	3	1.5
Total Group II	18	9.3
Group III:		
7/42E	7	3.6
77	7	3.6
53	3	1.5
7/54	3	1.5
6	2	1.05
75	1	0.5
Total Group III	23	11.75
Group IV	3	1.5
Mixed Group		
83A/53/77	3	1.5
3A/7	1	0.5
3C/55/71/75	2	1.05
57/52A/79/80/7	1	0.5
Total Group	7	3.55

Group	No. of strains	Percentage
Type 81	1	0.5
Type 187	2	1.05
Type 83A	3	1.5
Non typable	115	60.5
Total strains	190	

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TABLE 7. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM HANDS

Group	No. of strains	Percentage
Group I:		
52/52A/79/80	5	3.5
80/29	3	2.1
52	2	1.4
52A	1	0.7
Total Group I	11	7.7
Group II:		
71/3C	12	8.5
3A/3C	1	0.7
55/3A/71	5	3.5
Total Group II	18	12.7
Group III:		
77	3	2.1
6/7/42E/47/53	10	7.0
47/53	1	0.7
7/75	1	0.7
Total Group III	15	10.5
Group IV:	1	0.7
Mixed Group		
29/80/53	1	0.7
6/54/75/83A	1	0.7
55/71/7	1	0.7
6/7/42E/47/53	1	0.7
54/75/187	1	0.7
52/6	1	0.7
52/83A/75/53	2	1.4
42E/6	1	0.7
Total Group	9	6.3
Non Typable	87	61.7
Total Strains	141	

TABLE 8. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM THE THROAT

Group	No. of strains	Percentage
Group I:		
80	1	2.7
Group II:		
3A	2	5.5
71	3	8.3
3A/3C	2	5.5
3C/55/71	3	8.3
Total Group II	10	27.6
Group III:		
7	2	5.5
47/54/75/77	1	2.7
77	1	2.7
Total Group III	4	10.9
Group IV	0	0
Mixed Group		
52/53	1	2.7
75/81	1	2.7
Total Group	2	5.4
83 A	3	8.3
Non typable	16	44.4
Total strains	36	

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where close tolerances are required on assembled parts and where secure fastening is necessary for safety or proper continuing function, simple tools for disassembly are acceptable provided that all parts which require cleaning shall be accessible by use of the simple tools and the manufacturer's recommended cleaning procedures shall result in thorough cleaning and sanitizing of the equipment.

In addition to this amendment, a few minor changes incorporating, for the most part, previously approved wording or concepts in other Standards/Criteria were also recommended for approval by the Joint Committee.

Standard No. 2—Food service equipment

A review of the requirements of Item 4.10, General Design and Construction, of NSF Standard No. 2, as it related to corrugated transport carts was carried out. After discussion with the manufacturer's representatives and much deliberation, the public health representatives requested that an NSF Standards Task Committee be formed to further study this problem and recommend appropriate revisions in the Standard to delineate the design and construction requirements for this type of equipment.

According to the Foundation staff, the provision of Item 4.312 of NSF Standard No. 2 as it pertained to drain size of small bain maries and steam tables has created unnecessary problems for manufacturers. After a brief discussion, the public health representatives concurred in the concept of permitting drains as small as 1/2 inch for containers holding one gallon or less of liquid.

Standard No. 4—Commercial cooking and warming equipment

Item 4.01 of Standard No. 4 on cleanability of food contact surfaces was amended to read the same as stated under Criteria C-2.

The Foundation staff reviewed the need for clarification of the intent in the coverage of Standard No. 4 as it related to mobile barbecue equipment. After considerable discussion, it was agreed that Standard No. 4 was the appropriate document under which to evaluate such equipment, provided adequate attention was given to the need for protection of the equipment against dust during transportation via streets and highways from the basic commissary to the point of use.

The report of the Standards Task Committee for Fat Filters recommending deletion of requirements for removing particles

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