Enterotoxins and Phage Typing of *Staphylococcus aureus* Isolated from Clinical Material and Foods in Libya.

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ABSTRACT:

Enterotoxin was detected in 22 (61.1%) of the 36 *S. aureus* strains isolated from clinical materials and in 3 (13%) of the 23 *S. aureus* strains from food samples ($P<0.05$). On the basis of individual types of enterotoxin, staphylococcal enterotoxin A (SEA) was produced by 11.1%, SEB by 38.9% and SEC by 22.2% of *S. aureus* strains from clinical material. Of the food *S. aureus* strains, SEC and SED produced by 8.7% and 4.3% respectively. Of the clinical and food *S. aureus* strains 52.8% and 39.1%, respectively, were typeable by the 23 phages of International Phage Set. The majority of the typeable *S. aureus* strains from clinical and food sources belonged to group II being at 22.2% and 17.4% respectively. Furthermore, of the 14 SEB-producing *S. aureus*, 42.9% were of phage group II.

In conclusion the results obtained indicate that enterotoxin-producing *S. aureus* strains from clinical materials in Libya are not uncommon, however, certain foods appear not to be the source of such strains. Because of the low susceptibility to bacteriophages shown by *S. aureus* isolated in Libya, compared to reports from several countries, other methods of typing should be used in conjunction with phage typing in epidemiological investigations concerning this organism.

INTRODUCTION:

As a pathogen *Staphylococcus aureus* has an impressive armoury of extracellular virulence factors among which are the enterotoxins; the cause of food poisoning. These enterotoxins are designated A, B, C1, C2, C3, D and E (1). Because of their cross-reactivity the three C enterotoxins are therefore not usually analyzed separately (2).

As an epidemiological tool, phage typing of staphylococci has been used for many years in tracing the source of contamination in food poisoning outbreaks. In Europe and United States it is widely accepted that most of the *S. aureus* strains responsible for food poisoning (enterotoxin-producers) belong to types within phage group III and that the type of enterotoxin most frequently involved in food poisoning is staphylococcal enterotoxin A (3,4).

Although, production of enterotoxins and phage typing of *S. aureus* strains isolated from clinical materials and foods has been reported from many countries (5,6,7), to our knowledge, such information from Libya is lacking. Therefore, the aim of the present study is to provide, for the first time, data regarding the production of enterotoxins and phage types of *S. aureus* obtained from clinical and food samples in Tripoli, Libya.

MATERIALS AND METHODS:

1. Source of S aureus strains: Included in the present study, 36 *S. aureus* strains from clinical sources (ear, eye, blood, throat, umbilical and wound infections) and 23 strains from foods (ice cream, pizza, meat and tuna sandwitches). Throughout the study, the strains were kept on nutrient agar slants at room temperature.

2. Identification of S. aureus strains: Strains were grown on blood agar at 37^{o}C. After an overnight incubation the strains were examined for gram reaction and tested for catalase and coagulase using standard procedures as described previously (8). Coagulase-positive strains further tested by the Staphytect Plus kit (Oxoid, UK) as recommended by the manufacturer.

3. Detection of staphylococcal enterotoxins: Staphylococcal enterotoxins A (SEA), B (SEB), C (SEC), and D (SED) were detected by the SET-RPLA kit (Oxoid, UK). *S. aureus* strains were grown in tryptone soy broth (Oxoid) at 37^{o}C for 18-24 hours, with shaking. After growth, cultures were centrifuged at 900g for 20 minutes at 4^{o}C. Filtrates were then assayed immediately of toxin contents as recommended by the manufacturer. Positive and negative controls were included in all assays.

4. Bacteriophage typing: The International Phage Set (IPS) for typing human strains of *S. aureus* was used (9). This set contains 23 phages: 29, 52, 52A, 79, 80, 3A, 3C, 55, 71, 95, 6, 42E, 47, 53, 54, 75, 77, 83A, 85, 81, 94 and 96.

RESULTS:

Enterotoxin was detected in 22 (61.1%) of the 36 *S. aureus* strains isolated from clinical materials and in 3 (13%) of the 23 *S. aureus* strains from food samples. These differences are statistically significant ($P<0.05$, Chi-squares test). Of the clinical strains, 18 (50%) produced only a single enterotoxin and 4 (11.1%) produced two enterotoxin (Table 1). On the basis of individual types of enterotoxin, staphylococcal enterotoxin A (SEA) was produced by 4 (11.1%), SEB by 14 (38.9%) and SEC by 8 (22.2%) of *S. aureus* strains from clinical material. Staphylococcal enterotoxin D was not detected in the clinical strains. Of the food *S. aureus* strains, SEC and SED produced by 8.7% and 4.3% respectively. Staphylococcal enterotoxin B and SEC were not detected in *S. aureus* strains from food samples. The results obtained showed that SEB was present at a significantly higher rate among *S. aureus* strains from clinical than from food samples ($P<0.05$, Chi-squares test).

Nineteen (52.8%) of 36 clinical and 9(39.1%) of 23 food *S. aureus* strains were typeable by the IPS phages. The majority of the typeable *S. aureus* strains from clinical and food sources belonged to group II being at 22.2% and 17.4% respectively (Table 2). Furthermore, of the 14 SEB-producing *S. aureus*, 42.9% were of phage group II (Table 3).

DISCUSSION:

The role of staphylococcal enterotoxins in food poisoning is well established. However, their role in other infections is still controversial. Humphreys *et al* (10) found that enterotoxin production was higher among blood culture isolates, of *S. aureus*, from septicaemic patients than from nasal isolates from healthy individuals. Others (11) found no such difference. We detected enterotoxins in more than 61% of *S. aureus* strains isolated from clinical materials and it is worth mentioning that some of these strains were isolated from septicaemic patients and none of them were from healthy individuals. Recently, Al-Wali *et al* (12) compared enterotoxin production by *S. aureus* isolates from invasive infections and nasal carriers and reported that only SEB may be a significant factor in invasive infections. In the present study SEB was predominant; produced by nearly 40% of *S. aureus* strains isolated from clinical samples and it could be presumed that it played a role in the infections from where these strains were isolated.

Similar to findings of other workers (4), SEB was not detected in *S. aureus* strains from foods in the present work. However, a number of studies have shown that SEA is the predominant enterotoxin detected in *S. aureus* isolated from foods (7,13), and found in the food associated with approximately 75% of outbreaks due to *S. aureus* (3). Staphylococcal enterotoxin A was also not detected in our *S. aureus* strains from foods, which indicate that these types of foods (ice cream, pizza, meat and tuna sandwitches) may appear not to be the source of such isolates.

Contrary to reports from other countries (5,14), more than 50% of our *S. aureus* strains were untypeable by the IPS (Table 2). However, the non-

phage-typeable strains of *S. aureus* appear to be on the increase, particularly those from clinical material which lead typing centres in many countries to develop supplementary experimental phage sets (13). Several studies reported the predominance of phage group III among strains of *S. aureus* from clinical samples and foods (3,5,14). Among our strains of *S. aureus*, whether from foods or clinical materials, phage group II predominated. Furthermore, nearly 43% of the SEB-producing *S. aureus*, belonged to this phage group.

In conclusion the results obtained indicate that enterotoxin-producing *S. aureus* strains from clinical materials in Libya are not uncommon, however, certain foods appear not to be the source of such strains. Because of the low susceptibility to bacteriophages shown by *S. aureus* isolated in Libya, compared to reports from several countries, other methods of typing should be used in conjunction with phage typing in epidemiological investigations concerning this organism. More studies on a larger number of strains from other types of clinical materials and foods are needed to have a better idea on the role of enterotoxin-producing *S. aureus* in food-borne and other diseases in Libya.

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Table 1. Production of enterotoxins by *Staphylococcus aureus* strains isolated from clinical and food samples in Libya.

Source of *S. aureus*	No. tested	No. (%) positive for enterotoxin type: A	B	C	D	AB	BC	Total
Clinical	36	1(2.7)	10(27.8)	7(19.4)	--	3(8.3)	1(2.7)	22(61.1)
Food	23	--	--	2(8.7)	1(4.3)	--	--	3(13)
Total	59	1(1.7)	10(16.9)	9(15.3)	1(1.7)	3(5.1)	1(1.7)	25(42.4)

Table 2. Phage typing of *Staphylococcus aureus* isolated from clinical and food samples in Libya.

Source of *S. aureus*	No. tested	No. (%) of strains lysed by phage group: I	II	III	V	Miscellaneous	Mixed	Untypeable
Clinical	36	1(2.7)	8(22.2)	2(5.6)	3(8.3)	2(5.6)	3(8.3)	17(47.2)
Food	23	--	4(17.4)	3(13)	--	1(4.3)	1(4.3)	14(60.9)
Total	59	1(1.7)	12(20.3)	5(8.5)	3(5.1)	3(5.1)	4(6.8)	31(52.5)

Table 3. Phage typing and enterotoxin type of *S. aureus* strains from clinical material in Libya.

Enterotoxin type[a]	No. (%) of strains lysed by phage group: .					
	I	II	III	V	Miscellaneous	Mixed	Untypeable	Total
A	--	1(25)	1(25)	1(25)	1(25)	--	--	4(100)
B	1(7.1)	6(42.9)	1(7.1)	1(7.1)	--	--	5(35.7)	14(100)
C	--	1(12.5)	--	2(25)	--	1(12.5)	4(50)	8(100)

a= Staphylococcal enterotoxin D was not detected in *S. aureus* strains from clinical material.