

Enterotoxigenicity of *Staphylococcus aureus* Isolated from Food Handlers during Hajj Season in Saudi Arabia

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Abstract

Food poisoning during Hajj season is one of the main hazardous issues where most of the health services in Saudi Arabia are targeting to minimize every year during Hajj seasons. Ordinarily, food handlers are subjected to medical examination before assignment to work. However, they are mostly lacking proper training in food handling operations, mass feeding, and sanitary practices. This situation may encourage causing food poisoning especially with staphylococcus enterotoxins. 1516 clinical specimens from food handlers of different nationalities in Makkah were microbiologically investigated for bacterial pathogens during the hajj seasons of 2001-2002 in Makkah, Saudi Arabia. 129 *Staphylococcus aureus* isolates were isolated. Of which, 35% produced enterotoxins A, B, C and D singly or in pairs, when such enterotoxins were evaluated by Reversed Passive Latex Agglutination test (RPLA). Enterotoxins C and A, elaborated by 15.5% and 12.4%, isolates respectively, which showed the highest percentage. They were mostly isolated from nasal swabs than throat swabs. All isolates were resistant to Penicillin G. On the other hand, they were sensitive to Clindamycin, Oxacillin and Gentamicin when tested by Kirby-Bauer method. The (RPLA) method yielded satisfactory results.

Keywords: *Staphylococcus aureus*, Makkah, Hajj, Enterotoxin

1. Introduction

Staphylococcal intoxication worldwide stands out as one of the main food-borne diseases [1]. Ten Staphylococcal enterotoxins, enterotoxin ASEA [2], SEB [3] SEC 1 [4], SEC 2 [5], SEC 3 [6], SED [7], SEE [8], SEH [9], SEG (10), SEI [11], SEJ [12], SEK [13], SEL [1], SEM, SEN, SEO [14] have been identified and there is a possibility to identify more SEs [15]. The majority of those enterotoxin-positive strains most probably belong to human staphylococci [16]. Characterization of those SEs has been hindered because of the lack of reliable and rapid testing method. On the other hand, most foods implicated in staphylococcal food poisoning outbreaks contain low levels of enterotoxins; often less than 1 µg/100 g of food [6].

Food safety is one of the most important concern of health authorities in the Kingdom of Saudi Arabia during

Hajj. Hajj (pilgrimage season in Makkah) is a period when more than 2.5 million people gather in this Holy city for at least five days to perform religious rituals of worship. Previous unpublished data has been shown that Staphylococcal food intoxication is the most common food poisoning, followed by Salmonella gastroenteritis. On account of large public gathering with huge amounts of food prepared and consumed, food handlers may neglect hygienic practice and may be considered as a major source of food contamination; they may contaminate raw materials, equipments, and expired products [17] and often do not have visible lesions.

The primary aim of this study is to screen food handlers for coagulase positive staphylococci and to determine the enterotoxigenicity of these isolates by the RPLA test. Before the identifications of SEH, SEG and SEI about 5% of food-borne staphylococcal outbreaks are caused by unidentified SEs [8]. Therefore. The

RPLA test still the most reliable and rapid testing method especially when the time is an important factor. Also to use this isolated strains in further studies [18].

2. Materials and Methods

2.1. Organisms

A total of 1516 clinical specimens were isolated from different nationality food handlers during the Hajj seasons (2001-2002) in Makkah, Saudi Arabia including 428 nasal swabs, 428 throat swabs, 428 nail swabs, 228 stool samples and 4 wounds swabs, were examined for presence of *Staphylococcus aureus*. Organisms were isolated on sheep blood agar and mannitol salt agar for stool samples.

2.2. Characterization of Strains

Colonies resembling staphylococci and consisting of catalase-positive, gram-positive cocci in clusters were tested for clumping factor and staphylococcal protein A by Staphaurex kit purchased from Murex Diagnostics Limited.

2.3. Enterotoxin Detection

The strains were cultured in Brain Heart Infusion (BHI) at 37°C for 18 – 24 h, after centrifuging at 3000 rpm at 4°C for 20 min. The supernatant was used for enterotoxin evaluation. The staphylococcal enterotoxins A, B, C, and D were detected by using an RPLA diagnostic Kit from DENKA SEIKEN CO. Because these kits showed high specificity and sensitivity with a detection limit of 0.75 ng, the test was completed within 24 h and neither required complicated procedures nor expensive equipments [18].

2.4. Antimicrobial Sensitivity Determinations

A total of 45 *Staphylococcus aureus* enterotoxin produc-

ers cultures were tested for sensitivity to the following Antimicrobial agents :Penicillin G (PG), Oxytetracycline (OT), Gentamicin (GM), Erythromycin (E), Cotrimoxazole (TS), Oxacillin (OX), Clindamycin (CD), and Cephalothin (KF) by Kirby-Bauer method (12). (Sensitivity multi.Disk for Gram (+) microorganism were obtained from Mast Group Ltd.).

3. Results

Of the 129 *Staphylococcus aureus* isolates from 1516 clinical specimens from different nationalities foodhandlers in Makkah, 45 produced one or more enterotoxins. Most strains produced only one type of enterotoxin but few produced two enterotoxins simultaneously. The incidence of enterotoxins A, B, C, and D production in strains of *Staphylococcus aureus* is shown in **Table 1**. Thirty eight strains (29.5%) produced only a single enterotoxin; of these, 16 strains produced enterotoxin A, one produced B, 20 produced C and only one produced enterotoxin D. Seven strains (5.4%) produced > 1 enterotoxin; 3strains produced A with B, 3 strains produced A with C and only one strain produced C and D

The Sensitivity results of 45 *Staphylococcus aureus* enterotoxins producers tested in this study fell in to 7 groups. Every group has the same results as shown in **Table 2**.

4. Discussion

Enterotoxins were shown to be produced by 35% of the strains isolated from 29% of the total working food handlers who look healthy. These results are in agreement with those obtained by Wei and Chiou [18].

Enterotoxin C showed the highest incidence 15.5%. Some researchers have reported that the source of these enterotoxin C producing strains is unclear (6). On the other hand, some researchers have related Enterotoxins C to strains of animal origin [19]. But the interchange of the staphylococci between animals and man might explain the high incidence of SEC. Enterotoxins A, B and

Table 1. The incidence of enterotoxins A, B, C, and D production in isolated strains of *Staphylococcus aureus*.

Source of strains	No. of strains producing enterotoxins	Enterotoxins produced according to source						
		A	B	C	D	AB	AC	DC
Nasal swabs	29	11	1	11	1	2	2	1
Throat swabs	9	2	-	5	-	-	-	-
Nail swabs	6	3	-	3	-	-	-	-
Stool samples	1	-	-	1	-	-	-	-
Total	45	16	1	20	1	3	3	1

Table 2. *Staphylococcus aureus* isolates classified according to antimicrobial sensitivity test.

<i>S. aureus</i> strains No. with same sensitivity	Type of Enterotoxin	KF	CD	OX	TS	E	GM	OT	PG
11 Strains from No. 1 to 11	C								
14 Strains from No. 12 to 25	A								
3 Strains from No. 26 to 28	AB	S	S	S	S	S	S	S	R
2 Strains No. 29 & No. 30	AC								
Strain No. 31	D								
Strain No. 32	DC								
5 Strains from No. 33 to 37	C								
Strain No. 38	AC	S	S	S	R	S	S	S	R
Strain No. 39	B								
Strain No. 40	C	R	S	S	R	S	S	S	R
Strain No. 41	A	S	S	S	S	R	S	S	R
Strain No. 42	A	S	S	S	S	S	S	R	R
Strain No. 43	C								
Strain No. 44	A	R	S	S	S	S	S	S	R
Strain No. 45	C	S	S	S	R	S	S	R	R

Penicillin G (PG), Oxytetracycline (OT), Gentamicin (GM), Erythromycin (E), Cotrimoxazole (TS), Oxacillin (OX), Clindamycin (CD), and Cephalothin (KF).

AB elaborated by 12.2%, 0.7% and 2.3% respectively which agreed with the findings of other researchers [6]. An interesting observation is that we isolated some strains from different locations of three food handlers (Nasal, Throat, Stool) (Nasal, Throat, nails) (Nasal, nails), type C, A and A respectively with the same sensitivity results. On the other hand, we isolated three different strains from different locations (Nasal, Throat, nails) of one female food handler.

This figure of 35% may be on the lower side of the scale as other enterotoxins SEE, SHE, SEG and SEI were omitted from the study because simple detection method was not available. Also, coagulase negative staphylococci were not subjected to enterotoxin evaluation and they have been reported that few of those strains are enterotoxigenic [16].

So, it may be concluded that 50% of the isolates could be enterotoxigenic. Also, during Hajj periods food handlers become under pressure to prepare large quantities of food. Thus may neglect the instruction on hygienic standards. In addition, those with poor hygienic practices can contaminate the food which they handle [17] and some of visitors buy food to be consumed later after several hours allowing small numbers of staphylococci to multiply and thus produce enterotoxins. All that indicate that in the case of food poisoning, it's necessary to detect the food and food handlers for enterotoxigenicity of staphylococci because their presence does not necessary imply that enterotoxin was produced. Many strains are not enterotoxin producer. Similarly, the absence of viable staphylococci in food does not mean that toxin is also absent. Because enterotoxins are more heat stable than producing cells and can be present in heated foods, the

organisms isolated were sensitive to most of the Antimicrobial agents except Penicillin G (PG). No MRSA nor Oxacillin (OX) resistant strains were isolated.

5. Conclusions

This study shows that the total ratio of both Arabs and Asians carriers are nearly the same (8.7 and 8.9 respectively). This indicates that bad habits such as; picking nose (fingering the nose), nasal secretions and spitting on the ground could be the reason for increasing the ratio of staphylococcal intoxication. However, the predominance of specific Enterotoxins type among *S. aureus* isolates from human carriers is variable. Most of enterotoxigenic *S. aureus* were isolated from the nose which could facilitate the transmission of this organism from nose to hand during unhygienic cleaning habits. The sensitivity results indicate the susceptibility of one person to harbor or be infected with more than one strain of *S. aureus* which reflects the importance of proper microbiological investigation to achieve proper and successful treatment. This study emphasizes the importance of implementing a health certification process for food handlers supported with a proper training and educational agenda.

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7. References

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