National Veterinary Institute

How many *Staphylococcus aureus* isolates should be tested for entereotoxin production?

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Introduction

Staphylococcus aureus **intoxication is a common food- borne illness, but the number of cases reported is probably underestimated. The detection of *S. aureus* enterotoxin (SET) in food is often difficult. Most SET detection methods are immunological, such as the technique of reversed passive latex agglutination (SET-RPLA), commonly used for detection of SET A-D from bacterial strains. Usually, only one isolate per suspected sample is tested for SET production. The aim of the study was to find out if testing of up to ten colonies from each food sample might increase the identification of food sources for staphylococcal intoxication.**

Table 1. Number of enterotoxin producing *Staphlococcus aureus* isolates found in raw milk and raw milk cheeses by use of SET-RPLA and multiplex PCR.

Origin	No. of samples	No. of isolates	SET-RPLA		PCR	
			no. of SET positiv		no. of SET positiv	
			samples	isoaltes	samples	isolates
Goat milk	5	50	3	24	3	24
Cow milk	10	100	2	12	4	23
Cheese	7	70	2	2	6	32
Total	22	220	7	38	13	79

Results and discussion

A total of 220 *S. aureus* isolates obtained from 22 samples of raw cow and goats milk, and raw milk cheeses were investigated for SET production (Table 1). Results (Table 2) show various abilities of *S. aureus* to produce SET among isolates from the same sample depending on agar media (BP-RPF and BA) or method (SET-RPLA and multiplex PCR) used. For example in one sample of raw milk cheese sample (nr. 400) one out of five isolates obtained from BP-RPF produce SET D, while none of the isolates from blood agar produce SET by use of SET-RPLA. Multiplex PCR-technique confirmed SET-RPLA results and revealed more positive SET isolates (B, I, G, J) from both, RPF-BP and BA (Table 2, Fig. 1). Characterisation of these ten isolates by REA-PFGE (Fig. 2) showed that two isolates producing SETG,B and G had identical DNA restriction pattern (clone .). Four other isolates producing SETI and G, from the same cheese, also displayed identical pattern (clone .). The other isolates were different clones of *S. aureus*.

We know from previous studies that the same food sample can harbour different serovars and clones of the same bacterial species. In the present study, six samples had between two to six different SET producing *S. aureus* isolates and up to six different REA-PFGE patterns.

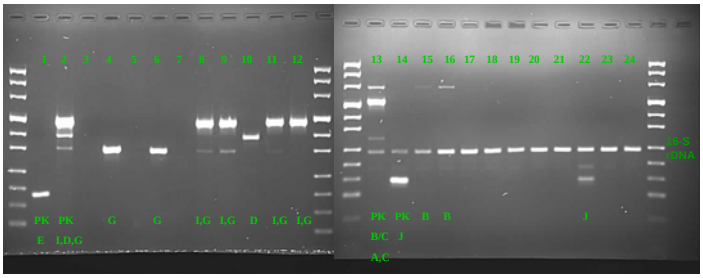

Fig. 1. SET genes in *S. aureus* isolates (line 3 to 24) from cheese sample nr. 400 obtained by multiplex PCR.

Materials and methods

Altogether 15 samples of raw cow (10) and goats (5) milk and 7 raw milk cheeses were analysed for *S. aureus* by use of Baird Parker agar with rabbit plasma fibrinogen supplement (Bio Mérieux) (BP-RPF) and blood agar (BA). From each sample and each agar plate (BP-RPF and BA) 5 *S. aureus* isolates were identified and examined for SET production by SET-RPLA.

Multiplex PCR-technique for detection of genes coding for SET types A-E and G-J was used to confirm results obtained by SET-RPLA, as well as for the presence of other SET than A-D. Restriction endonuclease analysis (REA) with Pulsed Field Gel Electrophoresis (PFGE) was used to find out if the isolates producing SET share the identical pattern as the isolates that do not produce SET.

Table 2. Number and type of *Staphlococcus aureus* enterotoxins of isolates obtained from BP-RPF and BA by use of SET-RPLA and multiplex PCR.

Origin	No.	SET-RPLA no. of positive isolates from							Multiplex PCR no. of positive isolates from														
		BP-RPF				BA			BP-RPF							BA							
		A	B	C	D	A	B	C	D	A	B	C	D	E	G	I	J	A	B	C	D	E	G	I	J
Goat milk	2394			4								4													
	2396			5				5				5								5					
	2399			5				5				5								5					
Cow milk	2204			5				5				5								5					
	SK254														1	5							3	5	
	SK262																	1							
	SK265			2								2													
Cheese	29-1														5	5							1		
	29-2														5	5									
	29-3														5	5									
	29-4														4	4							2	1	
	400				1								1		4	4	1		2				2		
	882-1							1												1					

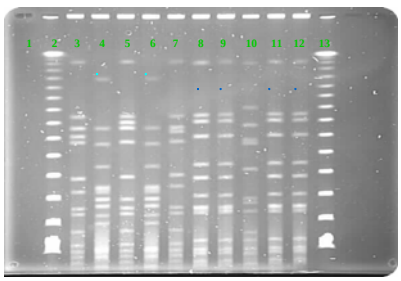

Fig. 2. PFGE profiles of *S. aureus* isolates obtained from cheese sample nr. 400. Isolates nr. 319 producing SETG and B (line 4) and 321 producing SETG (line 6) had identical DNA restriction patterns (clone .). Isolates nr. 323, 324, 326 and 327 producing SETI and SETG (lines 8, 9, 11 qnd 12) displayed identical DNA restriction patterns (clone .).

Conclusion

The results indicate that conventional testing of only one isolate per sample, could miss SET producing isolates of *S. aureus*. Therefore, it is necessary to test several isolates of *S. aureus* for toxin production in cases where typical symptoms of SET intoxication are observed. Methods with increased sensitivity for detection of SET and ability to reveal "new" SET (G-J), such as multiplex PCR, should be used. Furthermore, REA-PFGE was shown to be an effective tool for studying genetic relationship between *S. aureus* isolates producing SET in epidemiological investigations.