An evaluation of the enterotoxigenic status of bovine, ovine, human and food isolates of *Staphylococcus aureus*

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Background

- *Staphylococcus aureus* is an important food-borne pathogen because of its ability to produce a wide range of extracellular protein toxins and virulence factors that contribute to the pathogenicity of the organism.
- Of particular relevance to the food processing industry is the ability of some *S. aureus* strains to produce heat stable enterotoxins that cause staphylococcal food poisoning (SFP), which ranks as one of the most prevalent causes of gastroenteritis worldwide.

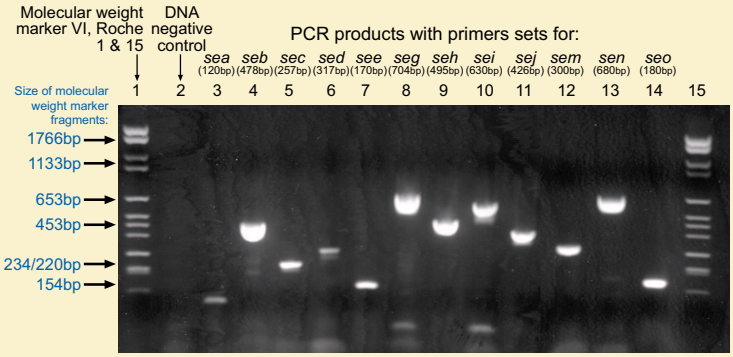
Aim

- **To investigate the prevalence of 'classic' (*sea* to *see*) and 'new' (*seg, seh, sei, sej, sem, sen* and *seo*) staphylococcal enterotoxin (*se*) genes from bovine, ovine, human and food isolates of *S. aureus* using PCR based procedures.**
- **To determine expression of the genetic determinants for SE enterotoxin (Types A-E) in a subset of isolates by immunological methods.**

Methodology

- A panel of 90 *S. aureus* strains were collected from bovine (hide, nose, carcass and meat cut), mastitis milk, ovine (nose, carcass, meat cut and processing surfaces), human (nose and clinical samples), and New Zealand Institute of Environmental Science and Research reference collection.
- PCR was performed with primers specific for 12 *S. aureus* enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei, sej, sem, sen* and *seo*).
- TECRA ELISA-based method was used with a subset of isolates to detect enterotoxin in isolates found to positively contain *se* genes by PCR.

Agarose gel electrophoresis of *se* gene PCR products



Results

- Overall, 61 (67.7%) *S. aureus* isolates were found to be positive for one or more *se* genes and 17 distinct *se* genotypes were identified with these isolates.

Detection of *se* genes encoding different SE types in *S. aureus* isolates from various sources

Result of PCR testing	Number of strains from: Human	Ovine	Bovine	Mastitis milk	Food poisoning	Total *se* genes detected
seo positive	16	5	1			22
sem positive	15	4	1			20
sen positive	15	4	1			20
sei positive	15	4	1			20
seg positive	15	4	1			20
seh positive	5	1	5	6		17
seb positive	5	3	7			15
sea positive	5	1	1	1	3	11
sec positive	2	8				10
sej positive	1	3			2	6
sed positive	1	3			2	6
see positive		1			1	2

Genes were found to co-exist in 17 different genotypes

seb
seg sei sem sen seo
seh
sec
sed seg sei sej sem sen seo
sea seh
sea
sea sed sej
seb seg sei sem sen seo
see
seg seh sei sem sen seo
sea seb
sea seg sei sem sen seo
seo
sea sec seg seh sei sem sen seo
sec seo
sec seg seh sei sem sen seo

- Thirtythree isolates possessed only one kind of *se* gene and 28 isolates harboured more than one *se* gene.
- Human strains more frequently harboured *se* genes (80.7%) than strains from bovine (61.9%) or ovine (63.0%) sources.
- The percentages of human, bovine, mastitis milk and ovine *S. aureus* strains that harboured *se* genes increased respectively from 42.3%, 38.1%, 8.3% and 59.2% to 80.7%, 61.9%, 50.0% and 63.0% when 'new' *se* genes were considered.
- 'New' *se* genes were detected in 37 isolates.
- The most commonly detected *se* genotype was *seb.*
- *seg, sei, sem, sen* and *seo* co-existed in all *seg-sei* positive isolates (n=20). This gene combination was significantly (p=<0.01) more frequently associated with human isolates (57.7%) than animal strains (10.4%).
- *sej* was found to co-exist in all isolates positive for *sed.*
- A 100% correlation was observed, between detection of enterotoxin genes A - E and expression of corresponding entertoxin proteins *in vitro.*

Elisa detection of SE's produced by pure cultures of *S. aureus* and enterotoxin genes by PCR

Isolate	Non-specific SE detection	Specific SE detection Type A, B, C, D & E	PCR SE gene detection *sea, seb, sec, sed, see, seg, seh, sei, sej, sem, sen & seo*
C1	+	A	*sea*
C2	-	-	*seg, sei, sem, sen, seo*
C3	+	A and D	*sea, sed, sej*
C4	+	B	*seb*
C5	+	C	*sec, seg, seh, sei, sem, sen, seo*
C6	+	E	*see*
B1	+	B	*seb*
B2	+	B	*seb*
B3	+	B	*seb*
B4	+	B	*seb*
B5	+	B	*seb*
B6	+	B	*seb*
B7	+	A	*sea, seh*
B8	+	B	*seb*
B9	-	NT	*seg, sei, sem, sen, seo*
B10	-	NT	*seh*
B11	-	NT	ND
B12	-	NT	ND
B13	-	NT	ND
B14	-	NT	ND
B15	-	NT	ND
B16	-	NT	ND
B17	-	NT	*seh*
B18	-	NT	ND

-, Negative +, Positive ND, None detected NT, Not tested

Conclusion

- **This study demonstrates that the newly described *se* genes are widely distributed among *S. aureus* strains and that human strains more frequently harboured *se* genes than bovine or ovine strains.**
- **Consideration of the 'new' SE's in this study resulted in a 23.3% increase in potentially enterotoxigenic strains.**
- **The presence of enterotoxin genes does not demonstate the ability to produce SE protein, however it is now thought likely that the role of 'new' SE's in SFP outbreaks has been underestimated.**
- **Currently, the detection of SE proteins is expensive and limited to only certain SE's (SEA-SEE). The DNA based approach used in this study was relatively inexpensive, efficient and capable of detection of all SE genetic determinants for which gene sequence data was available.**

Acknowledgments
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