

ORIGINAL ARTICLE

Molecular Detection of Virulence Associated Genes in Coagulase Negative Staphylococci Isolated from Blood Culture

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SUMMARY

Background: Coagulase-negative staphylococci (CoNS) are one of the most important causes of infections. Unlike *Staphylococcus aureus*, less is known about their pathogenic mechanisms. In the present study, we aimed to evaluate the presence of virulence genes among 98 CoNS isolated from blood cultures of inpatients.

Methods: The isolates were identified by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). PCR was performed to detect 29 virulence factors using specific primers for *icaA*, *icaB*, *icaC*, *icaD*, *icaADB*, *aap*, *fbe*, *aae*, *sesI*, *atIE*, *hla*, *hnb*, *hld*, *gehC*, *gehD*, *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *tst*, *eta*, *etb*, *etd*, *etx*, and *pvl* genes. The VITEK2 system (bio-Merieux, France) and the BD Phoenix™ System (Becton Dickinson, USA) were used for antimicrobial susceptibility testing.

Results: *Staphylococcus epidermidis* was found to be the most virulent CoNS species. All isolates were negative for *eta*, *etb*, *etd*, *sea*, *seb*, *sed*, *see*, *seg*, *sei*, and *pvl* virulence genes. We detected up to 15 virulence genes in a single isolate. The most common gene was *icaC* (73.5%), followed by *icaA* (57.1%), *icaD* (56.1%), *aap* (55.1%), *aae* (52.0%), *sesI* (51.0%), *gehC* (50.0%), *hld* (50.0%), *hnb* (49.0%), *fbe* (44.9%), *atIE* (37.8%), *icaADB* (37.8%), *gehD* (34.7%), *icaB* (31.6%), *hla* (30.6%), *etx* (2.0%), *sec* (1.0%), *seh* (1.0%), and *tst* (1.0%).

Conclusions: We determined high rates of genes encoding biofilm formation. Only four isolates did not possess either the *ica* operon or *aap* gene. Although we found low rates of toxin-related genes, our data indicates that apart from biofilm formation, the CoNS isolates could express various virulence genes similar to those of *Staphylococcus aureus*.

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Supplementary Tables and Figures

Supplemental Table 1. Primers used for the detection of virulence genes.

Target gene	Primers	Primer sequence (5' - 3')	Product size, bp	Reference
icaA	icaAF	ACAGTCGCTACGAAAAGAAA	103	[22]
	icaAR	GGAAATGCCATAATGAGAAC		
icaB	icaBF	CTGATCAAGAATTTAAATCACAAA	302	[22]
	icaBR	AAAGTCCCATAAGCCTGTTT		
icaC	icaCF	TAACTTTAGGCGCATATGTTT	400	[22]
	icaCR	TTCCAG TTAGGCTGGTATTG		
icaD	icaDF	ATGGTCAAGCCCAGACAGAG	198	[22]
	icaDR	CGTGTTTTCAACATTTAATGCAA		
icaADB	icaADBF	TTATCAATGCCGAGTTGTC	546	[9]
	icaADBR	GTTTAACGCGAGTGCGCTAT		
aap	aapF	ATACAACGGTGCAGATGGTTG	400	[9]
	aapR	GTAGCCGTCCAAGTTTTACCAG		
aae	aaeF	GAGGAGGATTTTAAAGTGC	858	[9]
	aaeR	AACATGACCATAGTAACC		
atIE	atIEF	CAACTGCTCAACCGAGAACA	682	[23]
	atIER	TTGTAGATGTTGTGCCCA		
fbe	fbeF	TAAACACCGACGATAATAACCAAA	496	[9]
	fbeR	GGTCTAGCCTTATTTTCATATTCA		
sea	sea-1	TTGAAAACGGTTAAAACGAA	120	[24]
	sea-2	GAACCTTCCCATCAAAAACA		
seb	seb-1	TCGCATCAAACCTGACAAACG	478	[24]
	seb-2	GCAGGTACTCTATAAGTGCC		
sec	sec-1	GACATAAAAGCTAGGAATTT	257	[24]
	sec-2	AAATCGGATTAACATTATCC		
sed	sed-1	CTAGTTTGGTAATATCTCCT	317	[24]
	sed-2	TAATGCTATATCTTATAGGG		
see	see-1	CAAAGAAATGCTTTAAGCAATCTTAGGCCAC	170	[25]
	see-2	CTTACCGCCAAAGCTG		
seg	seg-1	AATTATGTGAATGCTCAACCCGATC	642	[25]
	seg-2	AAACTTATATGGAACAAAAGGTACTAGTTC		
seh	seh-1	CAATCACATCATATGCGAAAGCAG	376	[25]
	seh-2	CATCTACCCAAACATTAGCACC		
sei	sei-1	CTCAAGGTGATATTGGTGTAGG	576	[25]
	sei-2	AAAAAACTTACAGGCAGTCCATCTC		
tst	tst-1	AGCATCTACAAACGATAATATAAAGG	481	[26]
	tst-2	CATTGTTATTTTCCAATAACCACCCG		
eta	ET-1	CTATTTACTGTAGGAGCTAG	741	[27]
	ET-2	ATTTATTTGATGCTCTCTAT		
etb	ET-3	ATACACACATTACGGATAAT	629	[27]
	ET-4	CAAAGTGTCTCCAAAAGTAT		
etd	ET-14	AACTATCATGTATCAAGG	376	[27]
	ET-15	CAGAATTTCCCGACTCAG		

Supplemental Table 1. Primers used for the detection of virulence genes (continued).

Target gene	Primers	Primer sequence (5' - 3')	Product size, bp	Reference
etx	ETxF	CAACCTGAACTATCTATAAG	810	this study
	ETxR	CAACTACTTTATTATCTCCG		
pvl	luk-pv1	ATCATTAGGTA AAAATGTCTGGACATGATCCA	433	[28]
	luk-pv2	GCATCAASTGTATTGGATAGCAAAAAGC		
gehC	gehCF	CAAAAGATAGCCAATCAACAG	830	this study
	gehCR	GCGTACAATCGCTTCGTTACC		
gehD	gehDF	TTTGAATTCTGCGCAAGCTCAATATAA	1179	[9]
	gehDR	TTTGCGGCCGCTATCGCTACTTACGTGTAA		
sesI	SesIF	GCTGATTATGTAATGACTCAAAT	408	[9]
	SesIR	AGCTTTTGTTGTTTGAGCTTC		
hla	hla_haem-1	TGGGCCATAAACTTCAATCGC	72	[25]
	hla-haem-2	ACGCCACCTACATGCAGATTT		
hlb	hlb_epid-1	TGGTGGCGTTGGTATTGTGA	541	[25]
	hlb_epid-2	ACCCCAAGATTTACGGACC		
hld	hld-epid-1	ATGGCAGCAGATATCATTTTC	444	[25]
	hld-epid-2	CGTGAGCTTGGGAGAGAC		