Prevalence of *Staphylococcus succinus* and *Staphylococcus equorum* in Nasal Swab Isolates

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Introduction

Staphylococcus equorum and *Staphylococcus succinus* are bacterial species commonly associated with livestock and certain food products. Human infection as a result of these species is rarely reported and there are currently no reports citing these species as components of the normal human flora. During the summer of 2010, nasal swabs were taken from volunteers at various hospitals and communities in Ecuador. Bacteria isolated from these samples were initially characterized using cultural methods. Isolates that were mannitol fermenters and oxacillin sensitive were further characterized using multiplex PCR and XapI restriction fragment length polymorphisms of the dnaJ gene. Approximately 17% of samples analyzed thus far contain either *S. succinus* or *S. equorum*. These preliminary data suggest that these species can at least be transient members of the human nasal flora and can possibly be established as a more stable component.

Methods

Urea Hydrolysis: Samples were grown in 3.87% urea broths containing phenol red indicator at 37º C for 24 hours to characterize urea hydrolysis.

Sugar Fermentation: Samples were grown in 1% solutions of sucrose, xylose, dextrose, sorbitol, and lactose with a phenol red indicator to characterize sugar fermentation.

Restriction Digest PCR of *dnaJ* Gene: Isolated DNA from the samples was used in a PCR with *dnaJ* primers to amplify the *dnaJ* gene, which was then digested with the enzyme XapI and run on a 2% agarose gel. Each species of *Staphylococcus* can be identified based on characteristic restriction fragment length polymorphisms. (1).

PCR Confirmation of Potential MRSA Isolates: DNA was isolated from potential MRSA isolates and used in a multiplex PCR assay to confirm culture based identifications. The multiplex PCR utilized three pairs of primers which amplify conserved portions of the *mecA*, *FemB*, and 16S *rRNA* genes. PCR products were analyzed on a 2% agarose gel (2).

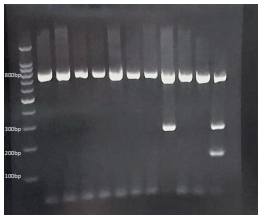

Figure 1. *Staphylococcus* species identification using Multiplex PCR. Lane 1- 100bp Molecular Weight Marker. Lane 2- IBM029. Lane 3- IBM032. Lane 4- IBM078. Lane 5- IBM100-L. Lane 6- IBM100-S. Lane 7 - IBM 107. Lane 8 – IBM126. Lane 9 – IBM002-S. Lane 10 – IBM097. Lane 11 - ICQ104. Lane 12 – FAA1026.

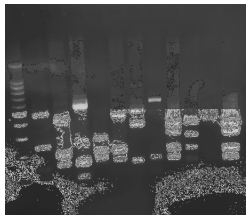

Figure 2. XapI digest of PCR amplification of dnaJ gene. Lane 1- 100bp Molecular Weight Marker. Lane 2- IBM029. Lane 3- IBM032. Lane 4- IBM078. Lane 5- IBM100-L. Lane 6- IBM100-S. Lane 7 - IBM 107. Lane 8 – IBM126. Lane 9 – IBM002-S. Lane 10 – IBM097. Lane 11 - ICQ104. Lane 12 – FAA1026.

	Species
IBM029	S. pasteuri
IBM032	S. saprophyticus
IBM078	S. scuiri
IBM100-L	S. hominis
IBM100-S	S. kloosi
IBM107	S. epidermidis
IBM126	S. ludgenisis
IBM002-S	S. aureus
IBM097	S. succinus
ICQ104	S. equorum
FAA1026	S. aureus (MRSA)

Table 1. Species identifications of isolates digested with the XapI restriction enzyme

Sugar Solution	H023 (*equorum*)	H109 (*succinus*)	H197–alpha (*equorum*)	H197-beta (*equorum*)	H274-S (*aureus*)	S. *aureus*
Sucrose	+	+	+	+	+	+
Xylose	-	+	-	-	-	-
Dextrose	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-
Lactose	+	+	+	+	+	+

Table 2. Sugar fermentation results of samples in 1% sugar solutions.

	H023	H109	H197-alpha	H197-beta	H274-S	S. *aureus*
Hydrolysis	-	+	-	-	-	-

Table 3. Urea hydrolysis

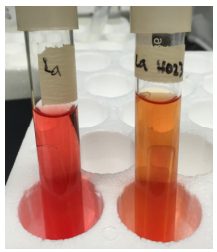

Figure 3. Lactose fermentation. Negative control shown on left; positive result (H023) shown on right.

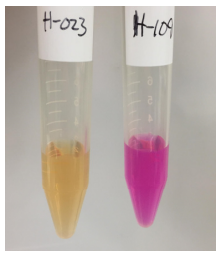

Figure 3. Urea hydrolysis. Negative result shown on left (H023); positive result shown on right (H109).

Discussion

We were able to confirm that each isolate belongs to the genus *Staphylococcus* by amplifying the 16s rRNA, *femB*, and *mecA* genes in a multiplex PCR reaction (Figure 1). Isolates which produced a single band at ~800bp contain the 16s rRNA that is characteristic of *Staphylococcus* species; isolates which also produced a band at ~300bp contain the *femB* gene which is characteristic of *S. aureus.* Finally, isolates that produced at band at ~150bp carry the *mecA* gene and are, therefore, resistant to methicillin (5). Species identification was done using a XapI restriction digest of the *dnaJ* gene. A total of 63 samples were analyzed, approximately11.1% were S. *succinus* and 1.5% were S. *equorum.*

Initial analysis of hospital samples were characterized using sugar fermentation and urea hydrolysis tests (3). Sugar fermentation tests revealed that all species analyzed can ferment sucrose, dextrose, and lactose. None of the species analyzed are capable of fermenting sorbitol. *S. succinus* (H109) is the only isolate capable of fermenting xylose. Urea hydrolysis tests were used to differentiate urease-positive bacteria from urease-negative bacteria. The results demonstrate that most samples are urease-negative, with the exception of H109, and thus cannot utilize urea as an energy source.

References

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Acknowledgements

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