

# Prevalence of *Staphylococcus succinus* and *Staphylococcus equorum* in the Anterior Nares of Volunteers from the Loja Community of Ecuador



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## Introduction:

*Staphylococcus equorum* and *Staphylococcus succinus* are bacterial species commonly associated with livestock including equine and avian species, also 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. The samples analyzed during Fall 2017 and Spring 2018 were from Loja community. 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 14% 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:

### Bacterial Growth:

Samples of the bacteria from nasal swabs that have been kept in a glycerol media at -80 degrees Celsius are inoculated into Mueller Hinton broth and incubated at 37C overnight, then onto mannitol salt agar plates. A single colony is selected from the plate and again grown overnight in Mueller Hinton broth for DNA isolation. Colonies are also inoculated onto plates containing oxacillin to determine whether or not the species is resistant to oxacillin.

### Isolation of DNA:

DNA is isolated from the cells using a lysostaphin based procedure (1). A spectrophotometer is then used to quantify the amount of DNA. Agarose gel electrophoresis is used to confirm the results of the spectrophotometer showing that DNA has successfully been isolated.

### Multiplex Polymerase Chain Reaction:

A PCR mixture containing three primer pairs are used to amplify the 16s *rRNA*, *femB*, and *mecA* genes (2). Agarose gel electrophoresis is used to separate the PCR products and the gel is imaged. This is used to confirm that the species are a type of *Staphylococcus*, *Staphylococcus aureus*, or Methicillin Resistant *Staphylococcus aureus* (MRSA).

### Restriction Fragment Length Polymorphism Analysis:

The genomic DNA isolated is then used in an additional PCR to amplify a portion of the *dnaJ* gene followed by a restriction enzyme XapI digest (1), and the fragments are separated on an agarose gel using electrophoresis. The pattern of fragments produced is unique to specific bacterial species and is compared to controls of known species for identification.

## Results:

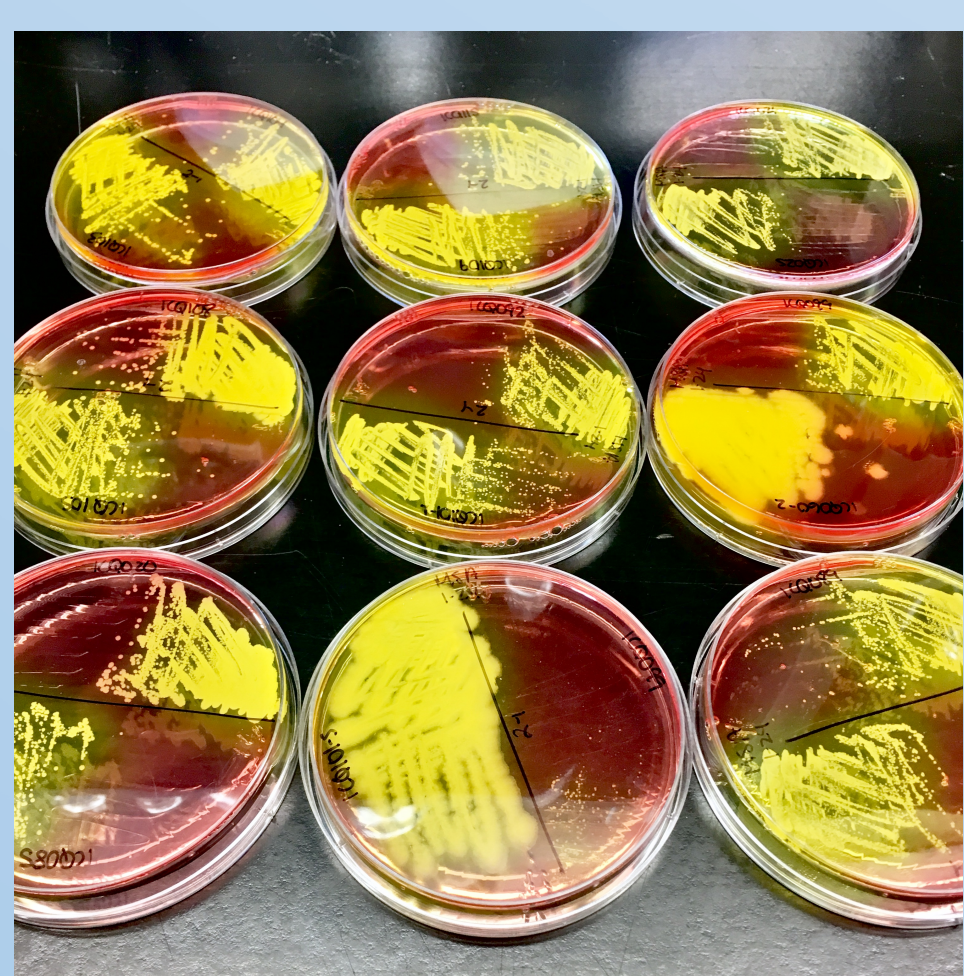


Figure 1: Bacterial growth on mannitol salt agar plates. Color change from red to yellow indicates fermentation of mannitol and limits the study to mannitol fermenters.

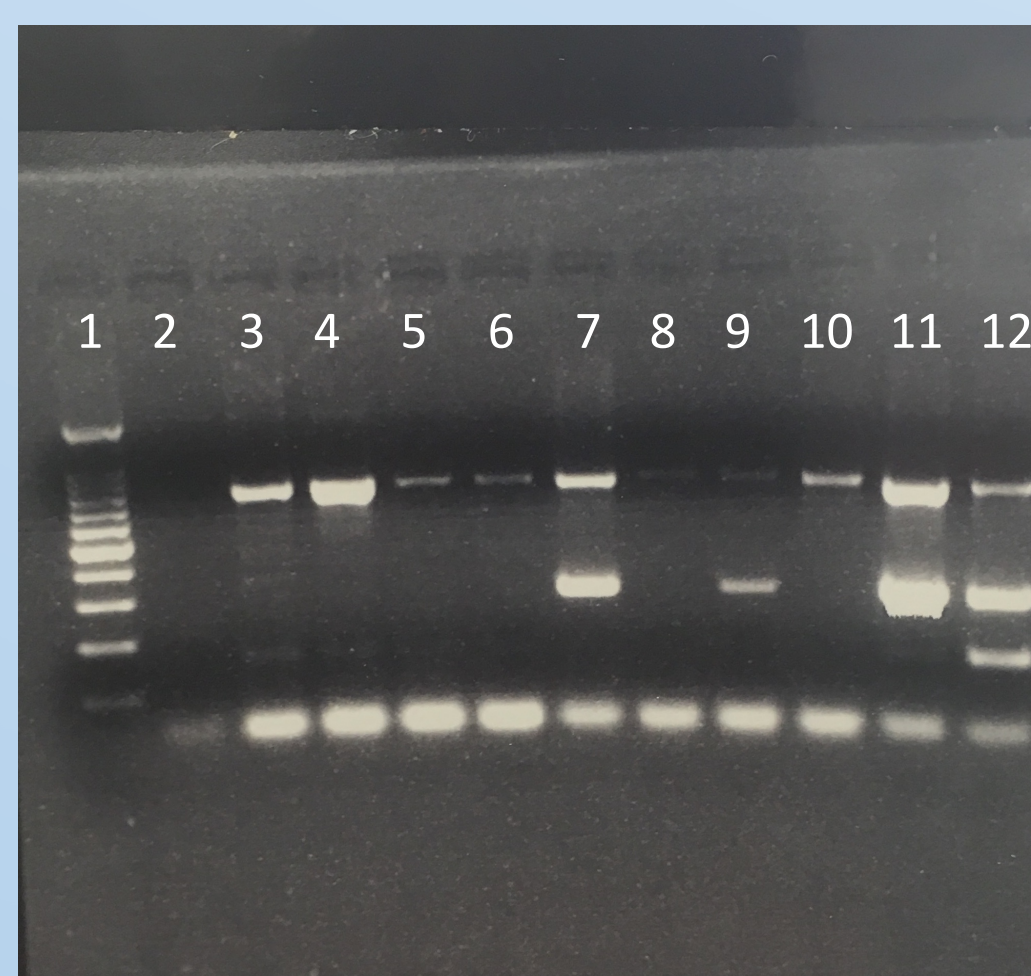


Figure 2: Multiplex PCR gel used to determine whether species are *Staphylococcus*, *S. aureus*, or MRSA. Lanes: 1- 100bp ladder, 2- ICQ103, 3- ICQ104, 4- ICQ108, 5- ICQ109, 6- ICQ115, 7- ICQ092, 8- ICQ101-5, 9- ICQ101-L, 10- ICQ105, 11- SA control, 12- MRSA control

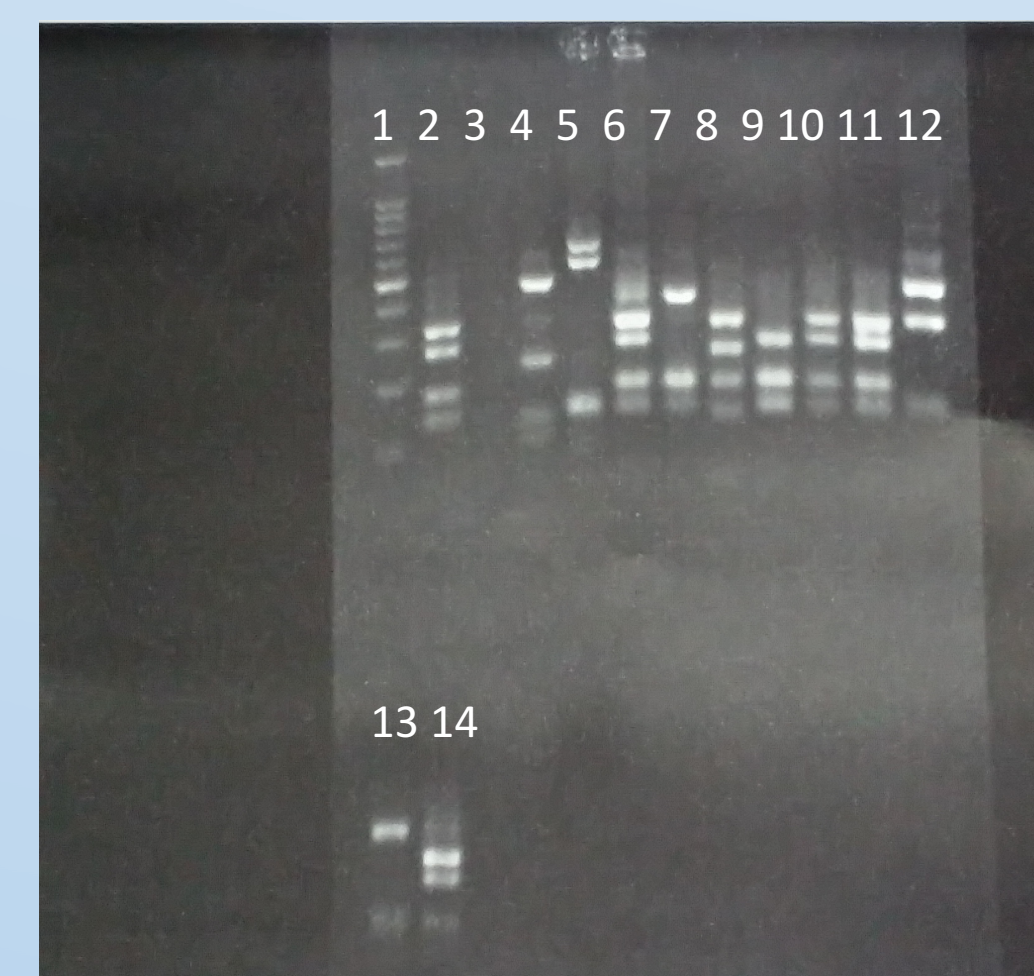


Figure 3: Xap1 digest gel for *dnaJ* PCR product. Lanes: 1- 100bp marker, 2- ICQ067, 3- H089, 4- H008, 5- IBM138, 6- ICQ083, 7 ICQ084, 8- ICQ117, 9- ICQ118, 10- ICQ124, 11- H082, 12- H087, 13- H107, 14. SA control

Sample ID number	Fermentation of Mannitol	Resistant to oxacillin	Multiplex PCR Results	Xap1 Digest Results	Percentage of total sample set
H075	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i> : 40 %
ICQ020	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
ICQ085	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
ICQ089	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
H029	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
IGA020	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
ICQ067	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
ICQ065-1	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
ICQ083	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
ICQ124	yes	no	<i>S. aureus</i>	<i>S. aureus</i>	
ICQ061	yes	yes	Methicillin Resistant <i>S. aureus</i>	<i>S. aureus</i>	Methicillin Resistant <i>S. aureus</i> : 12 %
H023	yes	yes	Methicillin Resistant <i>S. aureus</i>	<i>S. aureus</i>	
H082	yes	yes	Methicillin Resistant <i>S. aureus</i>	<i>S. aureus</i>	
IGA025	yes	no	<i>Staphylococcus</i>	<i>S. succinus</i>	<i>S. succinus</i> : 12 %
IGA019	yes	no	<i>Staphylococcus</i>	<i>S. succinus</i>	
ICQ117	yes	no	<i>Staphylococcus</i>	<i>S. succinus</i>	Other <i>Staphylococcus</i> species: 36%
ICQ104	yes	no	<i>Staphylococcus</i>	<i>S. scirui</i>	
ICQ105	yes	no	<i>Staphylococcus</i>	<i>S. scirui</i>	
ICQ065-2	yes	no	<i>Staphylococcus</i>	<i>S. nepalensis</i>	
H008	yes	no	<i>Staphylococcus</i>	<i>S. caprae</i>	
IBM138	yes	no	<i>Staphylococcus</i>	<i>S. caprae</i>	
ICQ084	yes	no	<i>Staphylococcus</i>	<i>S. kloosi</i>	
H017	yes	no	<i>Staphylococcus</i>	<i>S. kloosi</i>	
1CQ118	yes	no	<i>S. aureus</i>	<i>S. hominis</i>	
H087	yes	no	<i>Staphylococcus</i>	<i>S. cohnii</i>	

\*this data is representative of a larger sample set

## Discussion:

Multiplex PCR was used to amplify the 16s *rRNA* gene, *femB* gene and *mecA* gene in each sample. The results indicate all of the isolates belong to the genus *Staphylococcus* as demonstrated by the 16s *rRNA* band (Figure 2). *S. aureus* samples contained two bands at approximately 800bp and 300bp, showing the 16s *rRNA* and *femB* genes respectively. MRSA isolates showed an additional band at 150bp representing the *mecA* gene (Figure 2). *dnaJ* PCR results showed the amplification of the *dnaJ* gene in each of the samples. A restriction XapI enzyme digest was used to identify the species of the isolates (Figure 3). A total of 25 samples were analyzed during Spring 2018, and 12% of the isolates were found to be *S. succinus* such as sample ICQ117 (Figure 3). This is consistent with the entire sample set as 11.4% are *S. succinus*. *S. equorum* were not present in the samples identified during Spring 2018, however, they were found in 1.5% of samples within the total sample set. Although these results do not indicate that species besides MRSA have resistance to oxacillin, previous studies have shown antibiotic resistance in *S. succinus* and *S. equorum*. Specifically, *S. equorum* has shown to be vancomycin resistant (3). Because *S. equorum* can reside in nasal mucosa and is living within close proximity of *S. aureus*, there is concern regarding whether or not the gene for antibiotic resistance could transfer into *S. aureus*. Further research could be done to create antibiotic susceptibility profiles for *S. equorum* and *S. succinus*, as well as determining if genetic transfer between these species and *S. aureus* can occur.

## References:

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