Introduction

Staphylococcus equorum and Staphylococcus succinus are bacterial species commonly associated with animals (3,8) and certain food products (5,6,9). Only rarely are incidents of human infection due to these species reported and there are no reports of these species being a component of normal human flora (7). During the summer of 2010, nasal swabs were taken from 183 staff and patients at a hospital in Loja, Ecuador to determine MRSA (methicillin-resistant Staphylococcus aureus) prevalence. MALDI-TOF analysis of isolates from five individuals revealed the presence of either S. succinus or S. equorum. Confirmation of the MALDI-TOF identification for these isolates was performed by analyzing XapI restriction fragment length polymorphisms of the dnaI gene (2). The growth of the isolates were characterized to determine pH range, halotolerance, and sugar fermentation. In addition, MICs were performed on each isolate.

Materials and Methods

Salt and pH tolerance: Samples were grown on plates and in broths of 5%, 10%, 15%, 20%, and 30% NaCl as well as in broths of pH 3, 5, 7, 8, and 10 to characterize halotolerance and pH tolerance.

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

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

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 amplified conserved portions of the mecA, FemB, and 16S rRNA genes. PCR products were analyzed on a 2% agarose gel (4).

Antibiotic Resistance Characterization through MIC: Minimum Inhibitory Concentrations of resistance to various antibiotics were performed on each sample according to NCCLS protocol (1).

Discussion

After the initial identification of the staphylococcus species of each sample using the MALDI-TOF analysis, we were able to confirm that one isolate was Staphylococcus succinus and three isolates were Staphylococcus equorum through a multiplex PCR amplifying the 16S rRNA, FemB, and mecA genes and a PCR restriction analysis of the dnaI gene (Figures 1 and 2) however while running these tests it was discovered that the MALDI-TOF had incorrectly identified one sample as Staphylococcus succinus when in fact it was a Staphylococcus aureus. Following species identification, halotolerance and pH tolerance was tested. The data shows that S. equorum and S. succinus are able to grow in conditions of up to 15% NaCl and pH levels of 7 to 10 with the exception of S. aureus also being able to grow in a pH of 5. Sugar fermentation was also tested. All three species fermented sucrose and dextrose, S. succinus also fermented xylose, and S. aureus fermented lactose. Lastly, Minimum Inhibitory Concentration (MIC) data showed that each species has characteristic ranges of susceptibility or resistance to certain antibiotics. For erythromycin, all three species fell into the intermediate category. For tetracycline, S. equorum was either susceptible or intermediate, S. succinus was susceptible, and S. aureus was intermediate. For kanamycin, all species were resistant except one S. equorum isolate was susceptible and another had inconclusive data. For vancomycin, all species were susceptible. For chloramphenicol, S. equorum and S. succinus were susceptible while S. aureus was intermediate. For gentamycin, S. succinus and S. aureus were resistant, one isolate of S. equorum was susceptible, and the other two had inconclusive data. For streptomycin, S. succinus and S. aureus were resistant, one isolate of S. equorum was intermediate, and the other two had inconclusive data. For ampicillin, all species were resistant.

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References