Speciation of Methicillin-Resistant Staphylococci Isolated from Ecuadorian Hospitals and Communities

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Background

Staphylococcus aureus is a bacterium that commonly causes infections in the human population. Many patients' prognoses are worsened because some strains have acquired antibiotic resistance. Surveillance of methicillin-resistant Staphylococcus previously focused on the well characterized aureus species (MRSA).

Community and hospital samples collected from various regions of Ecuador from 2010-2012 revealed the majority of methicillin-resistant Staphylococcus (MRSA) are not of the aureus species. These species are speculated to be important in the acquisition of methicillin resistance in S. aureus by serving as a reservoir for the SCC-mec cassette, which is the genetic element responsible for methicillin resistance [1].

We tested a PCR protocol to identify the species of Staphylococcus that are resistant to methicillin. The protocol identifies species based on an intergenic region between the 16S and 23S rDNA regions on the chromosome [2]. The banding pattern from this intergenic region is specific to each species. It is our hope that speciation of the methicillin-resistant Staphylococcus isolates will allow us to develop a better understanding of how antibiotic resistance is transferred between Staphylococcus species.

Materials & Methods

Sample Collection: Nasal swabs were collected using StarSwab II ™ Platinum Series swabs (Staplex Scientific, Inc.) from community members and hospital staff and patients age 12 or older. These samples were collected from various regions in Ecuador from 2010-2012.

Primary PCR Analysis: Polymerase chain reaction (PCR) using three increasingly selective primers was performed on isolates that passed catalase, Gram stain, and latex agglutination tests. Samples were run on a 2% agarose gel containing 2 µl ethidium bromide. Samples were compared to a positive S. aureus control, a positive MRSA control, and a PCR negative control in order to accurately identify each isolate (Figure 1).

MRS Characterization: Positively identified MRS isolates were sent to Marshfield Clinic for further characterization using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF).

Speciation by PCR Analysis: PCR was performed on the characterized MRS isolates in order to develop a reliable speciation protocol. Isolated DNA from MRS samples was used as a template in the PCR recipe (Tables 1 & 2). These samples were originally run on a 3% agarose gel containing 2 µl ethidium bromide at 45V for 4 hours. The gel was used and adjusted until optimal separation was obtained consistently (Figures 2 & 3).

References


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