Verification of Methicillin Resistant *Staphylococcus aureus* in Ecuador Hospital Samplings by Use of Polymerase Chain Reaction

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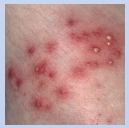
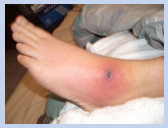
Abstract

There are very few published studies about the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Ecuador. Nasal swabs collected at a hospital in the Loja province were brought back to the University of Wisconsin-Eau Claire to be analyzed for MRSA. Nasal swabs were identified through a series of experiments shown in figure A. In order to confirm that these samples were *Staphylococcus aureus* and were in fact resistant to methicillin, DNA was isolated and polymerase chain reaction (PCR) was used to detect specific genes found only in *Staphylococcus aureus* and MRSA. PCR results confirmed which suspected samples contained the mecA gene found in MRSA. The results of studying MRSA prevalence have significant implications for public health policy and procedure in Ecuadorian hospitals and communities.

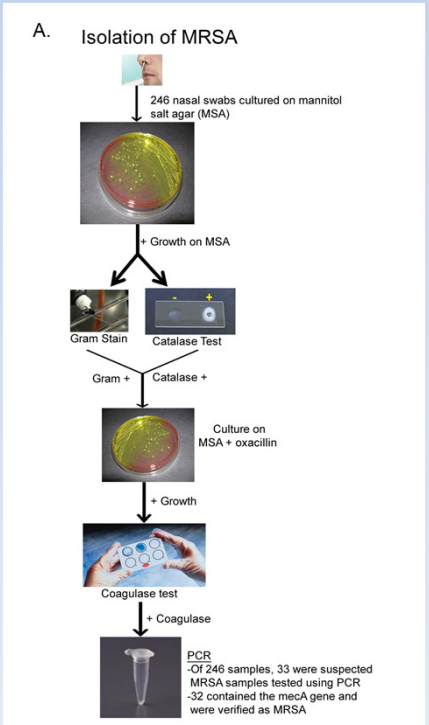
Background

MRSA is a pathogen that is commonly associated with nosocomial infections and current studies also reveal increasing prevalence in communities. MRSA infections cause a significant increase in morbidity and mortality in patients and also prolong hospital stays (Panhotra *et al.*, 2005). Infections can remain on the surface of the skin or move into tissues, bones, and organs (Mayo Clinic, 2011).



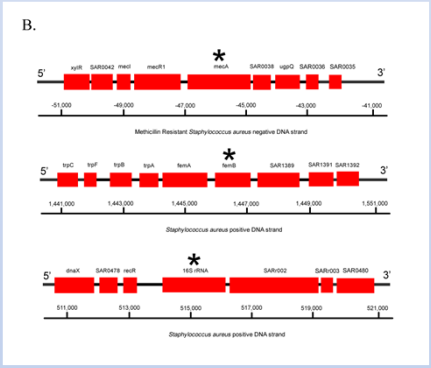


The results of our study allow for the verification of suspected MRSA isolates that grew on MSA + oxacillin. Fine tuning of methods for DNA isolation and PCR allows this protocol to be used in subsequent studies of MRSA to take place this summer and in the future.



Materials and Methods

Nasal swabs were taken from a hospital in the Loja province of Ecuador. Hospital samples included both patients and staff. Samples were processed as seen in figure A in order to identify suspected MRSA isolates. DNA was isolated from suspected MRSA samples and used for PCR. Primers were used to confirm presence of the mecA, femB, and 16S genes. PCR amplification products were analyzed using 2% agarose gel electrophoresis.



Primers

mecA gene: codes for penicillin binding protein 2a (PBP2a) which decreases the binding ability of beta lactam antibiotics, contributing to MRSA antibiotic resistance (Hiramatsu *et al.*, 1991).
femB gene: found only in Staphylococcus aureus and codes for a protein which assists in the formation of peptidoglycan cell wall structure (Hübscher *et al., 2007*).
16S rRNA gene: confirms that isolates belong to the genus *Staphylococcus*. *Staphylococcus* 16S rRNA codes for a protein that assists in translation.

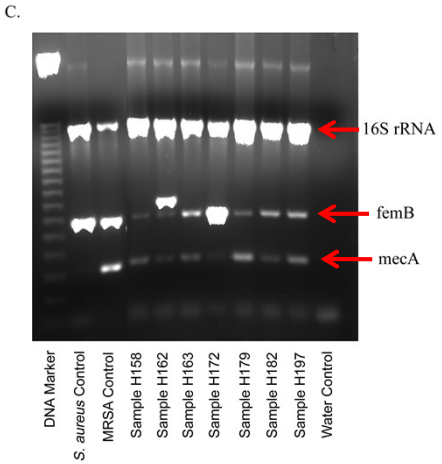


Figure C. PCR confirmed mecA gene presence in the MRSA control sample and suspected hospital MRSA isolates. *Staphylococcus aureus* control does not contain the mecA gene and is not resistant to methicillin.

Role of mecA in Methicillin Resistance

The mecA gene codes for penicillin binding protein 2a (PBP2a). All *Staphylococcus aureus* produce penicillin binding proteins(PBPs), but only MRSA produces PBP2a (Villegas-Estrada *et al.*, 2008).

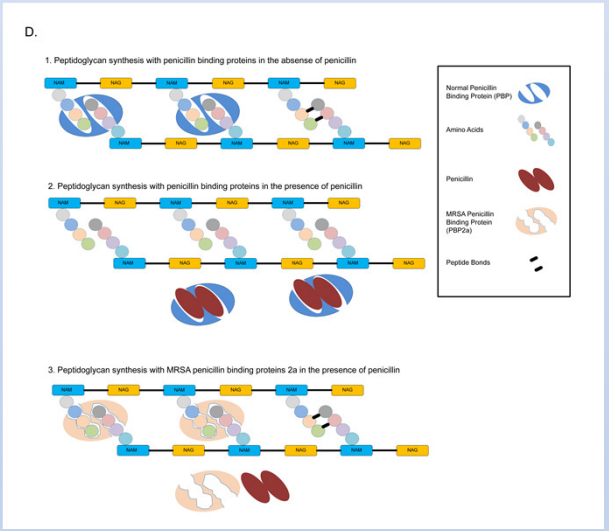


Figure D. The mechanism of general penicillin binding proteins and MRSA's penicillin binding protein 2a are show in the presence and absence of penicillin.

1. PBPs bind peptides in the absence of penicillin and use transpeptidase activity to create peptide bonds during synthesis of peptidoglycan in the cell wall.
2. PBPs are bound by penicillin, making them unavailable for peptidoglycan synthesis.
3. PBP2a found in MRSA has an altered active site which prevents the binding of penicillin. PBP2a is therefore free to associate with peptides and use transpeptidase activity to synthesize peptidoglycan.

Results and Conclusions

Of the 246 nasal swabs collected in the hospital, 33 have been suspected of being MRSA isolates. PCR has verified the presence of the mecA gene in 32 of these 33 isolates.

Early on there were many problems with primer concentrations and many samples often tested positive for 16s and mecA, but not femB. Trial and error with primer concentrations has created an optimized protocol for PCR with suspected MRSA samples and results have improved.

Future work includes the analyzing of community samples to confirm the prevalence of MRSA in communities. Studies will continue this summer in Ecuador to further examine MRSA prevalence in communities and hospitals.

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