Prevalence of Methicillin-Resistant *Staphylococcus aureus* in Loja Province, Ecuador

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic-resistant strain of the bacterium *Staphylococcus aureus* , which can cause serious and potentially fatal infections in humans. In recent decades incidence of MRSA infections has risen, and MRSA now accounts for a significant proportion of nosocomial infections and results in thousands of deaths annually both in the United States and globally[1]. Little published data exists on the prevalence of MRSA in Ecuador, however, and its health impact in Ecuador is therefore poorly understood. This study examined the prevalence of MRSA colonization in Loja Province, Ecuador. Nasal swabs were obtained from individuals in rural communities and from patients and staff at a regional public hospital to assess the prevalence of MRSA carriage. Health surveys were also conducted to assess risk factors for MRSA colonization. Preliminary results indicate that of the hospital samples, 54.1% were positive for *S. aureus*, and 15.4% were positive for MRSA. Of the community samples, 41.5% were positive for *S. aureus*, and 5.4% were positive for MRSA. These results indicate that MRSA is a potentially serious health threat in Loja Province that warrants further investigation.

Materials and Methods

Sample Collection Nasal swabs were collected using StarSwab II ™ Platinum Series swabs (Starplex Scientific, Inc.) from individuals 12 or older in the rural communities of Ashimingo, Coamine, Tacoranga, Santa Ester, Chirimoyos, Chaquizhca, and Guara . Nasal swabs were also collected from patients and staff 12 and older at Isidro Ayora Hospital in Loja City, Ecuador.
Mannitol Salt Agar (MSA) Samples were inoculated onto MSA and incubated at 37 degrees Celsius for 24 hours to select for halotolerant specimens and identify potential *S. aureus* isolates by colony morphology and mannitol fermentation.
Gram Stain Gram stains were conducted to identify isolates of Gram positive cocci.
Catalase Test Catalase tests were conducted on suspected *S. aureus* isolates to verify the presence of the catalase enzyme.
Latex Agglutination Test Latex agglutination tests were conducted on suspected *S. aureus* isolates using BactiStaph® Latex 150 Test Kits (Remel) according to the manufacturer's instructions to verify the presence of clumping factor and Protein A simultaneously.
Antibiotic Resistance Testing Suspected *S. aureus* isolates were inoculated onto MSA containing 4 µg/ml oxacillin and incubated at 37 degrees Celsius for 24 hours to identify potential methicillin-resistant specimens.
PCR Analysis DNA was isolated from suspected MRSA isolates and PCR was conducted using 16srRNA, femB, and mecA primers (Table 1). PCR products were analyzed using 2% agarose gel electrophoresis.
Data Analysis 95% confidence intervals and two-proportion z-tests were calculated using Minitab ® 14 Student software.

Table 1: Primers used in PCR analysis, primer sequences, and size of PCR product.

Primer	Sequence	Size/Reference or Gen Bank Acc. #
16srRNA F	5' CCTATAAGACTGGGATAACTTCGGG 3'	597 bp/ D83356
16sfRNA R	5' CTTTGAGTTTCAACCTTGCGGTCG 3'	
femB F	5' CGTGAAACTGAGAGCGTGC 3'	297 bp/ NCBI Primer Blast
femB R	5' AATTGGGCCGTCAGTTTTGC 3'	
mecA 1	5' TCCAGATTACAACTTCACCAGG 3'	162/ Y00688
mecA 2	5' CCACTTCATATCTTGTAACG 3'	

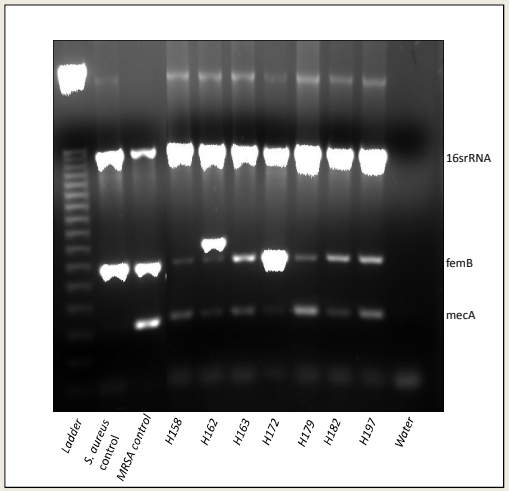

Figure 1. PCR results of hospital MRSA isolates.

Results

A total of 246 samples were collected from the hospital, and 258 samples were collected from the communities. Preliminary results of MSA, Gram stain, catalase, and latex agglutination tests indicate that 54.1% (95% CI 47.8% - 60.3%) of hospital samples and 41.5% (95% CI 35.5% - 47.5%) of community samples were positive for *S. aureus* (Figure 2). Prevalence of *S. aureus* was therefore significantly higher in the hospital than the communities (p=0.002). In addition, results of antibiotic-resistance tests indicate that 15.4% (95% CI 10.9% - 20.0%) of hospital samples and 5.4% (95% CI 2.7% - 8.2%) of community samples were positive for MRSA (Figure 2). MRSA prevalence was similarly significantly higher in the hospital than the communities (p<0.01). The hospital sample results were further categorized according to gender, age, and history of hospitalization and surgery to examine the prevalence of *S. aureus* and MRSA in these subgroups (Figure 3). Community samples were further categorized according to community (Figure 4). Differences in *S. aureus* and MRSA prevalence between these subcategories, however, were not significant.

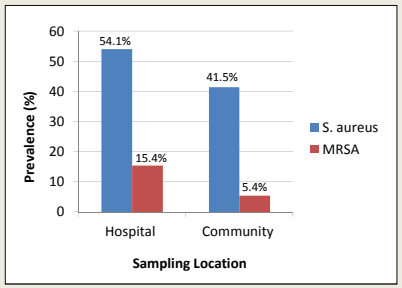

Figure 2. Prevalence of *S. aureus* and MRSA in total hospital and community samples.

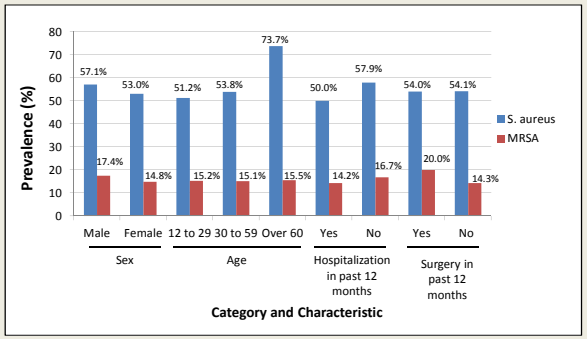

Figure 3. Prevalence of *S. aureus* and MRSA in hospital samples by sex, age, history of hospitalization, and history of surgery.

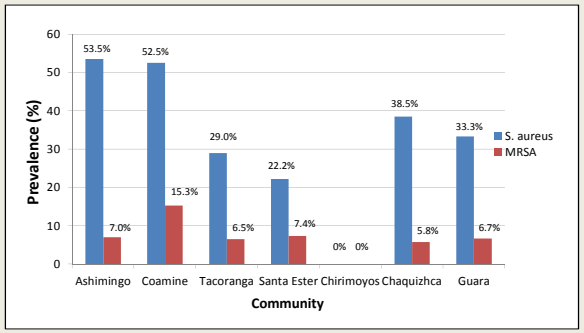

Figure 4. Prevalence of *S. aureus* and MRSA by community.

Discussion

The preliminary data indicate that MRSA is prevalent in Loja Province, suggesting that a significant proportion of the population is at risk for MRSA infections. Our findings therefore suggest that MRSA poses a potentially serious health threat in Loja Province that merits further study. Further work is being done to confirm these preliminary findings with PCR analysis to verify the presence of the *mecA* gene in suspected MRSA samples. Currently, 33 suspected MRSA samples have been tested, and 32 were *mecA* positive (Figure 1).

References

1. Klein, E., Smith, D.L., and R. Laxminarayan. 2007. Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999-2005. *Emerging Infectious Diseases* 13: 1840-1846.

Acknowledgements

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