

# Prevalence of Methicillin-resistant *Staphylococcus aureus* in El Oro Province, Ecuador

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## Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic-resistant strain of the bacterium *Staphylococcus aureus*. Infection by *S. aureus* causes soft tissue and skin infections, which are normally treated with antibiotics[1]. However, antibiotics are ineffective when the infection is caused by a resistant strain. MRSA poses serious health threats on the global scale because of its increased difficulty to treat, accounting for a significant portion of hospital-acquired infections and resulting in thousands of deaths annually[2].

Transmission of *S. aureus*/MRSA generally occurs through physical contact of colonized hospital personnel with patients. Risk factors associated with acquisition of *S. aureus*/MRSA are prolonged hospitalization, dialysis treatments, or heavy antibiotic treatment[3].

Little published data exists on the prevalence of MRSA in Ecuador, resulting in a poorly understood health impact for the country. The results indicate that MRSA is a potentially serious health threat in this hospital that warrants further investigation.

## Materials and Methods

**Sample Collection:** Nasal swabs were collected using StarSwab II™ Platinum Series swabs (Starplex Scientific, Inc.) from patients and staff age 12 or older in Machala Hospital, a regional public hospital in El Oro Province, Ecuador.

**Mannitol Salt Agar (MSA) & Oxacillin (OX):** 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 based on colony morphology and mannitol fermentation (Figure 1). Additionally, samples were inoculated onto MSA containing 4 µg/ml oxacillin and incubated under the same conditions to identify any methicillin-resistant isolates.

**Catalase Test:** Catalase tests were conducted on suspected *S. aureus* isolates to identify the presence of the catalase enzyme based on the isolates' ability to produce oxygen gas when exposed to hydrogen peroxide (Figure 2).

**Gram Stain:** Gram stains were conducted to distinguish potential *S. aureus* specimens by identifying samples containing Gram positive cocci (Figure 3).

**Latex Agglutination Test:** Latex agglutination tests were conducted using BactiStaph® Latex 150 Test Kits (Remel) according to the manufacturer's instructions to verify the presence of the coagulase enzyme in suspected *S. aureus* isolates.

**PCR Analysis:** Polymerase chain reaction (PCR) using three increasingly selective primers (Table 1) was performed on isolates that passed all three previous tests. Samples were run on 2% agarose gels and compared with a positive *S. aureus* control, a positive MRSA control, and a PCR negative control in order to accurately identify each isolate as either MRSA or *S. aureus* (Figure 4).

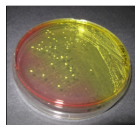


Figure 1: *S. aureus* colonies and mannitol fermentation on MSA.



Figure 2: Positive (left) and negative (right) catalase tests.

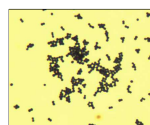


Figure 3: Gram stain of *S. aureus*.

## Results

Sample	Patient/Staff	Unit	Age	Sex	Surgery	Dialysis
MH114	Staff: Nurse	Surgery	30	Male	No	No
MH141	Staff: Nurse	Emergency	32	Male	No	No
MH155	Staff: Nurse	Hemodialysis	31	Female	No	No
MH175	Patient	Maternity Ward	29	Female	Yes	No
MH188	Patient	Maternity Ward	23	Female	No	No
MH203	Staff: Nurse	Neonatal	47	Female	No	No
MH208	Patient	Maternity Ward	17	Female	Yes	No
MH216	Staff: Doctor	Obstetrics	63	Male	No	No
MH229	Patient	Maternity Ward	29	Female	Yes	No
MH259	Staff: Nurse	Emergency	46	Female	No	No
MH298	Patient	Maternity Ward	16	Female	Yes	No
MH307	Staff: Nurse	Burn Unit	50	Female	No	No
MH371	Staff: Doctor	Pediatrics	41	Female	No	No
MH377	Staff: Administrative	Pediatrics	30	Male	No	No
MH387	Staff: Administrative	Burn Unit	54	Male	No	No
MH401	Patient	Obstetrics	26	Female	Yes	No
MH513	Staff: Nurse	External Consult	54	Female	No	No
MH532	Patient	Internal Medicine	34	Female	No	No
MH638	Patient	Hemodialysis	58	Male	No	Yes
MH664	Patient	Hemodialysis	49	Female	No	Yes
MH696	Staff: Administrative	Trauma	24	Female	No	No

Table 1: Demographic information of MRSA positive samples.

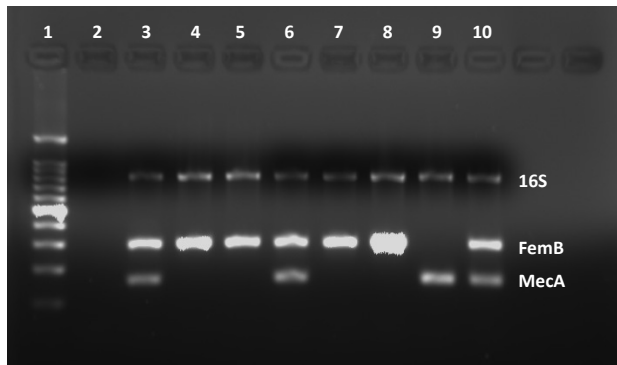


Figure 4: 2% agarose gel with a molecular ladder (lane 1) and negative, MRSA, and *S. aureus* controls (lanes 2-4, respectively) and isolates of interest.

Primer	16S	FemB	MecA
Selection	<i>Staphylococcus</i>	<i>Staphylococcus aureus</i>	Methicillin-resistance

Table 2: Three primers used in PCR analyses

- Total prevalence of *S. aureus*: **23%**
- Total prevalence of MRSA: **4%**
- Total prevalence of MRS: **15%**

- Ratio of MRSA to *S. aureus*: **19%**
- Ratio of MRSA + MRS to *S. aureus*: **83%**

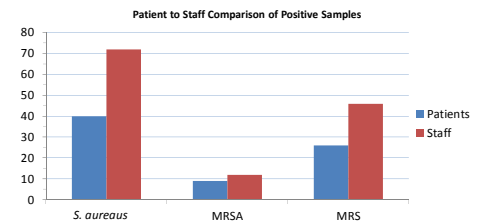


Figure 5: 112 positive *S. aureus* samples include 40 patients and 72 staff. 21 positive MRSA samples include 9 patients and 12 staff. 72 positive MRS samples include 26 patients and 46 staff.

## Discussion

Surprisingly, the most prominent group of methicillin-resistant *Staphylococcus* did not belong to the *aureus* species. This group of methicillin-resistant *Staphylococcus* (MRS) is poorly characterized in the literature. Since the methicillin-resistant cassette can be easily passed across the *Staphylococcus* family, we suspect that MRS may be an important source of methicillin-resistant genes for *S. aureus*.

The data indicate that *S. aureus*, MRSA, and MRS are all prevalent in Machala Hospital, suggesting that a significant number of patients and staff are at risk for *Staphylococcus* and MRSA related infections. Our findings therefore indicate that MRSA poses a potentially serious health threat in El Oro Province that merits further study.

## References

- [1] Barnes, B.E. and Sampson, D.A. (2011). A literature review on community-acquired methicillin-resistant *Staphylococcus aureus* in the United States: Clinical information for primary care nurse practitioners. *Journal of the American Academy of Nurse Practitioners*, 23, 23-32.
- [2] DeBonville, D. (2012). The impact of incorrect MRSA diagnoses. *Medical Library Observer*, 44(1), 26-28.
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## Acknowledgments

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