

Prevalence of MRSA Colonization of Patients and Staff in a Regional Hospital in Cuenca, Ecuador

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic-resistant strain of the bacterium *Staphylococcus aureus* that is responsible for many hospital-acquired infections world-wide. MRSA commonly colonizes individuals on the mucous membranes of the anterior nares, as well as the skin of the axilla and groin and can be spread between individuals via direct contact. Very little information is currently available on the prevalence of MRSA colonization of patients and staff in Ecuadorian hospitals.(1,3) During the summer of 2012, nasal swabs were collected from 494 volunteers in a regional public hospital in Cuenca, Ecuador to determine the prevalence of MRSA colonization within the hospital. The nasal swabs were inoculated onto mannitol salt agar supplemented with 4 µg/ml oxacillin to select for potential MRSA. Mannitol fermenting colonies were further examined using Gram stain and catalase tests. Colonies consisting of catalase positive Gram positive cocci were tested further using a latex agglutination test to detect bacteria producing coagulase and/or protein A. Isolates that were oxacillin-resistant, mannitol fermenting, catalase positive, Gram positive cocci with a positive latex agglutination reaction were presumptively identified as MRSA. To further confirm the bacteria presumed to be MRSA, a coagulase test was performed.(2,4) Thirty-two presumptive MRSA isolates were identified from the 494 volunteers, resulting in a sample prevalence of 6.5% for MRSA colonization. These results indicate that MRSA is present within the Ecuadorian hospital examined, and the potential for hospital-acquired infections exists.

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 Jose Carrasco Arteaga IESS Cuenca Hospital, a regional public hospital in Cuenca, Ecuador. All volunteers received and signed an informed consent and completed a brief survey to collect demographic information.

Mannitol Salt Agar (MSA) & Oxacillin (OX): Samples were inoculated onto MSA and incubated at 37 degrees Celsius for 24 hours to select for halotolerant bacteria, and identify potential *S. aureus* isolates based on colony morphology and mannitol fermentation. Additionally, samples were inoculated onto MSA containing 4 µg/ml oxacillin and incubated under the same conditions to identify any methicillin-resistant isolates (Figure 1).

Gram Stain: Gram stains were conducted to distinguish potential *S. aureus* specimens by identifying samples containing Gram positive cocci (Figure 2).

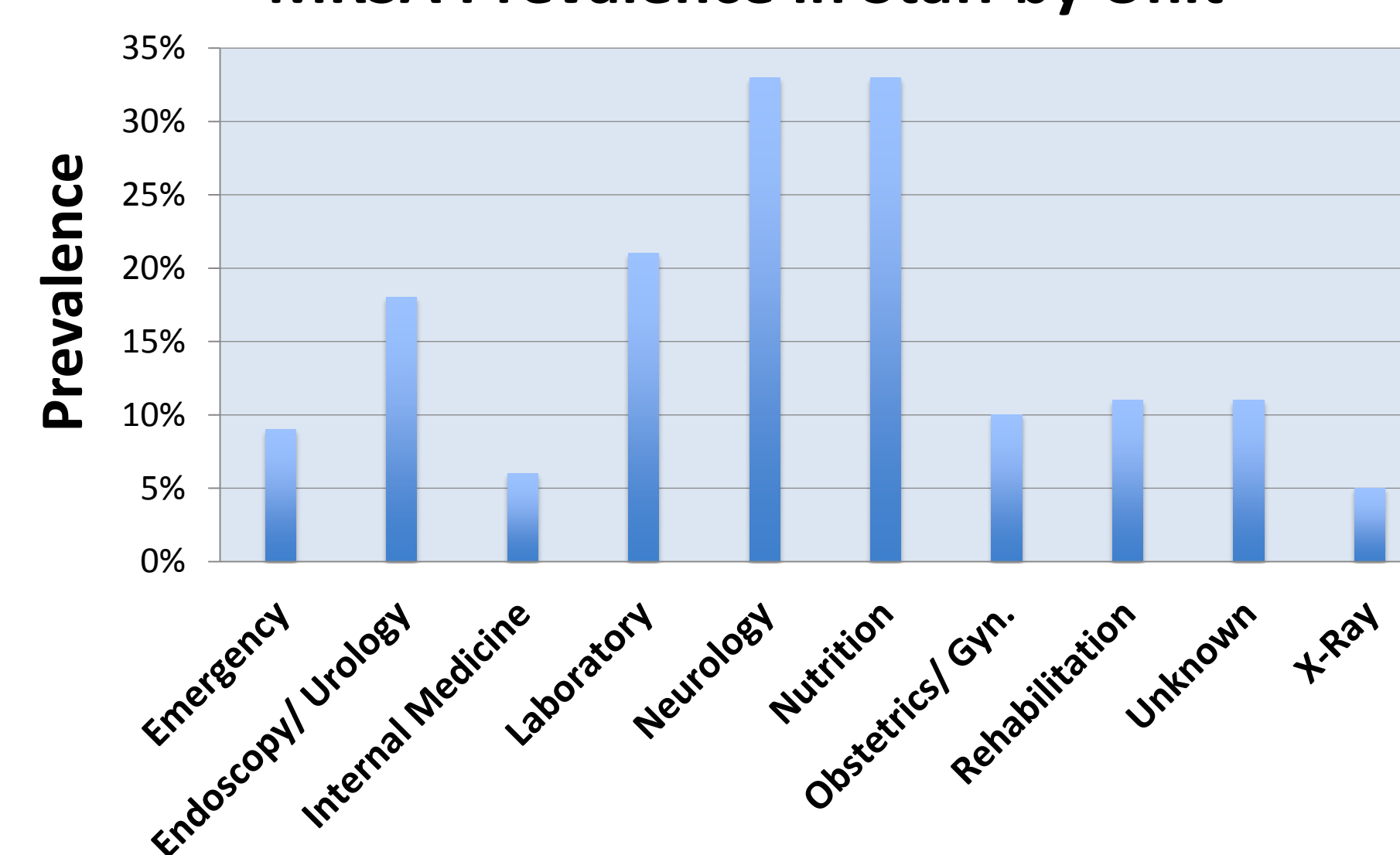
Catalase Test: Catalase tests were conducted on suspected *S. aureus* isolates to identify the presence of the catalase enzyme based on the isolates' ability to produce oxygen gas when exposed to hydrogen peroxide (Figure 3).

Latex Agglutination Test: Latex agglutination tests were conducted on suspected *S. aureus* using BactiStaph® Latex 450 Test Kits (Remel) according to the manufacturer's instructions to verify the presence of the clumping factor and Protein A simultaneously in suspected *S. aureus* isolates (Figure 4).

Slide Coagulase Test: Coagulase tests were conducted to confirm the presence of bound coagulase (clumping factor) using rabbit coagulase plasma. Presence of coagulase allows for degradation of fibrinogen and the formation of fibrin clots (Figure 5).

Results

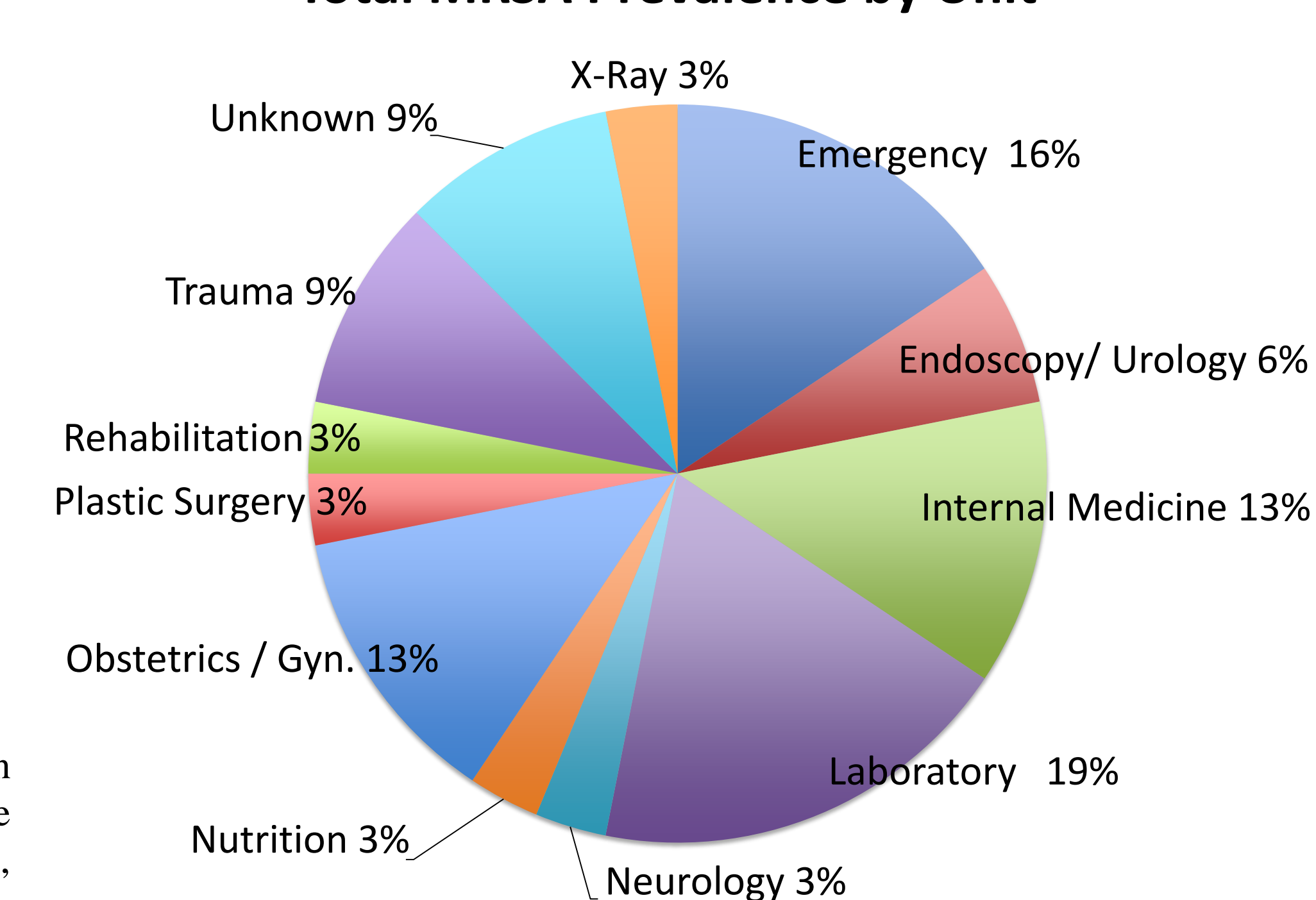
MRSA Prevalence in Staff by Unit



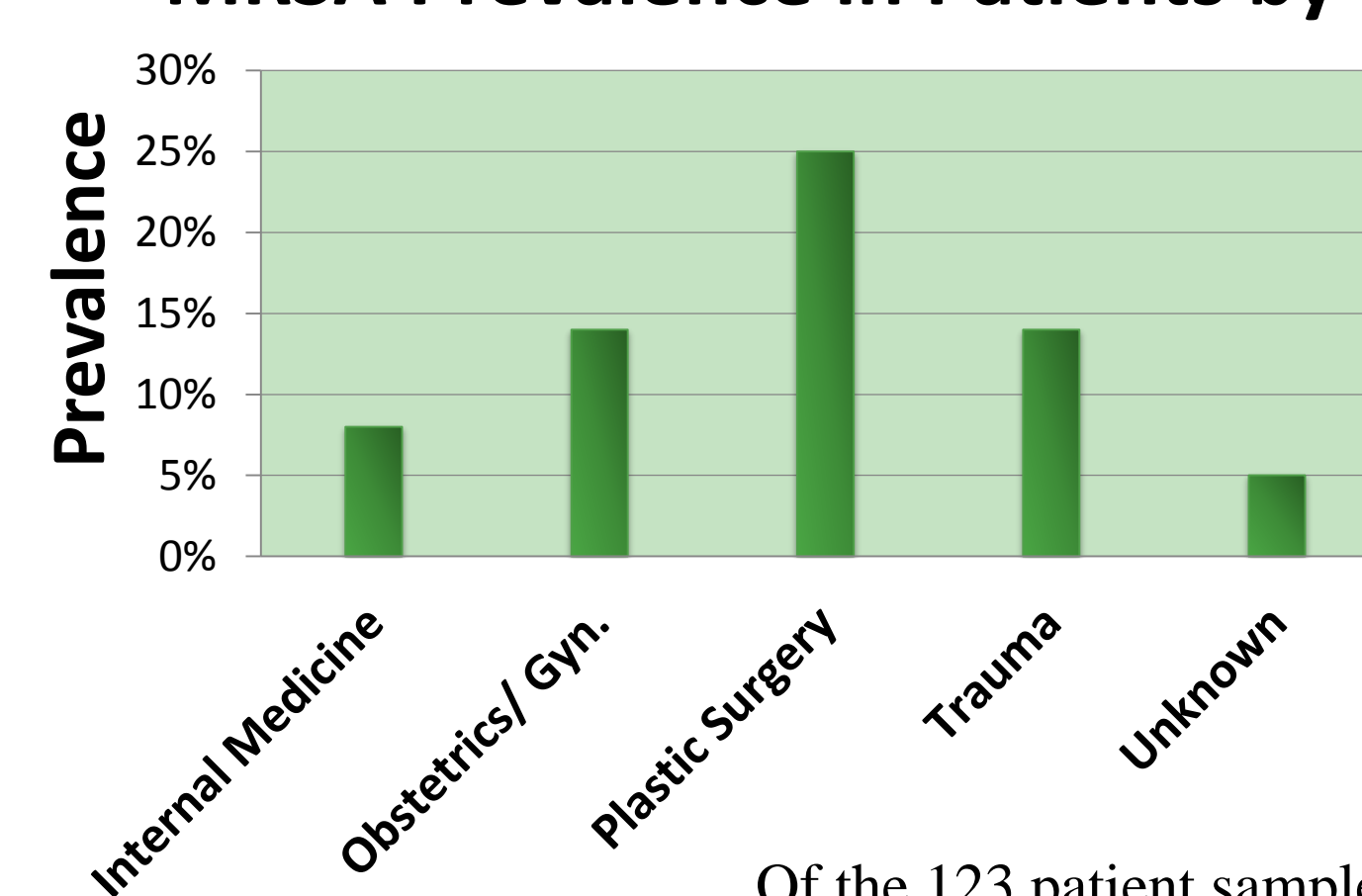
Unit	Total Prevalence
Emergency	5/54 (9%)
Endoscopy/ Urology	2/11 (18%)
Internal Medicine	2/31 (6%)
Laboratory	6/29 (21%)
Neurology	1/3 (33%)
Nutrition	1/7 (33%)
Obstetrics/ Gynecology	2/21 (10%)
Rehabilitation	1/9 (11%)
Unknown	2/19 (11%)
X-Ray	1/20 (5%)

Of the 371 staff samples there was an absence of MRSA in staff units: administration, anesthesiology, biosecurity, burn unit, cardiology, cashiers office, chemotherapy, gastroenterology, hemodialysis, hospital stores, housekeeping, insurance service, intensive care, maintenance, neonatal, neurosurgery, occupational health & safety, outpatient clinic, pediatrics, psychology clinic, security, social services, statistics, sterilization, surgery, trauma, urology.

Total MRSA Prevalence by Unit



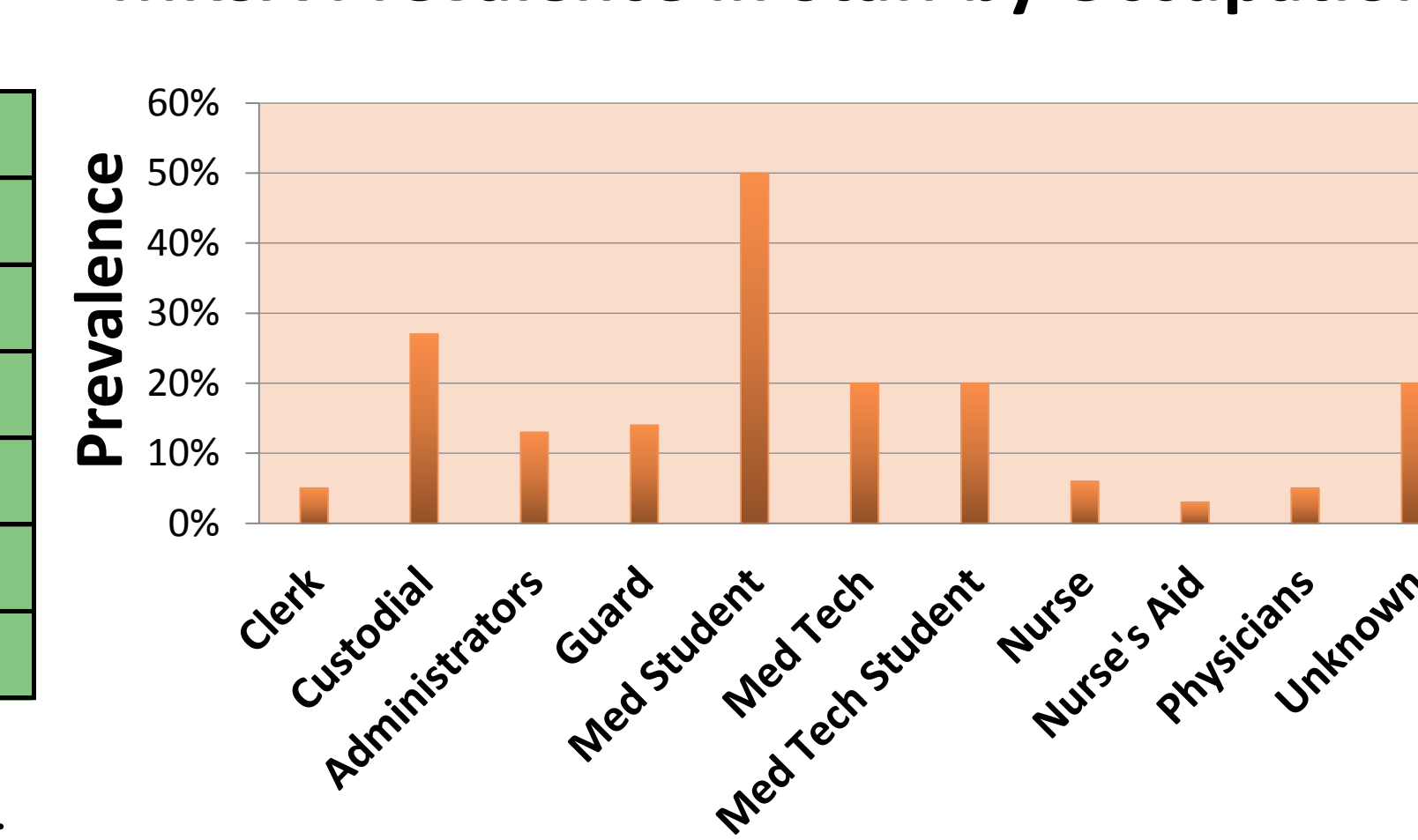
MRSA Prevalence in Patients by Unit



Unit	MRSA Prevalence
Internal Medicine	2/26 (8%)
Obstetrics/ Gyn.	2/14 (14%)
Plastic Surgery	1/4 (25%)
Trauma	3/21 (14%)
Unknown	1/19 (5%)

Of the 123 patient samples there was an absence of MRSA in patient units: hemodialysis, endoscopy, surgery, oncology, general, urology, rehabilitation, chemotherapy, neurology, biologicos.

MRSA Prevalence in Staff by Occupation



Occupation	MRSA Prevalence
Clerk	1/21 (5%)
Custodial	3/11 (27%)
Administrator	1/8 (13%)
Guard	1/7 (14%)
Med Student	2/4 (50%)
Med Tech	3/15 (20%)
Med Tech Student	1/5 (20%)
Nurse	6/95 (6%)
Nurse's Aid	2/67 (3%)
Physician	1/21 (5%)
Unknown	2/10 (20%)

Of the 371 staff samples there was an absence of MRSA in occupations: assistant, maintenance, maintenance aid, med intern/resident, nursing student, nutritionist, physical therapist, receptionist, security, service aid, social worker, students, therapist, x-ray techs.

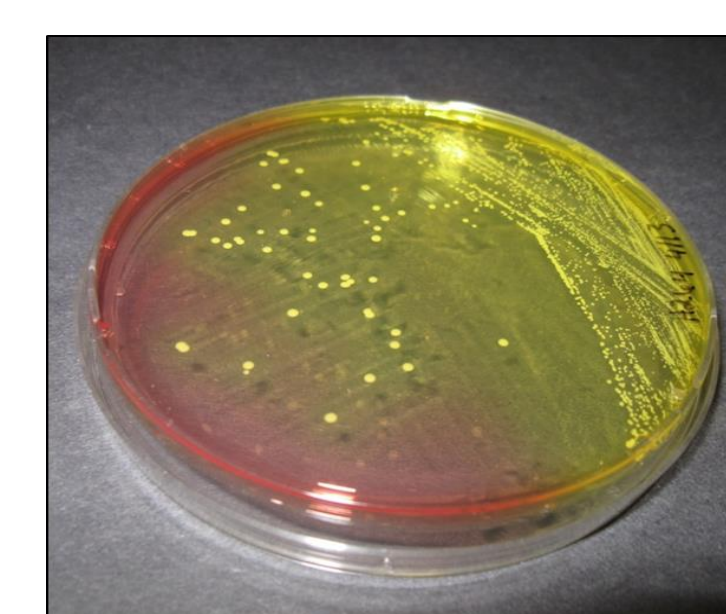


Figure 1: *S. aureus* colonies and mannitol Fermentation on MSA.



Figure 2: Gram stain of *S. aureus*.

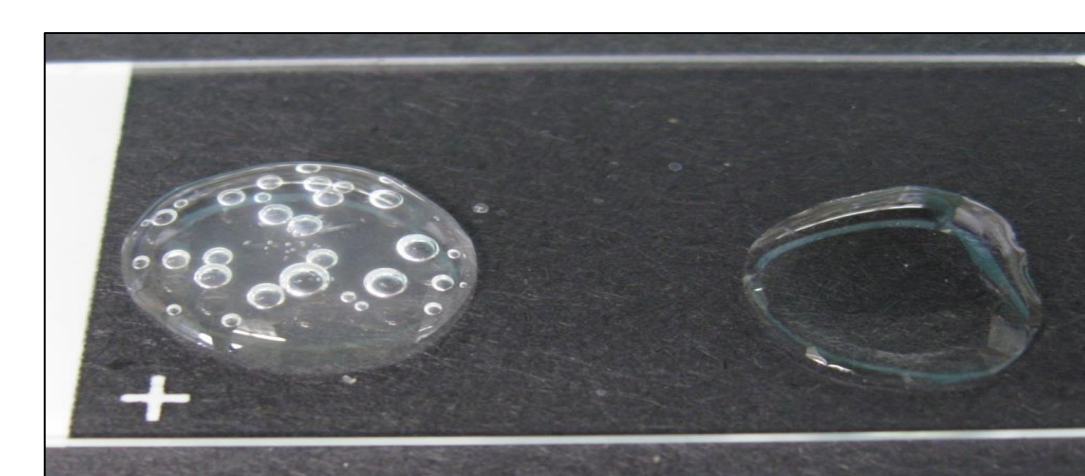


Figure 3. Positive (left) and negative (right) catalase tests.

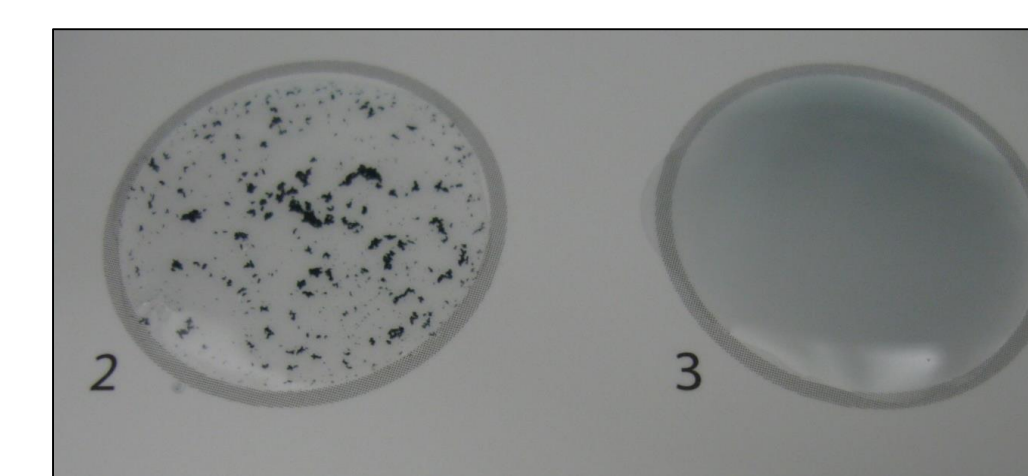


Figure 4. Positive (left) and negative (right) latex agglutination tests.

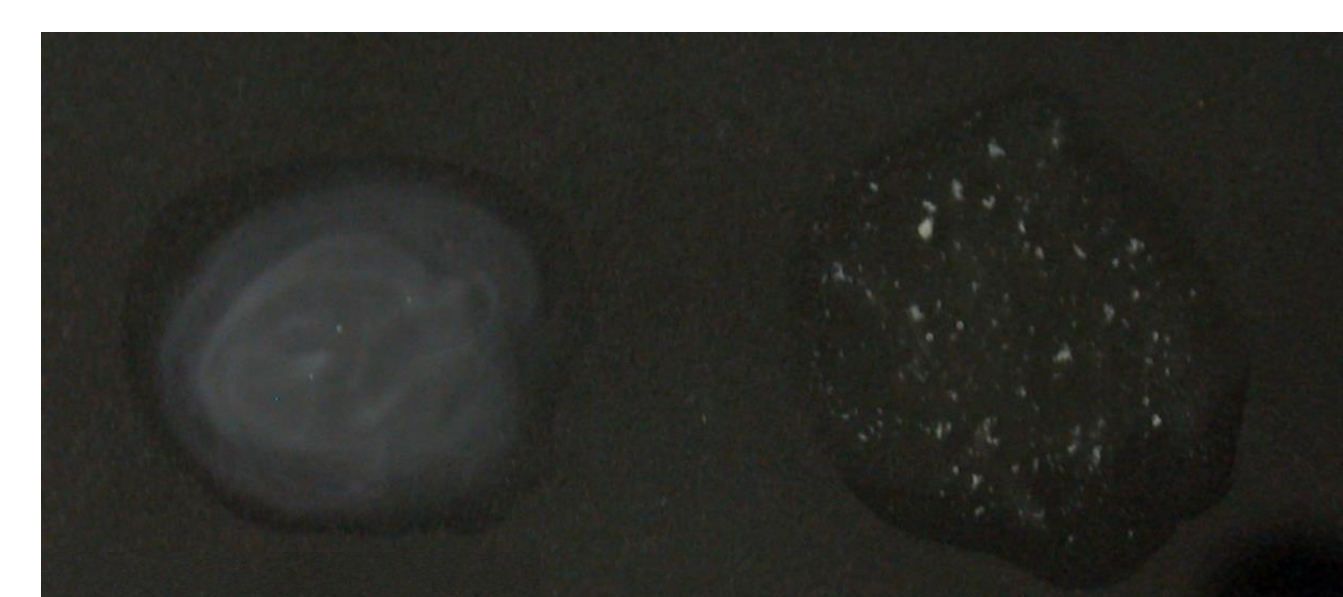


Figure 5. Negative (left) and positive (right) slide coagulase tests.(5)

Discussion

A total of 494 samples were collected from the local hospital in Cuenca, Ecuador, of those 32 (6.5%) were presumptive MRSA. The total prevalence of MRSA among the hospital staff was 6.2%, while the total prevalence among the patients was 7.3%. These preliminary data indicate that MRSA is present in Cuenca. The findings therefore suggest that the potential for hospital-acquired infections exists. Further work will utilize PCR to confirm culture-based MRSA ID's and to genetically classify the MRSA isolates.

Acknowledgment

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References

- Guzman-Blanco, M., Mejia, C., Isturiz, R., Alvarez, C. Bavestrello, L., Gotuzzo, E., Labarca, J., Luna, C.M., Robriquez-Noriega, E., Salles, M.J.C., Zurita, J., and Seas, C. 2009. Epidemiology of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Latin America. *International Journal of Antimicrobial Agents* 34: 304-308.
- MacFaddin, J.F. 2000. Page 78 in *Biochemical Tests for Identification of Medical Bacteria*, 3rd ed. Williams & Wilkins, Baltimore.
- Mejita, C., Zurita, J., and Guzman-Blanco, M. 2012. Epidemiology and Surveillance of Methicillin-resistant *Staphylococcus aureus* in Latin America. *Brazilian Journal of Infectious Diseases* 14: S79-S86.
- Norris, J. R. and Swain, H. 1971. Chapter II in *Methods in Microbiology*, Vol. 5A, edited by J.R. Norris and D. W. Ribbons. Academic Press, Ltd., London.
- Katz, Sue. Slide Test. 11 November 2010. American Society for Microbiology. ASM Microbe Library. Web. 22 April 2014.