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Molecular characterization of *Staphylococcus aureus* isolates obtained from paraguayan inmates: A pilot study

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Abstract

Introduction: Skin and soft tissue infection caused by *S. aureus* within the prison environment is particularly problematic because it involves a set of multiple risk factors for the colonization or infections due to this bacteria, in addition of the easy spread and the difficulty to contain and manage outbreaks that can result in serious morbidity and death.

Materials and Methods: This descriptive and cross-sectional study included 12 *S. aureus* isolates recovered from skin lesions and nasal swabs taken from minors inmates (130) of the Correctional Integral Educational Center of Itauguá (Central Department of Paraguay). The bacterial identification and antimicrobial susceptibility were carried out following the criteria recommended by the CLSI. The DNA extraction was performed using a commercial kit. The detection of methicillin resistance (*mecA*) and virulence factors: enterotoxins, hemolysins and exfoliative toxins genes carried out by PCR. The genetic variability was studied by Multi-Locus Variable Number Tandem Repeat Analysis. All the inmates voluntarily participated of the study giving their oral consent.

Results: All isolates were sensitive to ciprofloxacin, rifampicin and trimethoprim-sulfametoxazol, 8% were resistant to erythromycin and clindamycin, 67% were resistant to gentamycin, and 75% to methicillin and ceftriaxone, all MRSA carried out the *mecA* gene. PVL and *hlA* were the most frequent virulence genes detected (83%), followed by *seC* and *hlB*. 92% (11/12) isolates could be analyzed by MLVA, giving five different MLVA profiles among the isolates analyzed. Seven isolates were clustered in the same profile (A) and the other four were identified each one as unique profile.

Conclusion: CA-MRSA isolates with the same MLVA and virulence factors profile were identified in the correctional in different inmates, not only as carriers, but also causing infection. The fact of the identification of identical isolates reveals the importance of implementing educational interventions focused on improvements in personal hygiene practices to avoid outbreaks or more serious health-problems that could lead to infections by this pathogen.

Keywords: *Staphylococcus aureus*, MLVA, virulence profile, immates, prision

Introduction

In the last 50 years, methicillin-resistant *Staphylococcus aureus* (MRSA) has made a tremendous impact worldwide. Among the different forms of pathologic presentations, MRSA infections appear primarily as skin or soft tissue infection (SSTI), but also as a cause of death and serious morbidity such as pneumonia, osteomyelitis, cerebral abscess, and septicemia [1, 2].

It is not limited to medical facilities and healthcare institutions anymore, indeed since two decades ago, there has been a substantial increase in MRSA infections from the community among apparently healthy individuals. The MRSA isolated from individuals outside of the healthcare setting has been called community-associated (CA-MRSA) [1, 2]. There are particular risk groups of being infected by CA-MRSA, including healthy adolescents, sports teams members, military groups, intravenous drug users (IVDUs), dialysed patients and inmate populations [3-7].

Skin and soft tissue infection caused by *S. aureus* within the prison environment is particularly problematic because it envolves a set of multiple risk factors for the colonization or infections due to this bacteria as: high rates of IVDU, overcrowding, poor hygiene, lack of access to medical care, and high proportions of disadvantaged ethnic prisioners. In addition there may be other risks factors unique to the imprisonment environment such as the easily spreading and the difficulty to contain and manage outbreaks that can result in serious morbidity and death [2, 8].

The penitentiary system of Paraguay have many problems that include the lack of methods to achieve the reinsertion into society, but also there overcrowding of their facilities including those oriented to minor law offenders, according to a report made in 2016 by the National Mechanism for the Prevention of Torture (MNP, for its acronym in Spanish). Some key points of the country´s 16 penitentiaries are: an insufficient budget, the poor quality of food, almost no sports activities, prison overpopulation (most of these places harboring twice as many inmates of its current capacity), the difficulty of access to healthcare and medicines (due to the low number of staff assigned to this purpose), in addition, there are no records of medical history of the inmates [9-12].

This study aimed to analyze molecularly *S. aureus* isolates obtained from the minors inmates in the Correctional Integral Educational Center of Itauguá, Central Department of Paraguay, during a medical intervenion made to look after the general health conditions of inmates.

Materials and methods

Study design and population: This descriptive and cross-sectional study included *S. aureus* isolates recovered from clinical specimens that included skin lesions and nasal swabs taken from all those the minors inmates in the Correctional Integral Educational Center of Itauguá (Central Department of Paraguay) who had active skin lesions suggestive of pyoderma (currently, this correctional center has 130 male inmates aged between 14 and 18 years old and the totally of them was included in this study) [11]. The samples were collected within a medical intervention called ¨Medical attention to the interns of the Panchito López Juvenile Correctional¨, the medical assistance was made in august 2017 in response to a request from the medical director of the prison and it involved doctors, biochemists and microbiologists from the National Hospital of Itauguá and the Center for Dermatological Studies (CED, by it acronyms in Spanish, National Leprosy Control Program).

The bacterial identification and antimicrobial susceptibility testing were carried out in the microbiology laboratory of the CED. The identification was based on analyzing the morphology of the colony on sheep blood agar and the positive catalase and coagulase tests. The *in vitro* antimicrobials susceptibility was tested by the Kirby-Bauer diffusion method, using Oxoid/BBI discs following the criteria recommended by the CLSI (Clinical and Laboratory Standards Institute), 2017 [13]. Molecular analysis of the *S. aureus* isolates was made at Microbiology Department, Institute of Health Sciences Research (IICS in Spanish).

DNA extraction: Was performed using a commercial kit for genomic DNA extraction (Wizard Genomic, Promega, USA) following the manufacturer's instructions. The extracted DNA was subsequently preserved at -20°C. Molecular confirmation of the species was carried out by amplification of the 16S *rRNA* gene [14].

Detection of methicillin resistance gene (*mecA*) and virulence factors by PCR: The PCR to identified the methicillin resistance gene (*mecA*) was carry out following the instructions described previously [15, 16]. Oligonucleotides described by Manfredi *et al.* in 2010 were used for detection enterotoxins genes: *sea, seb, sec, sed, seh* [14]. Detection of hemolysins (*hla* and *hlb*) and exfoliative toxins (*eta* and *etb*) genes was carried out using oligonucleotides and protocols described by Wang *et al.*, 2011 and Johnson *et al*,. 1991 respectively [17, 18].

MLVA (Multi-Locus Variable Number Tandem Repeat): PCR amplification of seven VNTR loci (*clfA, clfB, sdrC, sdrD, sdrE, spA* and *sspA*) was performed using five pairs of oligonucleotides [19] followed by polyacrylamide gel electrophoresis (7.5% separator, 5% concentrator), staining with silver nitrate. Gels digitalization was carried out with the UN-SCAN IT GEL software and analysis of the band profile obtained by each isolate using the Tree CON software.

Statistical analysis: The data obtained were stored in an electronic datasheet and the statistical calculations were made using Microsoft Excel software, version 2013.

Ethical considerations: All the inmates received medical treatment when it was required and they voluntarily participated of the study giving their oral consent, previous to a verbal report them about the procedure to be carried out.

The samples were strictly managed by codes and the results obtained were exclusively used for epidemiological purposes. The codes implicates de acronyms ED plus one number identifies the convict, followed by a point and another number that indicates whether the isolate came from a nasal (1) or a skin lesion (2) swab.

This study was carry out in agreement between the Microbiology Department of the IICS and the Dermatological Specialties Center of the National Leprosy Control Program and the Dermatology Service of the National Hospital of Itauguá.

Results

We isolate 12 *S. aureus* from nasal swabs (42%, 5/12) and skin lesions swabs (58%, 7/12) from totally 9/130 (7%) convicted men participated of the study, all between 14 and 18 years old, inmates at the Correctional Integral Educational Center of Itauguá, 3 of them carried out *S. aureus* isolates both in nasal and skin lesions swabs. All isolates were sensitive to ciprofloxacin, rifampicin and trimethoprim-sulfametoxazol, 8% were resistant to erythromycin and clindamycin, 67% were resistant to gentamycin, and 75% to methicillin and ceftriaxone. All MRSA carried out the *mecA* gene.

PVL and *hlA* were the most frequent virulence genes detected, followed by *seC* and *hlB* (Table 1). 67% (8/12) of the isolates analyzed carrying simultaneously the Panton-Valentine leucocidin and the alfa hemolysin gene. None of the isolates carried out the gene *etA*, *etB*, *seA*, *seB*, *seD* neither *seH*.

92% (11/12) isolates could be analyzed by MLVA. Five different MLVA profiles were found among the isolates analyzed by MLVA. Seven isolates were clustered in the same profile (A), with slights differents in its virulence factors and antimicrobial susceptibility profiles. The others four isolates were identified each one as a unique profile by MLVA (B, C, D and E) (Figure 1). The samples ED1.1, ED1.2, ED3.2, ED5.2 and ED8.2 has the same susceptibility to antibiotics and virulence factor profile.

Table 1: Virulence factors of the *S. aureus* isolates (N=12)

Virulence Factor Gene	N (12)	Percentage (%)
Panton-Valentine leucocidin (PVL)	10	83
Alfa hemolysin (*hlA*)	10	83
Beta hemolysin (*hlB*)	1	8
Exfoliative toxin A (*etA*)	0	0
Exfoliative toxin B (*etB*)	0	0
Enterotoxin A (*seA*)	0	0
Enterotoxin B (*seB*)	0	0
Enterotoxin C (*seC*)	3	25
Enterotoxin D (*seD*)	0	0
Enterotoxin H (*seH*)	0	0

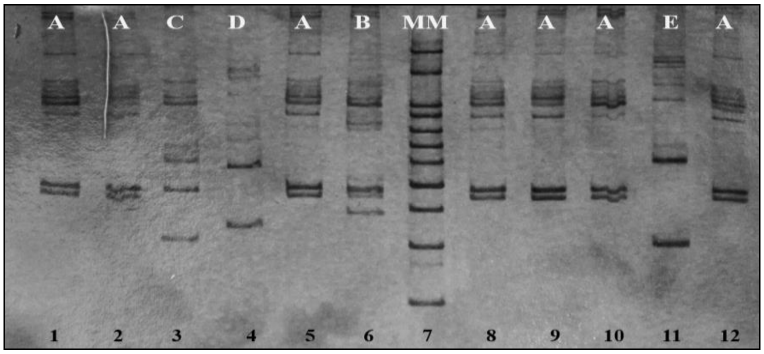

Fig 1: Polyacrylamide gel electrophoresis staining with silver nitrate, showing the different MLVA profiles (lanes). Molecular marker of 50 base pairs (Genbiotech, Argentina) in lane 7. Lanes/Isolates: 1/ED1.2, 2/ED2.2, 3/ED3.1, 4/ED3.2, 5/ED4.1, 6/ED5.2, 8/ED6.2, 9/ED7.1, 10/ED7.2, 11/ED8.2 and 12/ED9.1.

Table 2: Phenotypic and molecular characteristics of *S. aureus* isolates (N=12)

		Susceptibility to Antibiotics					**Molecular Analysis**					
Code	**Material**	**ERY**	**CLN**	**OXA**	**CEF**	**GEN**	***mecA***	***pvl***	***hlA***	***hlB***	***seC***	**MLVA**
ED1.1	Nasal swab	S	S	R	R	R	P	P	P	N	N	Not amplified
ED1.2	Skin Lesion swab	S	S	R	R	R	P	P	P	N	N	A
ED2.2	Skin Lesion swab	S	S	S	S	S	N	N	P	P	N	A
ED3.1	Nasal swab	R	R	R	R	S	P	N	P	N	N	C
ED3.2	Skin Lesion swab	S	S	R	R	R	P	P	P	N	N	D
ED4.1	Nasal swab	S	S	R	R	R	P	P	P	N	P	A
ED5.2	Skin Lesion swab	S	S	R	R	R	P	P	P	N	N	B
ED6.2	Skin Lesion swab	S	S	R	R	R	P	P	N	N	N	A
ED7.1	Nasal swab	S	S	R	R	R	P	P	N	N	N	A
ED7.2	Skin Lesion swab	S	S	S	S	S	N	P	P	N	P	A
ED8.2	Skin Lesion swab	S	S	R	R	R	P	P	P	N	N	E
ED9.1	Nasal swab	S	S	S	S	S	N	P	P	N	P	A

Abreviations: ERY: Erythromycin, CLN: Clindamycin, OXA: oxacillin, CEF: Cephalothin, GEN: Gentamicin, *mecA:* methicillin resistance gene, *pvl*: PVL gene, *hlA:* alfa hemolysin gene, *hlB*: beta hemolysin gene, *seC*: entherotoxin C gene, MLVA: Multi-Locus Variable Number Tandem Repeat Analysis. The isolates codes implicates de acronyms ED plus one number identifies the convict, followed by a point and another number that indicates whether the isolate came from a nasal (1) or a skin lesion (2) swab.

In someone of the inmates whose skin lesion swabs samples were negative for *S. aureus*, other pathogens as dermatophytes were found (5%, 7/130) among them: *Malassezia sp, Trichophyton rubrum* and *Epidermophytom flocosum*. Also in one of the positive sample for *S. aureus*, the presence of the mite *Sarcoptes scabiei* was detected simultaneously.

Discussion

S. aureus isolates with the same MLVA and virulence profile were identified in the correctional in different inmates, not only as carriers, but also causing infection. In spite of the identification of identical isolates, we cannot speak of an outbreak, due to the low frequency detection of *S. aureus* isolates in the study.

However our data suggest that the transmission of *S. aureus* is common in this place, because we found many isolates with the same profile in differents prisoners. About this, some studies support our assumption denoting that the correctional usually have a high turnover rate of people than the jail, it could be an extremely important contributing factor to the rising number of Community adquired-Methicillin resistant *S. aureus* (CA-MRSA) infections in certain communities [20, 21].

The main *S. aureus* isolates described in this study was methicillin-resistant with the PVL virulence factor, characteristic from isolates collected in the community environment (CA-MRSA) [22]. There are some populations with higher risk of CA-MRSA infections: closed communities, isolated rural population, aboriginal, sports club (principally contact sports): football, rugby, fighting. In the USA, Canada, Uruguay and another countries was identified the CA-MRSA, initially, preferably in closed communities (prisons) and settlements (peri-urban belt), but at present the great diffusibility and transmissibility of these infections make it present in various social strata [20, 23–28].

Although the objective of the present study was to molecularly analyze the isolates of *S. aureus* obtained from the prisoners, other pathogens were also been detected, such as mites and dermatophytes, which are known to be risk factors for bacterial dermal infections, that allow the entry of bacteria to the skin tissue and causing bacterial infections and aggravating them. A clear example of this situation was the CA-MRSA outbreak in Uruguayan prisons that occurred in 2003, very well documented, which started as an outbreak of scabies, reaching more than 1,000 people (among inmates, prison officials and relatives), leading to the death of 12 people. Subsequently, the outbreak spread to the population became an epidemic, affecting at least 100,000 people [25].

The correctional usually are a single facility often harboring inmates who require care that is consistent with that provided in hospitals, mental health facilities, assisted living and residential care facilities, and our findings suggests that exists faults in the prison system, between them the bad hygienic practices in the enclosure and the overcrowding, both constitutes risks factors for the bacterial spreading. To face this situation it´s necessary implement some specific measures that involved the disruption of transmission in jails: such as the increment of hand washing (one of the most basic measures in infection control), it use of isolation rooms and personal protective equipment. Another key point to take care in jails in order to the control infection is the control of syringe supplies and the correct way of descart them [23, 29].

The bacterial spreading and the emergence of an outbreak of CA-MRSA generates expenses and a substantial public economic burden, greater than other acute infectious diseases that have garnered the attention of policy makers, scientists and manufacturers. The costs per CA-MRSA infection ($7,070 – $20,489) is two to five times greater that an influenza case or three to five times more expensive that of foodborne illness. This numbers help us to justify the investment in effective CA-MRSA prevention and control and imply that even minimal investment could generate favorable returns for policy makers, military and jail systems [30].

Therefore it reveals the importance of implementing educational interventions focused on improvements in personal hygiene practices to avoid outbreaks or more serious health-problems that could lead to infections by this pathogen.

The perspective of the present study is to perform other molecular studies like the Multi-locus sequencing typing (MLST) and the *cassette SCC mec* detection that allows the identification of the circulating clones in this risk population.

References

1. Waness A. Revisiting Methicillin - Resistant *Staphylococcus aureus* Infections. J Glob Infect Dis. 2010; 2(1):49-56.
2. Haysom L, Cross M, Anastasas R, Moore E, Hampton S. Prevalence and Risk Factors for Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infections in Custodial Populations: A Systematic Review. J Correct Health Care. 2018; 24(2):197-213.
3. David MZ, Daum RS. Community-Associated Methicillin-Resistant *Staphylococcus aureus*: Epidemiology and Clinical Consequences of an Emerging Epidemic. Clin Microbiol Rev. 2010; 23(3):616-87.
4. Gorwitz RJ. Community-associated methicillin-resistant *Staphylococcus aureus:* epidemiology and update. Pediatr Infect Dis J. 2008; 27(10):925-6.
5. Tong SYC, McDonald MI, Holt DC, Currie BJ. Global implications of the emergence of community-associated methicillin-resistant *Staphylococcus aureus* in Indigenous populations. Clin Infect Dis Off Publ Infect Dis Soc Am. 2008; 46(12):1871-8.
6. May L, Porter C, Tribble D, Armstrong A, Mostafa M, Riddle M. Self-reported incidence of skin and soft tissue infections among deployed US military. Travel Med Infect Dis. 2011; 9(4):213-20.
7. Rubén Arturo Silvero Isidre, Acosta FR, Vargas CRC, Romero GAV, Perrota JFP, Fretes RMG. Molecular Characterization of *Staphylococcus aureus* Isolates Obtained from Hemodialyzed Patients at the Hospital de Clínicas of Paraguay: A pilot study. Int. J Med Stud. 2017; 5(1):14-9.
8. Binswanger IA, Redmond N, Steiner JF, Hicks LS. Health Disparities and the Criminal Justice System: An Agenda for Further Research and Action. J Urban Health Bull N Y Acad Med. 2012; 89(1):98-107.
9. ABC Color. Salud penitenciaria en agonía - Especiales - ABC Color [Internet]. 2017-2018 [cited]. Available from: http://www.abc.com.py/especiales/agoniza-la-salud-penitenciaria-1590699.html
10. Última Hora. Justicia reconoce que prisiones nacionales no están pensadas para reinserción [Internet]. ultimahora.com, 2017-2018 [cited]. Available from: https://www.ultimahora.com/justicia-reconoce-que-prisiones-paraguayas-no-estan-pensadas-reinsercion-n1114267.html
11. Mecanismo Nacional de Prevención de la Tortura M. Informes de Monitoreo y Seguimiento: Centros Educativos. [Internet], 2016-2018, [cited Jul 2]. Available from: http://www.mnp.gov.py/
12. Rodríguez Ruíz Díaz RA. Falencias del sistema penitenciario paraguayo [Internet]. El Independiente Paraguay. 2015-2018 [cited Jul 2]. Available from: http://www.elindependiente.com.py/ciencias-sociales/falencias-del-sistema-penitenciario-paraguayo/
13. Clinical Laboratory Standard Institute. Performance Standards for Antimicrobial susceptibility testing; fourteenth Informational Supplements, M100-S27 [Internet]. 2017. Available from: https://clsi.org/media/1469/m100s27_sample.pdf
14. Manfredi EA, Leotta GA, Rivas M. PCR múltiple para la detección de los genes sea, seb, sec, sed y see de *Staphylococcus aureus*: Caracterización de aislamientos de origen alimentario. Rev Argent Microbiol. 2010; 42(3):212-5.
15. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, *et al*. Involvement of Panton-Valentine Leukocidin-Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia. Clin Infect Dis. 1999; 29(5):1128-32.
16. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of Methicillin-Resistant Strains of Staphylococci by Polymerase Chain Reaction. J Clin Microbiol. 1991; 29(10):2240-4.
17. Wang D, Chen H, Li H, He Q, Ding X, Deng L. Detection of *Staphylococcus aureus* Carrying the Gene for Toxic Shock Syndrome Toxin 1 by Quantum-Dot-Probe Complexes. J Fluoresc. 2011; 21(4):1525-30.
18. Johnson WM, Tyler SD, Ewan EP, Ashton FE, Pollard DR, Rozee KR. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. J Clin Microbiol. 1991; 29(3):426-30.
19. Sabat A, Krzyszton-Russjan J, Strzalka W, Filipek R, Kosowska K, Hryniewicz W, *et al*. New Method for Typing *Staphylococcus aureus* Strains: Multiple-Locus Variable-Number Tandem Repeat Analysis of Polymorphism and Genetic Relationships of Clinical Isolates. J Clin Microbiol. 2003; 41(4):1801-4.
20. David MZ, Siegel JD, Henderson J, Leos G, Lo K, Iwuora J, *et al*. Hand and nasal carriage of discordant *Staphylococcus aureus* isolates among urban jail detainees. J Clin Microbiol. 2014; 52(9):3422-5.

21. Kajita E, Okano JT, Bodine EN, Layne SP, Blower S. Modelling an outbreak of an emerging pathogen. Nat Rev Microbiol. 2007; 5(9):700-9.
22. Rodríguez-Noriega E, Seas C, Guzmán-Blanco M, Mejía C, Alvarez C, Bavestrello L, *et al*. Evolution of methicillin-resistant *Staphylococcus aureus* clones in Latin America. Int J Infect Dis. 2010; 14(7):e560-6.
23. Bick JA. Infection control in jails and prisons. Clin Infect Dis Off Publ Infect Dis Soc Am. 2007; 45(8):1047-55.
24. Main CL, Jayaratne P, Haley A, Rutherford C, Smaill F, Fisman DN. Outbreaks of infection caused by community-acquired methicillin-resistant *Staphylococcus aureus* in a Canadian correctional facility. Can J Infect Dis Med Microbiol J Can Mal Infect Microbiol Medicale. 2005; 16(6):343-8.
25. Ma XX, Galiana A, Pedreira W, Mowszowicz M, Christophersen I, Machiavello S, *et al*. Community-acquired Methicillin-resistant *Staphylococcus aureus*, Uruguay. Emerg Infect Dis. 2005; 11(6):973-6.
26. Lee CJ, Sankaran S, Mukherjee DV, Apa ZL, Hafer CA, Wright L, *et al*. *Staphylococcus aureus* Oropharyngeal Carriage in a Prison Population. Clin Infect Dis Off Publ Infect Dis Soc Am. 2011; 52(6):775-8.
27. Maree CL, Eells SJ, Tan J, Bancroft EA, Malek M, Harawa NT, *et al*. Risk Factors for Infection and Colonization with Community-Associated Methicillin-Resistant *Staphylococcus aureus* in the Los Angeles County Jail: A Case-Control Study. Clin Infect Dis Off Publ Infect Dis Soc Am. 2010; 51(11):1248-57.
28. Tanner J, Lin Y, Kornblum J, Herzig CTA, Bystritsky R, Uhlemann AC, *et al*. Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* Clinical Isolates Obtained from the Rikers Island Jail System from 2009 to 2013. J Clin Microbiol. 2014; 52(8):3091-4.
29. Alvarez C, Labarca J, Salles M. Prevention strategies for methicillin-resistant *Staphylococcus aureus* (MRSA) in Latin America. Braz J Infect Dis. 2010; 14:107-8.
30. Lee BY, Singh A, David MZ, Bartsch SM, Slayton RB, Huang SS, *et al*. The economic burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Clin Microbiol Infect Off Publ Eur Soc. Clin Microbiol Infect Dis. 2013; 19(6):528-36.