Acquisition and transferability mechanisms of mercury resistance genes in Latin-American *Staphylococcus aureus* strains.

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Abstract

Background: Latin-American (LA) countries are among the largest mercury (Hg) polluters in the world. Fittingly, a significant high frequency (>50%) of Hg resistance genes (MRG) has been observed in LA MRSA genomes, including USA300-LV clone, which contain the genomic element COMER, encoding for copper and Hg resistance genes adjacent to SCC*mec*IVc/E. Co-selection of MRG and antibiotic resistance genes may be facilitated by shared transferable genetic elements, nevertheless, analyses of the genetic MRG context in strains other than USA300-LV are lacking. In this study, we aimed to characterize possible mechanisms of acquisition and transfer of MRG in LA *S. aureus*.

Methods: We sequenced 6 MRSA and 2 MSSA clinical isolates harboring MRG from Colombia, Ecuador, Peru and Chile using short-read (Illumina) and long-read (ONT) sequencing. Hybrid assemblies were constructed using Flye and iterative polishing with Medaka and Racon. Identification of insertion sequences, rearrangements and assessment of the genomic context was investigated using ISfinder, MAUVE, PlasmidFinder and SnapGene.

Results: Highly contiguous genome assemblies allowed us to identify the localization and genetic background of MRG. For MRSA belonging to USA300-LV (SCC*mec*IVc/E) and Brazilian (SCCmecIII) clones, we confirmed the presence of MRG within SCC*mec*. In contrast, for the 4 MRSA belonging to Chilean/Cordobes clone (SCC*mec*I), collected from Colombia, Chile and Peru, MRG were located on ~30kbp plasmids genetically related that also contained the *blaZ* beta-lactamase and cadmium/arsenic resistance genes. In MSSA strains, we observed both plasmidic and chromosomal localizations of MGR. Interestingly, in one of the MSSA, MRG were inserted downstream of orfX, along with repA, suggesting a plasmidic origin. In all these cases, MRG were flanked by IS6 family elements.

Conclusion: Genomic architecture of SCCmec types IVc/E and III might facilitate MRG transferability, whereas for the highly prevalent Chilean/Cordobes clone (SCCmecI) MRG acquisition occurs through plasmids. Our findings underscore the mechanisms of MRG transference in LA *S. aureus* likely related to antibiotic resistance co-selection.

Background

USA300-LV is the predominant MRSA clone in Colombia, and contains a genomic island designated "COMER" with genes for copper (Cu) and mercury (Hg) tolerance, adjacent to the SCC*mec* element[1]. We have observed a high prevalence of Heavy Metal Resistance (HMR) genes (Cu and Hg) in clinical isolates of *S. aureus* from Colombia (USA300-LV and Chilean/Cordobes clones), which suggest that the environment could be driving the evolution of this pathogen in our country[2].

HM environmental contamination is a serious threat to public health in developing countries[3] and could also influence the selection and evolution of HM resistance genes in MRSA. In this context, our country is ranked 3rd behind China and Indonesia, in terms of amount of Hg released to the environment. However, how these genes were acquired by *S. aureus* and how these genes are transmitted is still unknown.

Therefore, our hypothesis is that the HMR genes were acquired by the USA300-LV clone providing it an advantage that might be related with the co-acquisition of antibiotic resistance genes.

Aim

To characterize the genetic environment of mercury resistance genes in *S. aureus* from Latin America

Methods

Table 1. Selected strains included in this study

ID	Country	Strain	Clone	SCC*mec*	MLST
CA12[4]	Colombia	MRSA	USA300-LV	IVc	ST8
UE1097[5]	Ecuador	MRSA	Brazilian	III	ST1341
UC45[5]	Colombia	MRSA	Chilean/Cordobes	I	ST5
UCL420[5]	Chile	MRSA	Chilean/Cordobes	I	ST5
UCL417[5]	Chile	MRSA	Chilean/Cordobes	I	ST5
UP788[5]	Perú	MRSA	Chilean/Cordobes	I	ST5
UCL464[5]	Chile	MSSA	N/A	N/A	ST1093
UC327[5]	Colombia	MSSA	N/A	N/A	ST8

Sequencing by
Illumina
Oxford Nanopore

Hybrid assemblies and polishing
Flye
Minimap2
Racon

Genomic context
ISfinder MAUVE,
PlasmidFinder
SnapGene

Funding

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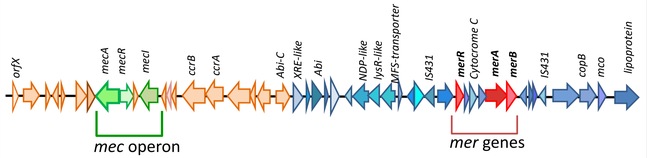
References

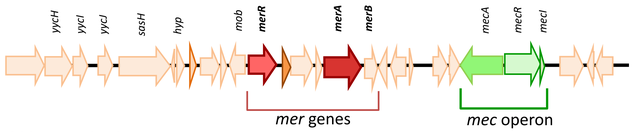
1. Planet PJ, Diaz L, Kolokotronis SO, et al. Parallel Epidemics of Community-Associated Methicillin-Resistant Staphylococcus aureus USA300 Infection in North and South America. J Infect Dis. 2015;212(12):1874–1882. doi:10.1093/infdis/jiv320
2. Diaz L, Solano J, Rios R, et al. 1214. High Frequency of Genes Encoding Resistance to Heavy Metals in Methicillin-Resistant Staphylococcus aureus (MRSA) Endemic Lineages From South America. Open Forum Infect Dis. 2018;5(Suppl 1):S368. Published 2018 Nov 26. doi:10.1093/ofid/ofy210.1047
3. Palacios-Torres Caballero-Gallardo K, Olivero-Verbel J. Mercury pollution by gold mining in a global biodiversity hotspot, the Choco biogeographic region, Colombia. Chemosphere. 2018;193:421–430.

Results

1. Localization of the *mer* genes in USA300 LV and Brazilian clones



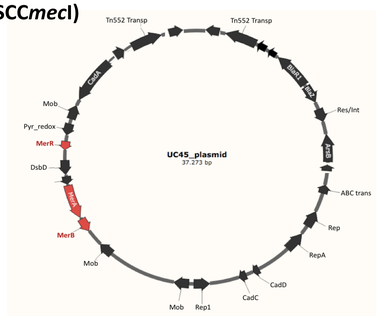
SCC*mec*IVc- ST8



***SCC*mec*III- ST1341**

As described, the chromosomal location of the *mer* genes in USA300LV and Brazililan clones is associated with the SCC*mec* elements (types SCC*mec*IVc and SCC*mec*III, respectively).

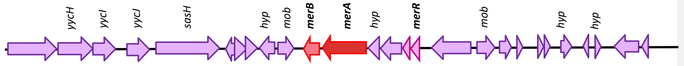
2. Genetic environment of the *mer* genes in the Chilean/Cordobes clone (ST5 –SCC*mec*I)



in the four strains belonging to the Chilean/cordobes clone, we consistenly found the *mer* genes on conserved plasmids

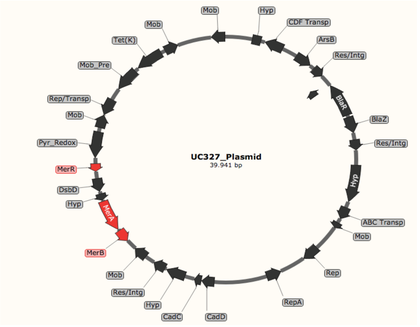
3. The *mer* genes in MSSA isolates

- **Chromosomal location in a Chilean MSSA strain**



ST1093

- **Plasmid location in a Colombian MSSA**



ST8

In MSSA, the *mer* genes were found on the chromosome close to the orfX and on a plasmid with antibiotic resistance genes.

Conclusions

- The mobile elements SCC*mec*IVc y SCC*mec*III seem to facilitate the mobilization of the *mer* resistance genes.
- The acquisition of mercury genes in the Chilean/Cordobés-I-ST5 clone occurred through conserved plasmids.
- Even though the prevalence of *mer* genes is low in MSSA, we were able to observe a less conserved mobilization of these resistance genes.
- Our results suggest an evolutive adaptation of MRSA to environmental conditions such as the mercury pollution in our region.
- The mercury resistance genes have been found along with both, antibiotic and other heavy metal resistance genes. Thus, our findings suggest that multiple environmental factors are contributing for the selection and establishment of successful MRSA lineages in the region.