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Vancomycin heteroresistant community associated methicillin-resistant *Staphylococcus aureus* ST72-SCCmecIVa strain colonizing the nostrils of a five-year-old Spanish girl

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ABSTRACT

Background and objectives: During a community methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization study, an MRSA strain with vancomycin hetero-resistance (h-VISA) was isolated from a five year-old girl with tetralogy of Fallot without previous exposure to vancomycin. An extended nasal colonization study was performed on all her close relatives.
Results: Only the patient and her sister were colonized by an h-VISA MRSA strain (clone USA 700, ST72, t148, agr 1 and SCCmec IVa). Mupirocin decolonisation was effective in the elder sister. A new nasal decolonisation in the younger girl using fusidic acid was also successful. However, after decolonisation both sisters were colonized by a methicillin-susceptible *S. aureus* (ST30, t012 and agr 3) previously isolated from their mother's nostrils.
Conclusion: As *S. aureus* have a great capacity to spread among people in close contact, knowledge of a patients' colonization status, tracing contacts, and a correct management are critical issues for the successful containment of multiresistant staphylococci.

Cepa de *Staphylococcus aureus* ST72-SCC*mec*IVa comunitaria, resistente a meticilina y heterorresistente a vancomicina, colonizadora de las fosas nasales de una niña española de 5 años de edad

Palabras clave:
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Colonización nasal
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Mupirocina

RESUMEN

Fundamento y objetivo: Durante un estudio comunitario de colonización nasal, hemos aislado *Staphylococcus aureus* resistente a la meticilina (SARM) con heterorresistencia a vancomicina (hVISA) en una niña de 5 años, que padecía una tetralogía de Fallot, que no había sido tratada previamente con vancomicina.
Resultados: Este hallazgo nos llevó a extender el estudio de colonización a sus familiares más cercanos. De estos, solo su hermana mayor fue colonizada por esta cepa SARM hVISA (clon USA 700, ST72, t148, agr 1 y SCC*mec*IVa). La descolonización con mupirocina fue eficaz en el caso de la hermana, pero un tratamiento con ácido fusídico fue necesario para eliminar la colonización nasal de la paciente. Sin embargo, después de la descolonización, ambas hermanas fueron colonizadas por una cepa de *S. aureus* sensible a meticilina (ST30, t012 y agr 3), que previamente había sido aislada de las fosas nasales de su madre.

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Conclusión: S. aureus tiene una gran capacidad de diseminación entre personas en estrecho contacto, por lo que el conocimiento del estado de colonización de los pacientes, la evaluación de la colonización nasal de los contactos y una aproximación terapéutica correcta son esenciales para la contención de la diseminación de cepas de estafilococos multirresistentes.

Staphylococcus aureus is a Gram-positive coccus that frequently colonizes human skin and mucosa, and it is associated to many life-threatening infections. Besides, this microorganism produces numerous virulence factors, toxins such as Panton-Valentine leukocidin (LPV) and the toxic shock syndrome toxin 1 (TSST-1), and it has a great ability to evolve rapidly acquiring resistance to current antimicrobial drugs which confers an additional capacity for survival. Methicillin-resistant *S. aureus* (MRSA) is prevalent in health care associated infections and it also can be a relevant aetiological agent in community infections. Vancomycin has been the drug of choice to treat infections caused by MRSA strains until 1996 when a community acquired pneumonia case due to a vancomycin intermediate *S. aureus* (VISA) was reported in Japan.[1] This strain had a thicker cell wall, produced a larger amount of penicillin binding protein (PBP) 2 and 2a, and showed a vancomycin minimum inhibitory concentration (MIC) of 4 μg/ml. However, this MRSA strain did not present *vanA*, *vanB* or *vanC* genes that confer high-level resistance to vancomycin in *Enterococcus*.[2] Later on, some strains have been referred as heteroresistant vancomycin-intermediate S. *aureus* (h-VISA) for including subpopulations with vancomycin MICs between 1 and 2 μg/ml mixed with other VISA subpopulations. Strains with vancomycin MICs ≥ 2 μg/ml have a decreased susceptibility and have been associated with therapeutic failures, despite the use of high doses of vancomycin.[3] The emergence of vancomycin resistant *S. aureus* (VRSA) is mostly associated with the acquisition of *vanA* gen, which probably transferred by conjugation from a vancomycin-resistant *Enterococcus* strain. However, VISA and h-VISA strains are not linked with these genes and it is probably related to complex genetic and cell wall changes.[4–6] However, Hiramatsu et al.,[7] consider that h-VISA is a precursor of VISA after a selective pressure by the use of betalactams and glycopeptides. h-VISA and VISA reports have increased in Europe, Asia and USA since 1996 and most of them are MRSA.[8–10]

Patients at greatest risk include those who have previously been treated with glycopeptides, or underwent to cardiovascular surgery, non-vascular prosthesis implantation surgery and dialysis.[11,12] Treatment for these strains with reduced susceptibility to vancomycin includes alternatives such as linezolid, daptomycin, and trimethoprim/sulfamethoxazole.[13]

The aim of this study was to report a case of intra–familiar transmission of an unusual h-VISA MRSA clone, colonizing two sisters without previous exposition to vancomycin.

Patients, materials and methods

Patients

During a community based colonization study, conducted between 2010 and 2012 in the villages of Plentzia, Gorliz and Barrika (Bizkaia, Basque Country, North of Spain), a MRSA strain was isolated from the anterior nares of a five-year-old Spanish girl suffering from tetralogy of Fallot and cardiovascular surgery. No other co-morbidities were reported in her clinical history. The girl received oral amoxicillin and intravenous cefuroxime in the past. To confirm these results and following the existing infection control program, new samples were obtained from the child and her close relatives (father, mother, grandparents and a seven years old sister): Twenty-five samples were studied from both anterior nares of the two sisters and their close relatives.

Sample collection and Staphylococcus aureus *isolation and identification*

Nasal samples were collected in Amies Portagerm transport (BioMérieux, France) and inoculated as soon as they arrived at the laboratory onto salt-mannitol agar plates (BBL, France). After 48 h incubation at 36 ± 1 °C, all the strains with different morphology were subcultured onto chromogenic media – chromID *S. aureus* (BioMérieux) and CHROMagar Staph aureus (CHROMagar, France) – and incubated for another 48 h at 36 ± 1 °C. All presumptive *S. aureus* strains were plated onto blood agar (BioMérieux) and subsequently identified using Gram staining, catalase, tube coagulase (Staph-ase, BioMérieux) and latex agglutination (Slidex Staph Plus, BioMérieux) tests, and the ID 32 Staph biochemical gallery (BioMérieux).

MRSA detection and antimicrobial susceptibility testing

Methicillin resistance was studied using chromogenic medium CHROMagar MRSA (CHROMagar), a latex agglutination test (Slidex MRSA, BioMérieux), and cefoxitin disks according to the Clinical Laboratory and Standards Institute guidelines (CLSI). Moreover, the presence of *mecA* gene was detected by using conventional polymerase chain reaction (PCR).[14] Disk diffusion and broth microdilution antimicrobial susceptibility testing of *S. aureus* isolates was performed following the M02 and M07 CLSI guidelines.[15,16] For testing high and low level resistance to mupirocin, two disks of 5 and 200 μg (Oxoid, UK) were used.[15] Gram-positive CLSI Microscan® panel (Siemens Healthcare, Germany) was used for the broth microdilution testing.[16] In addition, to identify h-VISA, VISA or VRSA strains, teicoplanin and vancomycin MICs were also determined by a macro E-test procedure (BioMérieux).

Toxin production

Presence of *luk-PV* and *tsst* gene that encode the PVL and the TSST-1 toxins, respectively, were studied by PCR[17,18] using ATCC 49775 and NCTC 7428 strains as positive controls for each technique.

Molecular typing

Molecular typing of all *S. aureus* isolates was performed using several techniques which included Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), staphylococcal protein A typing (spa typing), accessory gene regulator (*agr*) typing and staphylococcal cassette chromosome *mec* (*SCCmec*) typing.[19–23]