

Short Communication

Detection of *Staphylococcus aureus* with an intermediate profile to vancomycin (VISA) isolate from Santa Maria, RS

Jaciane Baggio Marques¹, Tanise Vendruscolo Dalmolin²,
Pauline Cordenonsi Bonez², Vanessa Albertina Agertt²,
Marli Matiko Anraku de Campos², Roberto Christ Vianna Santos¹

¹Laboratório de Microbiologia, Centro Universitário Franciscano, Santa Maria, RS, Brazil.

²Laboratório de Micobacteriologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

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Abstract

Twenty-three isolates of *Staphylococcus aureus* resistant to methicillin have been analyzed, being found a clinical isolate of VISA through microdilution technique. The others techniques were unable to detect such isolates. This is the first study that shows the presence of VISA in clinical isolates in the city of Santa Maria-RS.

Key words: *Staphylococcus aureus*, vancomycin, microdilution.

Staphylococcus aureus is one of the most important microorganisms found in the environment as an element of the microbiota. In some conditions it can become a pathogen, causing skin infections, respiratory infections, and sepsis (Koneman *et al.*, 2001).

The profile Vancomycin-intermediate *Staphylococcus aureus* (VISA) has become increasingly frequent. The identification of these strains led to the modification of the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2006, 2008, 2009) because there are flaws in the identification and therefore in the therapy with vancomycin. There is the patient exposure to unnecessary treatment, leading to therapeutic failure and increased costs associated with treatment (CLSI, 2009).

The resistance found in strains of *S. aureus* began to be isolated and identified, limiting the use of several drugs over the years (Rossi and Andreazzi, 2005). In 1960, methicillin raised, a year later resistant strains were found (Koneman 2001; Rossi and Andreazzi, 2005). With the emergence of Methicillin-resistant *S. aureus* (MRSA), the options for the treatment of infections by MRSA have become limited. Glycopeptides (vancomycin and teicoplanin) remained as the last resource for treatment of MRSA until recently (Cui *et al.*, 2006).

In 1996 it was first reported the emergence of VISA and it has since then been a major concern among all health

professionals. The VISA would soon spread around the world and this intermediate sensitivity to vancomycin might be associated with prolonged contact of the organism to this antimicrobial agent (Rossi and Andreazzi, 2005; Cui *et al.*, 2006). Although some strains are of intermediate aspect, there is no clinical response to treatment with vancomycin. Even with appropriate therapy, patients do not show signs of improvement. According to Rossi *et al.*, 2005 (Rossi and Andreazzi, 2005), all clinical isolates of VISA were recovered from patients who had treatment failure in the presence of vancomycin. Furthermore, all strains were previously reported as MRSA (Rossi and Andreazzi, 2005).

By failures in the identification of VISA, the CLSI in January 2006 changed the criteria and methodology for identification of these strains, decreasing the values of inhibition zones. To reach the current values, the method of reference applied was microdilution. After several tests with the VISA strains it was discovered that the disk diffusion, the method used in most laboratories is unable to identify strains with intermediate resistance to vancomycin, not distinguishing the strains of intermediate sensitivity. It was then discarded the possibility of using the disk diffusion technique as a method of identification of VISA, reinforcing the use of additional tests such as E-test or even the reference method, the microdilution (CLSI, 2006).

Given the clinical importance and difficulty of detection of VISA, this study aimed to detect VISA in clinical isolates from patients hospitalized in Santa Maria-RS.

Twenty-three isolates of methicillin-resistant *Staphylococcus aureus* were analyzed in a clinical laboratory of analysis in the city of Santa Maria-RS in the period from August to October 2010. Being all from clinical specimens of patients hospitalized and kept (Amies or Stuart) under refrigeration. Swabs were taken from the transport and inoculated in Blood Agar culture medium and incubated at 36, 5 °C to 37 °C for 24 hours. After this time, bacterial suspensions were prepared in sterile saline to obtain a turbidity of 0.5 Mac Farland scale, equivalent to 1.5×10^8 cfu/mL.

The methods recommended by CLSI 2008 (disk diffusion) and CLSI 2009 (microdilution) and the technology of E-test (Biomérieux, 2011) were applied. To carry out the techniques of disk diffusion and E-test were used Petri dishes with Mueller-Hinton (Merck®). Swabs after dipped in bacterial suspension were seeded, being implanted disks and E-test strips. For the microdilution, the medium was Mueller-Hinton broth in microdilution plates with the bacterial suspension and increasing concentrations of vancomycin and incubated for 24 hours at $35 \text{ °C} \pm 2 \text{ °C}$. The microdilution followed the concentrations recommended by the CLSI in 2009 on the plates and the tests were performed in quintuplicate (CLSI 2008, 2009).

The Table 1 shows the sites of isolation of strains of Methicillin-resistant *Staphylococcus aureus* from hospitalized patients in Santa Maria-RS (Table 1).

According to Swenson *et al.*, 2008 (Swenaon *et al.*, 2009), disk diffusion is ineffective in detecting VISAs and frequently way is proving to be a weak test. It is highly likely, however, that such failures are widely under-reported, since most of the laboratories in the past would have considered these strains as “susceptible” to vancomycin leading to failure with the use of antimicrobials; it means that the laboratory mistake is strongly associated with treatment failure (Tenover and Mollering, 2007; Swenaon *et al.*, 2009).

Currently there is a growing number of VISA mainly found in countries like Japan, United States, France, Britain and Germany, where its incidence is increasing causing a significant spread occurs worldwide and it is necessary more control done by surveillance systems and Centers for Disease Control and Prevention, which have increasingly received reports of isolation of VISA (Tenover *et al.*, 2006; Gyungtae *et al.*, 2010)

Most isolates with reduced susceptibility to vancomycin appear to have developed from pre-existing infections with MRSA after prolonged treatment with vancomycin are insulated with vancomycin intermediate profile (Tenover *et al.*, 2006).

In France, studies performed in a hospital show a prevalence of 0.07% VISA. In the U.S.A., the prevalence increased from 0.3% to 2.3% in just 22 years and in Asia,

Table 1 - Results of disk diffusion, E-test, and microdilution.

Strain number	Disk diffusion	E-test	Microdilution
1	20 mm	1.0 µg/mL	2 µg/mL
2	26 mm	1.0 µg/mL	2 µg/mL
3	21 mm	1.0 µg/mL	2 µg/mL
4	20 mm	1.0 µg/mL	2 µg/mL
5	22 mm	0.75 µg/mL	2 µg/mL
6	22 mm	1.0 µg/mL	2 µg/mL
7	26 mm	0.50 µg/mL	4 µg/mL
8	20 mm	1.0 µg/mL	2 µg/mL
9	20 mm	1.5 µg/mL	2 µg/mL
10	20 mm	1.0 µg/mL	2 µg/mL
11	18 mm	0.75 µg/mL	2 µg/mL
12	21 mm	1.0 µg/mL	2 µg/mL
13	20 mm	1.5 µg/mL	2 µg/mL
14	22 mm	1.5 µg/mL	2 µg/mL
15	18 mm	1.0 µg/mL	2 µg/mL
16	16 mm	0.75 µg/mL	2 µg/mL
17	18 mm	0.50 µg/mL	2 µg/mL
18	22 mm	1.5 µg/mL	2 µg/mL
19	18 mm	0.38 µg/mL	2 µg/mL
20	20 mm	1.0 µg/mL	2 µg/mL
21	18 mm	0.50 µg/mL	2 µg/mL
22	20 mm	0.75 µg/mL	2 µg/mL
23	19 mm	0.75 µg/mL	2 µg/mL
Quality control ATCC 29213	19 mm	0.5-2 µg/mL	2 µg/mL

countries like Thailand have rates of 0.8% and Japan 0.24% (Adam *et al.*, 2010 Gyungtae *et al.*, 2010).

In Brazil, in the state of Minas Gerais, in the city of Uberlandia a few cases was found of patients colonized with MRSA. With prolonged use of vancomycin among these patients, there was the isolation of a VISA strain (Melo *et al.*, 2005).

In this study, a total of 23 strains tested by disk diffusion test, microdilution, and E-test one isolated VISA were detected, according to Table 1. Disk diffusion and E-test methods were unable to detect VISA, collaborating with other studies (Wang *et al.*, 2006). Therefore it is necessary to encourage clinical laboratories to use microdilution technique for the detection of VISA, as recommended by CLSI 2009 (CLSI, 2009).

This is the first study that shows the presence of VISA in clinical isolates in the city of Santa Maria-RS, Brazil. We detected a strain of vancomycin intermediate, showing the presence of this microorganism in our midst, as it happens in the U.S.A., Japan, France, and Germany where there is an increasing number of strains with intermediate vanco-

mycin profile. Given the importance of this clinical VISA isolate, becomes important then surveillance studies, thus preventing the emergence of VISA.

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