REVIEW

Staphylococcus aureus with reduced susceptibility to vancomycin in healthcare settings

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Key words

Staphylococcus aureus • MRSA, GISA• h-GISA • Glycopeptide • Vancomycin

Summary

Glycopeptide resistance in Staphylococcus aureus is a source of great concern because, especially in hospitals, this class of antibiotics, particularly vancomycin, is one of the main resources for combating infections caused by methicillin-resistant Staphylococcus aureus strains (MRSA).
Reduced susceptibility to vancomycin (VISA) was first described in 1996 in Japan; since then, a phenotype with heterogeneous resistance to vancomycin (h-VISA) has emerged.
H-VISA isolates are characterised by the presence of a resistant subpopulation, typically at a rate of 1 in 10^5 organisms, which constitutes the intermediate stage between fully vancomycin-susceptible S. aureus (VSSA) and VISA isolates. As VISA phenotypes are almost uniformly cross-resistant to teicoplanin, they are also called Glycopeptides-intermediate Staphylococcus aureus strains (GISA) and, in the case of heterogeneous resistance to glycopeptides, h-GISA.
The overall prevalence of h-VISA is low, accounting for approximately 1.3% of all MRSA isolates tested.
Mortality due to h-GISA infections is very high (about 70%), especially among patients hospitalised in high-risk departments, such as intensive care units (ICU).
Given the great clinical relevance of strains that are heteroresistant to glycopeptides and the possible negative impact on treatment choices, it is important to draw up and implement infection control practices, including surveillance, the appropriate use of isolation precautions, staff training, hand hygiene, environmental cleansing and good antibiotic stewardship.

Introduction

Since the 1970s, the selective pressure exerted by antibiotics has given rise to increasingly resistant bacterial species and the last 20 years have seen a marked increase in multi-resistant pathogenic strains [1].
Staphylococcus aureus (S. aureus), human commensal bacterium involved in an array of pathologies, from minor dermatological diseases to severe disorders, such as pneumonia, endocarditis, meningitis or sepsis, continues to be one of the main causes of hospital and community infections worldwide [2]. The emergence of resistance to penicillin, followed by the spread of strains resistant penicillins penicillinases resistant (headed by methicillin, macrolides, tetracyclines, aminoglycosides and, recently, glycopeptides has turned the therapy of staphylococcal infections into a global challenge.
Glycopeptide resistance in *S. aureus* is a source of great concern because, especially in hospitals, this class of antibiotics, particularly vancomycin, is one of the main resources for combating infections caused by methicillin-resistant *S. aureus* strains (MRSA).

Methicillin-resistant *S. aureus*

The rate of mortality due to *S. aureus* infections was drastically reduced by the introduction of penicillin in the early 1940s. A few years later, however, strains of *S. aureus* that had developed plasmid-mediated resistance to penicillin appeared; this resistance was due to the production of penicillinase, a ß-lactamase capable of breaking down the drug before it could reach its target.
Methicillin, the first semisynthetic penicillin resistant to penicillinases, was introduced into clinical practice in 1959. This antibiotic proved efficacious in combating infections due to ß-Lactam antibiotic-resistant *S. aureus* strains until the appearance of methicillin-resistant strains of *S. aureus*, which soon became one of the main causes of infection in hospitals.
The first report of MRSA strains was made in England in 1961 [3], not long after the introduction of methicillin, and epidemics caused by MRSA were already being recorded in the early 1960s [4, 5]. Since then, MRSA strains have spread throughout the world and their prevalence has increased in both hospital and community settings. The epidemiology of MRSA has therefore changed in recent years, in that infections are no longer confined to the hospital environment, but also involve healthy subjects without particular risk factors in the community setting [6].
In the USA, MRSA account for more than 60% of all *S. aureus* isolates in intensive care units (ICU) [7]. In Europe, it has been estimated that MRSA cause 171,200 nosocomial infections each year, corresponding to 44% of all hospital infections [8]. In Italy, the percentage of MRSA strains isolated in hospitals is around 40%, with peaks of up to 80% in some hospitals [9].

These strains generally display multi-resistance, which considerably limits therapeutic options. A study conducted in Canada revealed that the mortality associated with bacteraemia due to MRSA was 39%, as opposed to 24% due to strains of Methicillin-sensitive Staphylococcus aureus (MSSA) [6].

Mechanism of methicillin resistance

All strains of *S. aureus* produce 4 main membrane proteins capable of binding penicillin and other ß-Lactam antibiotics (penicillin-binding proteins, PBP). ß-Lactam antibiotics are substrate analogues, which covalently bind to the serine-active sites of the penicillin-binding proteins (PBPs), inactivating the enzyme at concentrations roughly comparable to the minimum inhibiting concentrations (MIC). PBPs 1, 2 and 3, which have a high affinity for most ß-Lactam antibiotics, are essential to the development of the cell and to the survival of sensitive strains; the binding of ß-Lactam antibiotics to these PBP can kill the bacterial cell [4, 10].
The *mec*A gene, the expression of which is generally regulated by the *mecI* and *mecR1* genes, codifies for PBP type 2a (PBP2a), a low-affinity PBP on which resistance itself depends. PBP2a is a 78 kDa protein which, in methicillin-resistant strains, owing precisely to its low affinity for most ß-Lactam antibiotics, is not saturated (and thus functionally blocked) by otherwise lethal concentrations of these antibiotics. In such conditions, not only does it continue to function, it is also able to vicariously carry out the functions normally performed by the other (functionally blocked) high-affinity PBPs [11].
The *mec*A gene (2.1 kb) participates in a broader block of DNA (up to 60 kb), called staphylococcal chromosomal cassette (SCC*mec*), containing the determinants of resistance to the various non- ß-Lactam antibiotics. *MecA* is normally regulated by the genes *mecI* (repression) and *mecR1* (induction) [4, 10].

Resistance to glycopeptides and epidemiology of h- glycopeptide intermediate-resistant *S. aureus* (GISA) strains

Following the global rise in infections caused by multi-resistant MRSA strains, glycopeptides have become the antibiotics of choice for the therapy of nosocomial staphylococcal infections in the last 20 years. The glycopeptides in clinical use are vancomycin, the co-founder drug that came onto the market at the end of the 1950s, and teicoplanin, which was introduced into clinical practice in the second half of the 1980s.
The glycopeptide antibiotics are large rigid molecules, which inhibit the last stages of peptidoglycan biosynthesis. Their antimicrobial activity, which is limited to Gram-positive bacteria owing to their inability to penetrate the external membrane, is due to their particular affinity for the D-alanyl-D-alanine (D-Ala-D-Ala) dimer of the lateral chain of the peptidoglycan precursor, to which they strongly bind, albeit non-covalently [10]. Although this antibiotic has been widely used in the last two decades, most MRSA strains are still sensitive to vancomycin. Indeed, the first MRSA isolates with reduced sensitivity to glycopeptides took about 40 years to emerge [12].
The first MRSA isolates displaying reduced sensitivity to vancomycin (VISA) were reported in Japan in 1996 [13]; soon afterwards, a phenotype of *S. aureus* with acquired heterogeneous resistance to vancomycin (h-VISA) emerged [14, 15]. h-VISA isolates are characterised by the presence of a subpopulation (1 per 10^5 bacterial cells) resistant to vancomycin and represent the intermediate stage between total sensitivity to vancomycin (VSSA) and VISA isolates [10, 16-18]. Following the appearance of the first VISA (Mu50) and h-VISA (Mu3) strains reported in Japan [13, 14], both phenotypes were described worldwide. However, the exact prevalence of h-VISA strains is difficult to determine, owing to the wide range of methodological tests used, of definitions and of modifications in the breakpoints of susceptibility to vancomycin. This might explain the considerable variability in the prevalence of h-VISA strains in the various institutions, geographic regions and patient populations.
Very recently, a further phenotype was found and characterized in Mu3-6R-P strain: slow vancomycin-intermediate S. aureus (s-VISA) strains [19]. h-VISA may escape vancomycin therapy temporarily converting into s-VISA and later returning to the previous stage as soon as therapy is suspended. Therefore, the passage from h-VISA to s-VISA and viceversa can be interpreted as an oscillating, reversible switch mechanism.
Nevertheless, the overall prevalence of h-VISA remains low: about 1.3% of all methicillin-resistant Staphylococcus aureus (MRSA) isolates tested [16]. Di Gregorio et al. computed h-VISA to be 4.5% of MRSA strains [20]. Hanaki and coauthors estimated that h-VISA represent 6.5% of MRSA strains [21], while Chaudhari and collaborators estimated h-VISA to represent 6.9% of 58 clinical isolates of MRSA [22]. Monaco and coworkers carried out a study in order to assess the presence of h-VISA strains in Italy: they found h-VISA to be 13.6% of MRSA strains and 6.1% of all the studied S. aureus strains [23].
As VISA strains generally display cross-resistance to teicoplanin, they are also called glycopeptide intermediate-resistant *S. aureus* (GISA) [24] and, in the case of heteroresistance, h-GISA. In the USA, however, where teicoplanin is not available, the terms VISA and h-VISA are currently used.
International data from the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) involving 20,004 *S. aureus* isolates show that the proportion of MRSA with vancomycin MICs ≥ 2 mg/L increased from 5.6% in 2004 to 11.1% in 2009 ($P < 0.001$) [8].
A study conducted in the metropolitan area of Detroit in the USA documented a significant increase in the prev-

alence of h-VISA over 20 years: from 2.27% between 1986 and 1993 to 8.2% between 2003 and 2006 [25].
VISA strains tend to develop multi-resistance to a large number of commonly used antibiotics, thereby determining a reduction in possible therapeutic options and increasing the risk of administering inadequate antibiotic therapy [26]. An increase in the resistance of MRSA strains leads to increased morbidity and mortality due to severe infections such as bacteraemia, endocarditis and osteomyelitis [27, 28].
Concern over the development of vancomycin resistance in staphylococci is destined to grow dramatically following reports of vancomycin-resistant strains of MRSA (VRSA). The first strain was reported in the United States in 2002, isolated from a haemodialysis patient; this strain proved to be highly resistant to vancomycin and was also resistant to teicoplanin. It was isolated from the patient together with an enterococcus, VanA, and was found to contain in its genome not only the *mecA* gene of methicillin-resistance, but also the vanA gene, which is responsible for the most widespread form of vancomycin-resistance in enterococci. The DNA sequence of the *vanA* gene of the Staphylococcus was identical to that of the vanA gene of the *E. faecalis* isolated from an infected ulcer in the same patient. This strain, the first clinical isolate of *S. aureus* highly resistant to vancomycin, therefore seems to be the result of the spread of VanA resistance from the enterococcus to the *S. aureus* [10, 29]. To date, strains displaying high levels of resistance to vancomycin (acquired through the *vanA* gene) are rare, though cases have been reported in the USA, India and Iran [8].
The results of a study conducted by Maor [30] revealed that 6% of patients affected by MRSA presented h-VISA strains and that the mortality rate among all the h-VISA patients was 75%. This study suggests that h-VISA infection is associated with unsatisfactory clinical outcomes despite the adequate administration of vancomycin.
A study conducted on 86 patients from whom MRSA strains with reduced susceptibility to teicoplanin were isolated revealed that 3.4% of patients were colonised by h-GISA and that 2.5% had bacteraemia caused by h-GISA. The results of this study suggest that recurrent bacteraemia in a patient who has previously undergone antibiotic therapy with glycopeptides is an important indicator of the presence of h-GISA [31].
Mortality due to h-GISA infections is very high (about 70%), especially among patients hospitalised in high-risk wards, such as intensive care units (ICU), where the vulnerability of the patient is exacerbated by such contingencies as invasive medical procedures, the insertion of prosthetic devices or of central venous catheters, the high frequency of nursing procedures, and the ample use of broad-spectrum antibiotic therapy [32].
The hospital environment can play an essential role in the transmission of multidrug-resistant pathogens, and environmental monitoring can reveal the degree of microbial contamination [33]. Environmental contamination by MRSA strains tends to be very persistent (up to 38 weeks) [34], which means that surfaces in wards can become veritable reservoirs and vehicles for the spread of infection [35, 36]. h-GISA strains are characterised by thickening of the peptidoglycan wall [14, 15], which is proportional to the degree of resistance to glycopeptides; this ultrastructural feature may favour adhesion to surfaces, with important implications for the type of sanitation measures that need to be implemented.
A study conducted by Perdelli et al. [37] evaluated the percentage of MRSA with reduced susceptibility to glycopeptides in four ICU by means of environmental sampling of air and representative surfaces. The antibiogram performed on the colonies of *S. aureus* revealed that, in the air of the four ICU sampled, 88.8% of the strains proved to be resistant to methicillin and that 91.9% of these displayed reduced susceptibility to glycopeptides. A similar situation emerged with regard to the surfaces sampled (72.0% MRSA, 81.1% of which h-GISA).
The prevalence of notified infections due to h-GISA strains is low. However, as mentioned previously, this might be due to the routine use of laboratory screening techniques that have low sensitivity and specificity. It would therefore be useful to implement quality controls in order to verify the reliability of results and to unmask any possible underestimation of the phenomenon [38].
Given the great clinical relevance of strains that are heteroresistant to glycopeptides, and their possible negative impact on therapeutic choices, measures for prevention and control should be implemented both on the clinical front and with regard to hygiene/behavior.

Treatment and management

As vancomycin and other glycopeptides, such as teicoplanin, have constituted the treatment of choice for infections due to MRSA, their excessive use may have led to the appearance of h-VISA, VISA and VRSA strains. Moreover, it is likely that the true magnitude of the problem has been underestimated and that many cases of h-VISA, VISA and perhaps VRSA have gone undetected owing to the implementation of suboptimal screening programs and the shortcomings of current diagnostic techniques [30]. As yet, the proportion of MRSA strains with reduced susceptibility to vancomycin and teicoplanin in the hospital setting is not known [26]. Such knowledge, however, would be extremely important for the purposes of prevention and control [39], in that strains heteroresistant to glycopeptides (h-GISA) are the direct precursors of vancomycin-resistant *S. aureus* (VRSA) strains and seem to be directly implicated in the failure of antibiotic therapy in MRSA infections that spread to deep layers [40, 41].
An alternative to vancomycin is daptomycin, an antibiotic belonging to the class of lipopeptides, which disrupts the functioning of the cell membrane through a calcium-dependent bond. Its bactericidal activity depends on the concentration. The breakpoint of sensitivity to daptomycin for *S. aureus* is ≤ 1 μg/ml. Non-susceptible strains have appeared during treatment with this antibi-

otic. Although the mechanism of resistance has not been clarified, these strains often display point mutations of mprF, the gene for lysophosphatidylglycerol synthetase. Previous exposure to vancomycin and a high MIC of vancomycin have been associated to the increase in the MIC of daptomycin, an observation that seems to indicate possible cross-resistance [42].

Prevention and control

In recent years, several international scientific associations and institutions have drawn up recommendations aimed at reducing the spread of MRSA infections in the healthcare setting [43-47]. These recommendations are concordant with regard to some essential aspects, such as the use of specific surveillance tools, the adoption of contact precautions (hand hygiene, use of barrier measures) to limit the spread of any cross-infection, and policies aimed at promoting the proper use of antibiotics. With regard to this last aspect, it is important to rationalise the administration and use of glycopeptides in relation not only to therapeutic results but also to phenomena of resistance.

However, antibiotic policy must not be limited only to this class of antibiotics; it must also involve cephalosporins and carbapenems, since the heterogeneous expression of glycopeptide resistance is also influenced by exposure to almost all ß-Lactam antibiotics, even when administered at optimal concentrations [15]. The issues of the active detection of colonised patients and their decolonisation are more controversial [48-53]. This latter question has been the subject of recently published systematic reviews [54, 55]. In 1997, the Centers for Disease Control and Prevention (CDC) in Atlanta drew up a document containing recommendations for preventing the spread of vancomycin resistance [56]. Further considerations on the control of infections due to vancomycin-resistant *S. aureus* strains were made by Wenzel and Edmond [57], particularly with regard to the utility of conducting studies on the prevalence of antibiotic resistance, implementing control strategies and, especially, contact precautions (hand-washing, use of gloves, isolation, etc.), and immediate notification to the Infections Committee of the hospital.

It is also important to utilise appropriate diagnostic techniques in order to minimise recourse to prolonged empirical therapy; for example to use venous catheters only for the time strictly necessary, and to remove prosthetic materials infected by *S. aureus*. It is well known that MRSA can spread easily in the hospital environment, and it is reasonable to suppose that VISA strains have the same potential for transmission [10]. Measures for the prevention and control of the spread of these microorganisms have recently been revised in a document endorsed by several European countries. This underscores a few key points: proper hand hygiene and routine cleansing and decontamination of environments; the use of personal protection devices by healthcare personnel when attending to MRSA-positive patients; the implementation of MRSA surveillance programs, and the screening of patients at risk [58]. It has been demonstrated that controlling the spread of MRSA in hospitals requires the simultaneous implementation of both "horizontal" and "vertical" strategies. Horizontal strategies are those aimed at preventing the spread of infections due to all possible pathogens [37, 59-62] through interventions such as hand hygiene, environmental cleansing, antibiotic stewardship and proper management of vascular catheters; vertical strategies are those aimed at controlling a specific pathogen (MRSA) [63]. An approach that combines these two strategies – horizontal and vertical – can optimise the results [57]. In Italy, the Ministry of Health has recently drawn up a document which identifies the priority measures to be adopted in order to reduce the risk of healthcare-related infections (HAIs) caused by MRSA, as indicated in the most recent international scientific literature [63].

The main measures are listed below:

Surveillance

Organising a system of surveillance is useful only if data analysis leads to the adoption of suitable provisions. Thus, identifying patients infected/colonised by MRSA is useful if the system prescribes the subsequent isolation of the positive patient and the implementation of contact precautions.

Surveillance can allow the spread of MRSA inside health facilities to be detected and monitored over time, in order to plan adequate intervention. To ensure optimal cooperation on the part of the various departments, surveillance data must be provided periodically.

Handling information on MRSA positivity

The correct and timely transmission of information on MRSA positivity is important in order to ensure that the necessary interventions and/or decisions be taken to address the problem.

At the moment of hospitalisation, the availability of information on previous colonisation by MRSA can enable the patient to be placed pre-emptively in isolation, thereby reducing the spread of the microorganism in the hospital.

Hand hygiene

Proper hand hygiene is deemed to be the main means of reducing HAIs. Compliance with this measure on the part of healthcare personnel is generally less than 40%; this low percentage has been associated with the use of gloves, a practice erroneously regarded as a substitute for hand hygiene.

Kapil and collaborators carried out a survey among health-care workers (HCWs) and found that 70% had bacterial counts ≥ 100 CFUs. Hand hygiene reduced the count of 95-99% among doctors and nurses, 70% among hospital attendants and 50% among sanitary attendants. *S. aureus* was present on the hands of 8 HCWs of which three were MRSA [64]. Similar findings were obtained by Monistrol and coworkers who found that *S. aureus* is the most common contaminant in health settings and

that, isolated from the hands of healthcare workers, after an educational intervention, the MRSA count decreased from 1.96 ± 1.2 log10 CFU/ml to 0.89 ± 1.2 log10 CFU/ml [65]. Al-Tawfiq and coauthors observed a marked decrease in the rate of MRSA cases per 1,000 patient-days from 0.42 to 0.08, with an increase in the hand hygiene compliance [66].
82% of patients colonized by MRSA had positive hand cultures for MRSA, which reduced after a single application of alcohol gel [67]. Besides HCWs hand hygiene compliance, also patient hand disinfection plays a major role [68].
The use of alcohol gels and solutions for hand hygiene has overcome many of the problems of non-compliance, especially when time is short owing to heavy workloads.

Contact precautions

The spread of infections in healthcare facilities is made possible by the interaction of three principal elements: a source (reservoir) of pathogenic microorganisms; a susceptible host and a suitable means of entry for that specific microorganism.
The main reservoir of infection is constituted by persons (patients, healthcare workers, visitors and family members). Human reservoirs may be subjects who are colonised or have active infections. The environment may also be involved in the spread of microorganisms, through contaminated environmental sources or vehicles (equipment, instruments, medical devices, solutions for infusion, etc.).
As MRSA is chiefly spread through contact (direct or indirect), contact precautions must be taken in order to reduce the risk of transmission to a susceptible patient. These precautions include:

- isolation in a single room or, if this is not possible, isolation by cohort;
- the use of dedicated materials;
- hand hygiene;
- the use of disposable gloves and overalls;
- the use of protective barriers;
- proper management of equipment;
- environmental hygiene;
- proper handling of bedding and crockery;
- healthcare education, and staff training.

Environmental hygiene

Healthcare facilities need to draw up regulations for environmental cleansing (frequency, methods) and to appoint a person to be responsible for ensuring that these regulations are respected.
The environmental surfaces in healthcare facilities can contribute to the spread of cross-infections, in that they constitute a possible site for the accumulation of microorganisms [69]. Like medical devices, surfaces must therefore be thoroughly cleaned and disinfected regularly; disinfectants must be appropriate and used in conformity with the manufacturers' recommendations and the indications of the Hospital Infections Committee, and particular attention should be paid to surfaces that are touched frequently.

Screening

In departments with a high incidence of MRSA or in those accommodating patients at risk of severe MRSA infections, it is advisable to carry out active screening of high-risk patients. However, the implementation of an MRSA screening system is meaningful only if the results of screening are used to enact infection control measures.

Decolonisation

Care bundles recommend that nasal decolonisation be carried out with mupirocin in all patients identified as MRSA-positive, according to the screening strategies identified, and skin decolonisation with 4% chlorhexidine, 7.5% iodopovidone or 2% triclosan.
Universal decolonization is cost-saving [70] in that prevents 44% of MRSA colonizations and 45% of MRSA infections. Also the REDUCE MRSA trial confirmed this finding, showing that compared with screening and isolation, universal decolonization could save $171,000 and prevent 9 additional bloodstream infections for every 1,000 ICU admissions [71].

Personnel

The screening of personal is recommended only when there is a strong suspicion that staff may be a source of transmission, as in the case of an uncontrolled epidemic.

Antibiotic stewardship

According to the international recommendations, in order to reduce or at least contain the problem of antibiotic resistance, antibiotic policies, such as the following, should be implemented:

1. Avoid inappropriate or excessive antibiotic therapies and prophylaxes.

Pay attention to the diagnosis and ensure that the therapy is appropriate.

2. Ensure that the dose and duration of antibiotic therapy are correct.
3. Reduce as far as possible the use of broad-spectrum antibiotics, in particular third-generation cephalosporins and quinolones.
4. Limit the use of glycopeptides and check therapeutic levels.

It is also important to check that preoperative antibiotic prophylaxis is appropriate in terms of indication, choice of drug, dose and duration of prophylaxis, and to monitor the consumption of antibiotics, at least in critical departments at high risk of MRSA.
Antibiotic stewardship is particularly helpful in reducing MRSA cases and has long-term effect, as shown by studies carried out in a secondary-care hospital in Germany [72], and in a tertiary-care teaching hospital in the USA [73].
It is also important to educate junior doctors about the importance of preserving the effectiveness of the available armamentarium against S. aureus, as demonstrated by an interventional study performed at two teaching hospitals in France and Scotland [74].

New strategies and forms of antibiotic stewardship have been recently implemented for raising awareness of the importance of a correct and proper antibiotic policy among the HCWs.

New technologies can help in making antibiotic stewardship highly sustainable, strengthening its impact and preserving high quality care while reducing the costs [75].

In conclusion, given the great clinical relevance of strains that are heteroresistant to glycopeptides and the possible negative impact on treatment choices, it is important to draw up and implement infection control practices, including surveillance, the appropriate use of isolation precautions, staff training, hand hygiene, environmental cleansing and good antibiotic stewardship.

References

[1] Acar JF, Kaplan EL, O'Brien TF. *Monitoring and management of bacterial resistance to antimicrobial agents, a World Health Organization symposium.* Clin Infect Dis 1997;24:S1-176.

[2] Cristina ML, Spagnolo AM, Orlando P, et al. *The role of the environment in the spread of emerging pathogens in at-risk hospital wards*. Rev Med Microbiol 2013;24:104-12.

[3] Jevons MP, Coe AW, Parker MT. *Methicillin resistance in staphylococci*. Lancet 1963;1:904-7.

[4] Manso E, Varaldo PE. *La resistenza agli antibiotici negli stafilococchi*. Website: http://www.masciabrunelli.it/biolife/upload/file/Documenti/Documenta_imp_2002.PDF

[5] *Epidemiology of methicillin-resistant Staphylococcus aureus infection in adults*. Website: http://www.uptodate.com/contents/epidemiology-of-methicillin-resistant-Staphylococcus-aureus-infection-in-adults.

[6] Kluytmans J, Struelens M. *Meticillin resistant Staphylococcus aureus in the hospital*. BMJ 2009;338:b364.

[7] Boucher HW, Corey GR. *Epidemiology of methicillin-resistant Staphylococcus aureus*. Clin Infect Dis 2008;46: S344-9.

[8] Gould IM, David MZ, Esposito S, et al. *New insights into methicillin-resistant Staphylococcus aureus (MRSA) pathogenesis, treatment and resistance.* Int J Antimicrob Agents 2012;39:96-104.

[9] *Staphylococcus aureus*. Website: http://www.iss.it/chem/index.php?lang=1&id=70&tipo=11.

[10] Howden BP, Davies JK, Johnson PD, et al. *Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications.* Clin Microbiol Rev 2010;23:99-139.

[11] Tonin E, Tomasz A. *Beta-lactam-specific resistant mutants of Staphylococcus aureus.* Antimicrob Agents Chemother 1986;30:577-83.

[12] van Hal SJ, Lodise TP, Paterson DL. *The clinical significance of vancomycin minimum inhibitory concentration in Staphylococcus aureus infections: a systematic review and meta-analysis.* Clin Infect Dis 2012;54:755-71.

[13] Hiramatsu K, Hanaki H, Ino T, et al. *Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility.* J Antimicrob Chemother 1997;40:135-6.

[14] Hiramatsu K, Aritaka N, Hanaki H, et al. *Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin.* Lancet 1997;350:1670-3.

[15] Hiramatsu K. *Vancomycin–resistant Staphylococcus aureus: a new model of antibiotic resistance.* Lancet Infect Dis 2001;1:147-55.

[16] van Hal SJ, Paterson DL. S*ystematic review and meta-analysis of the significance of heterogeneous vancomycin-intermediate Staphylococcus aureus isolates.* Antimicrob Agents Chemother 2011;55:405-10.

[17] van Hal SJ, Wehrhahn MC, Barbagiannakos T, et al. *Performance of various testing methodologies for detection of heteroresistant vancomycin-intermediate Staphylococcus aureus in bloodstream isolates.* J Clin Microbiol 2011;49:1489-94.

[18] van Hal SJ, Jones M, Gosbell IB, et al. *Vancomycin heteroresistance is associated with reduced mortality in ST239 methicillin-resistant Staphylococcus aureus blood stream infections.* PLoS One 2011;6:e21217.

[19] Saito M, Katayama Y, Hishinuma T, et al. "*Slow VISA," a novel phenotype of vancomycin resistance, found in vitro in heterogeneous vancomycin-intermediate Staphylococcus aureus strain Mu3*. Antimicrob Agents Chemother 2014;58:5024-35.

[20] Di Gregorio S, Perazzi B, Ordoñez AM, et al. *Clinical, microbiological, and genetic characteristics of heteroresistant vancomycin-intermediate Staphylococcus aureus bacteremia in a teaching hospital*. Microb Drug Resist 2015;21:25-34.

[21] Hanaki H, Cui L, Ikeda-Dantsuji Y, et al. *Antibiotic susceptibility survey of blood-borne MRSA isolates in Japan from 2008 through 2011*. J Infect Chemother 2014;20:527-34.

[22] Chaudhari CN, Tandel K, Grover N, et al. *Heterogeneous vancomycin-intermediate among methicillin resistant Staphylococcus aureus.* Med J Armed Forces India 2015;71:15-8.

[23] Monaco M, Sanchini A, Grundmann H, et al. *Vancomycin-heteroresistant phenotype in invasive methicillin-resistant Staphylococcus aureus isolates belonging to spa type 041*. Eur J Clin Microbiol Infect Dis 2010;29:771-7.

[24] Renzoni A, Kelley WL, Vaudaux P, et al. *Exploring innate glycopeptide resistance mechanisms in Staphylococcus aureus.* Trends Microbiol 2010;18:55-6.

[25] Rybak M, Chin JN, Lau K, et al. *Increasing prevalence of glycopeptide hetero-resistant S. aureus from the Detroit metropolitan area over a 20-year period (1986–2006).* In: *17th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) and the 25th International Congress of Chemotherapy (ICC).* 2007 [Abstract O32].

[26] Appelbaum PC. *MRSA-the tip of the iceberg.* Clin Microbiol Infect 2006;12:3-10.

[27] Blot SI, Vandewoude KH, Hoste EA, et al. *Outcome and attributable mortality in critically ill patients with bacteremia involving methicillin-susceptible and methicillin-resistant Staphylococcus aureus.* Arch Intern Med 2002;162:2229-35.

[28] Chang FY, MacDonald BB, Peacock Jr JE, et al. *A prospective multicenter study of Staphylococcus aureus bacteremia: incidence of endocarditis, risk factors for mortality, and clinical impact of methicillin resistance.* Medicine (Baltimore) 2003;82:322-32.

[29] Chang S, Sievert DM, Hageman JC, et al. *Infection with vancomycin-resistant Staphylococcus aureus containing the vanA resistance gene.* N Engl J Med 2003;348:1342-7.

[30] Maor Y, Rahav G, Belausov N, et al. *Prevalence and characteristics of heteroresistant vancomycin intermediate Staphylococcus aureus bacteremia in a tertiary care center.* J Clin Microbiol 2007;45:1511-4.

[31] Kirby A, Graham R, Williams NJ, et al. *Staphylococcus aureus with reduced glycopeptide susceptibility in Liverpool, UK*. J Antimicrob Chemother 2010;65:721-4.

[32] Linares J. *VISA/GISA problem: therapeutic implications.* Clin Microbiol Infect 2001;7:8-15.

[33] Ottria G, Dallera M, Aresu O, et al. *Environmental monitoring programme in the cell therapy facility of a research centre: Preliminary investigation.* J Prev Med Hyg 2010;51:133-8.

[34] Dietz B, Raht A, Wendt C, et al. *Survival of MRSA on sterile goods packaging.* J Hosp Infect 2001;49:255-61.

[35] Centers for Disease Control and Prevention/Healthcare Infection Control Practices Advisory Committee (HICPAC). 2001. *Draft Guidelines for Environmental Infection Control in Healthcare Facilities. Atlanta, GA: Centers for disease Control and Prevention.* Available at www.cdc.gov/ncidod/hip/enviro/guide.htm.

[36] Farr BM, Salgado CD, Karchmer TB, et al. *Can antibiotic-resistant nosocomial infections be controlled?* Lancet Infect Dis 2001;1:38-45.

[37] Perdelli F, Dallera M, Cristina ML, et al. *A new microbiological problem in intensive care units: environmental contamination by MRSA with reduced susceptibility to glycopeptides*. Int J Hyg Environ Health 2008;211:213-8.

[38] Boccia D, D'Ancona F, Pantosti A. *Resistenza alla Vancomicina in ceppi invasivi di Enterococcus faecalis ed enterococcus faecium.* Dati preliminari Bollettino Epidemiologico Nazionale – Notiziario ISS – 2002;15(5).

[39] Walsh T R, Howe R A, Wootton M, et al. *Detection of glycopeptide resistance in Staphylococcus aureus.* J Antimicrob Chemother 2001;47:357-8.

[40] Ariza J, Pujol M, Cabo J, et al. *Vancomycin in surgical infections due to methicillin-resistant Staphylococcus aureus with heterogeneous resistance to vancomycin.* Lancet 1999;353:1587-8.

[41] Moore M R, Perdreau-Remington F, Chambers HF. *Vancomycin treatment failure associated with heterogeneous vancomycin-intermediate Staphylococcus aureus in patient with endocarditis and in the rabbit model of endocarditis.* Antimicrob Agents Chemother 2003;47:1262-6.

[42] Liu C, Bayer A, Cosgrove SE, et al. *Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant staphylococcus aureus infections in adults and children.* Clin Infect Dis 2011;52:e18-55.

[43] Infection Prevention Working Party. *MRSA Hospital.* The Netherlands, 2007.

[44] Rodríguez-Baño J, Bischofberger C, Álvarez-Lerma F, et al. *Vigilancia y el control de Staphylococcus aureus resistente a meticilina en hospitales españoles. Documento de consenso GEIH-SEIMC y SEMPSPH.* Enferm Infecc Microbiol Clin 2008;26:285-98.

[45] Calfee DP, Salgado CD, Classen D, et al. SHEA/IDSA. *Strategies to prevent transmission of methicillin-resistant Staphylococcus aureus in acute care hospitals.* Infect Control Hosp Epidemiol 2008;29:62-80.

[46] Cystic Fibrosis Trust. *Methicillin-resistant Staphylococcus aureus (MRSA).* Report of the UK Cystic Fibrosis Trust Infection Control Working Group 2008.

[47] Borgman S, Stark M, Kaiser P, et al. *MRSA in praxis, pflegeheim und häuslichem umfeld.* Bayerisches Ärzteblatt 2008;3:176-81.

[48] Tacconelli E. *Screening and isolation for infection control.* J Hosp Infect 2009;73:371-7.

[49] Harbarth S, Fankhauser C, Schrenzel J, et al. *Universal screening for methicillin-resistant Staphylococcus aureus at hospital admission and nosocomial infection in surgical patients.* JAMA 2008;299:1149-57.

[50] Robicsek A, Beaumont JL, Paule SM, et al. *Universal surveillance for methicillin-resistant Staphylococcus aureus in 3 affiliated hospitals.* Ann Intern Med 2008;148:409-18.

[51] Jeyaratnam D, Whitty CJ, Phillips K, et al. *Impact of rapid screening tests on acquisition of methicillin resistant Staphylococcus aureus: cluster randomised crossover trial.* BMJ 2008;336:927-30.

[52] Raineri E, Crema L, De Silvestri A, et al. *Methicillin-resistant Staphylococcus aureus control in an intensive care unit: a 10 year analysis.* J Hosp Infect 2007;67:308-15.

[53] Keshtgar MR, Khalili A, Coen PG, et al. *Impact of rapid molecular screening for methicillin-resistant Staphylococcus aureus in surgical wards.* Br J Surg 2008;95:381-6.

[54] Tacconelli E, De Angelis G, de Waure C, et al. *Rapid screening tests for methicillin-resistant Staphylococcus aureus at hospital admission: systematic review and meta-analysis.* Lancet Infect Dis 2009;9:546-54.

[55] van Rijen M, Bonten M, Wenzel R, Kluytmans J. *Mupirocin ointment for preventing Staphylococcus aureus infections in nasal carriers.* Cochrane Database Syst Rev 2008;4:CD006216.

[56] Centers for Disease Control and Prevention. *Interim guidelines for prevention and control of staphylococcal infection associated with reduced susceptibility to vancomycin.* MMWR Morb Mortal Weekly Rep 1997;46:626-35.

[57] Wenzel RP, Edmond MB. *Vancomycin-resistant Staphylococcus aureus: infection control considerations.* Clin Infect Dis 1998;27:245-51.

[58] Kalenic S, Cookson B, Gallagher R, et al. *Comparison of recommendations in national/regional Guidelines for the prevention and control of MRSA in thirteen European countries.* Int J Infect Control 2010;v6:i2.

[59] Spagnolo AM, Orlando P, Panatto D, et al. *An overview of carbapenem-resistant Klebsiella pneumoniae: Epidemiology and control measures.* Rev Med Microbiol 2014;25:7-14.

[60] Cristina ML, Spagnolo AM, Cenderello N, et al. *Multidrug-resistant Acinetobacter baumannii outbreak: an investigation of the possible routes of transmission.* Public Health 2013;127:386-91.

[61] Cristina ML, Spagnolo AM, Ottria G, et al. *Spread of multidrug carbapenem-resistant Acinetobacter baumannii in different wards of an Italian hospital.* Am J Infect Control 2011;39:790-4.

[62] Cristina ML, Spagnolo AM, Sartini M, et al. *Clostridium difficile infections: an emerging problem in healthcare facilities.* Rev Med Microbiol 2012;23:67-75.

[63] *Raccomandazioni sul controllo della diffusione nosocomiale dello Staphylococcus aureus resistente alla meticillina (MRSA) 2011.* A cura di: Istituto Superiore di Sanità, Agenzia sanitaria e sociale regionale dell'Emilia-Romagna, Ministero della salute – Ufficio V Malattie infettive, Direzione generale della prevenzione sanitaria. Disponibile sul sito: http://asr.regione.emilia-romagna.it/wcm/asr/aree_di_programma/rischioinfettivo/gr_ist/pr_staph/pubblicazioni/report_mrsa/link/mrsa.pdf.

[64] Kapil R, Bhavsar HK, Madan M. *Hand hygiene in reducing transient flora on the hands of healthcare workers: an educational intervention.* Indian J Med Microbiol 2015;33:125-8.

[65] Monistrol O, López ML, Riera M, et al. *Hand contamination during routine care in medical wards: the role of hand hygiene compliance.* J Med Microbiol 2013;62:623-9.

[66] Al-Tawfiq JA, Abed MS, Al-Yami N, et al. *Promoting and sustaining a hospital-wide, multifaceted hand hygiene program resulted in significant reduction in health care-associated infections.* Am J Infect Control 2013;41:482-6.

[67] Sunkesula V, Kundrapu S, Macinga DR, et al. *Efficacy of alcohol gel for removal of methicillin-resistant Staphylococcus aureus from hands of colonized patients*. Infect Control Hosp Epidemiol 2015;36:229-31.

[68] Hedin G, Blomkvist A, Janson M, Lindblom A. *Occurrence of potentially pathogenic bacteria on the hands of hospital patients before and after the introduction of patient hand disinfection*. APMIS 2012;120:802-7.

[69] Orlando P, Cristina ML, Dallera M, et al. *Surface disinfection: evaluation of the efficacy of a nebulization system spraying hydrogen peroxide.* J Prev Med Hyg 2008;49:116-9.

[70] Gidengil CA, Gay C, Huang SS, et al. *Cost-effectiveness of strategies to prevent methicillin-resistant Staphylococcus aureus transmission and infection in an intensive care unit.* Infect Control Hosp Epidemiol 2015;36:17-27.

[71] Huang SS, Septimus E, Avery TR, et al. *Cost savings of universal decolonization to prevent intensive care unit infection: implications of the REDUCE MRSA trial*. Infect Control Hosp Epidemiol 2014;35(Suppl 3):S23-31.

[72] Borde JP, Batin N, Rieg S, et al. *Adherence to an antibiotic stewardship bundle targeting Staphylococcus aureus blood stream infections at a 200-bed community hospital*. Infection 2014;42:713-9.

[73] Cook PP, Gooch M. *Long-term effects of an antimicrobial stewardship programme at a tertiary-care teaching hospital*. Int J Antimicrob Agents 2015;45:262-7.

[74] Pulcini C, Williams F, Molinari N, Davey P, Nathwani D. *Junior doctors' knowledge and perceptions of antibiotic resistance and prescribing: a survey in France and Scotland*. Clin Microbiol Infect 2011;17:80-7.

[75] Chan YY, Lin TY, Huang CT, et al. *Implementation and outcomes of a hospital-wide computerised antimicrobial stewardship programme in a large medical centre in Taiwan*. Int J Antimicrob Agents 2011;38:486-92.

Abbreviations

CDC: Centers for Disease Control and Prevention; D-ala-D-ala: D-alanyl-D-alanine; eDNA: extracellular DNA; GISA: glycopeptides-intermediate Staphylococcus aureus strains; HAIs: healthcare-associated infections; HCAAS: hospital-wide computerised antimicrobial approval system; h-GISA: heterogeneous glycopeptides-intermediate Staphylococcus aureus strains; h-VISA: heterogeneous vancomycin-intermediate Staphylococcus aureus strains; h-VRSA: heterogeneous vancomycin-resistant Staphylococcus aureus strains; ICU: intensive care unit; MGEs: mobile genetic elements; MIC: minimum inhibitory concentration; MRSA: methicillin-resistant Staphylococcus aureus strains; MSSA: Methicillin-sensitive Staphylococcus aureus; *PBPs: penicillin-binding proteins; PBP2a: PBP type 2a; PBP4: PBP type 4, a transpeptidase involved in crosslinking peptidoglycans; PRPs: penicillinase-resistant penicillins; SCCmec: staphylococcal chromosomal cassette; s-VISA: slow vancomycin-intermediate Staphylococcus aureus strains; T.E.S.T.: Tigecycline Evaluation and Surveillance Trial; TOMMs: thiazole/oxazole-modified microcins; VISA: vancomycin-intermdiate Staphylococcus aureus strains; VRSA: vancomycin-resistant Staphylococcus aureus strains; VSSA: vancomycin-susceptible Staphylococcus aureus strains.*

■ Received on November 13, 2014. Accepted on December 15, 2014.

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