



Decrease in vancomycin MICs and prevalence of hGISA in MRSA and MSSA isolates from a German pediatric tertiary care center

Katharina Haas¹ · Melanie Meyer-Buehn¹ · Ulrich von Both^{1,2} · Johannes Hübner¹ · Tilmann Schober^{1,3,4}

Received: 27 January 2023 / Accepted: 5 April 2023 / Published online: 18 April 2023
© The Author(s) 2023

Abstract

Purpose Resistance of *Staphylococcus aureus* to vancomycin includes a general increase of minimal inhibitory concentrations (MIC) within the susceptible range over time (Vancomycin MIC Creep) and the presence of a subset of the bacterial population that expresses resistance (heterogeneous glycopeptide-intermediate *S. aureus*; hGISA). Increased MICs have been associated with adverse clinical outcomes. However, the vancomycin MIC creep is not a uniform trend suggesting the importance of regional surveys.

Methods We performed a retrospective analysis at a German pediatric tertiary care hospital. Isolates from 2002 to 2017 were selected which were newly identified methicillin-resistant *S. aureus* (MRSA) or samples from invasive methicillin-susceptible *S. aureus* (MSSA) or MRSA infections. Vancomycin and oxacillin MICs as well as GISA/hGISA were measured using MIC test strips and resistance was evaluated over time.

Results A total of 540 samples were tested, 200 from the early (2002–2009) and 340 from the later period (2010–2017). All samples were vancomycin susceptible, but the MIC was higher for the earlier samples as compared to the later ones (1.11 vs 0.99; $p < 0.001$). 14% of the samples were hGISA, GISA strains were not detected. Again, vancomycin resistance decreased over time with 28 vs. 6% hGISA ($p < 0.001$). There was no significant difference between MRSA and MSSA samples with respect to vancomycin MIC and hGISA prevalence.

Conclusion This study shows a decreasing trend for both MIC values and presence of hGISA strains highlighting the importance of monitoring local susceptibilities. Vancomycin remains a first-line treatment option for suspected severe infection with Gram-positive cocci and proven infection with MRSA.

Keywords Vancomycin · Minimum inhibitory concentration · Heterogeneous glycopeptide-intermediate *S. aureus* · hGISA · MRSA

Introduction

Staphylococcus aureus (*S. aureus*) is regarded as one of the most important pathogens in all age groups [1]. Vancomycin is a first-line treatment option for several indications due to the worldwide spread of Methicillin-resistant *S. aureus* (MRSA) infections [2]. In 1996, the first MRSA strain with reduced susceptibility to vancomycin was described in Japan [3]. The Clinical and Laboratory Standards Institute (CLSI) breakpoints defines strains with a MIC of 4–8 µg/mL as intermediate and ≥ 16 µg/mL as resistant [4]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is more stringent and considers a MIC of ≥ 4 µg/mL as resistant since increasing the dose of vancomycin is limited by toxicities [5].

✉ Tilmann Schober
tilmann.schober@med.uni-muenchen.de

¹ Division of Pediatric Infectious Diseases, Dr. Von Hauner Children's Hospital, Ludwig-Maximilians-University Munich, Munich, Germany

² German Center for Infection Research (DZIF), Partner Site Munich, Munich, Germany

³ Division of Pediatric Infectious Diseases, Montreal Children's Hospital, McGill University, McGill University Health Centre, 1001 Décarie Blvd, Montréal, QC H4A 3J1, Canada

⁴ Division of Medical Microbiology, McGill University, Montreal, Canada

Although the occurrence of truly vancomycin-resistant strains remains extremely rare, there is concern about *S. aureus* strains that show heteroresistance to vancomycin [6]. In these cases, there is a subpopulation with a MIC of $> 2 \mu\text{g/mL}$ for vancomycin within an otherwise susceptible strain. In the presence of vancomycin, selection pressure favors the proliferation of intermediate-resistant clones. Persistent vancomycin exposure can lead to a uniform GISA population [7]. Importantly, this mechanism is different from fully vancomycin-resistant strains that have acquired plasmid-encoded *vanA* from *Enterococcus* species [8].

Clinically, the treatment of hGISA poses a major problem, as it is not possible to detect the resistance using standard methods. In addition, infections with hGISA are associated with poor treatment outcome [9].

Another problem in the treatment of infections with MRSA is a phenomenon known as "Vancomycin Minimal Inhibitory Concentration Creep" (Vancomycin MIC Creep) [6]. This refers to a progressively increasing tendency of MIC in *S. aureus* strains, which are in the susceptibility range according to current guidelines [10]. Elevated vancomycin MIC levels even within the susceptibility range are associated with poor treatment outcomes, including delayed drug response, prolonged hospitalization and increased mortality rates [11, 12]. In addition, higher vancomycin MICs are strongly correlated with the likelihood of becoming a hGISA strain [13].

Importantly, vancomycin MIC Creep is not a uniform trend globally [14]. It exists in some centers, whereas most centers report unchanged or even declining vancomycin MICs in *S. aureus* [15]. Pediatric studies have shown similarly conflicting results and data—especially from Europe—is scarce [10, 16].

The primary aim of this study was to evaluate the temporal trend of vancomycin MIC values and the potential presence of a vancomycin MIC creep in a central European tertiary care children's hospital. Secondly, the strains were tested for heterogeneous and intermediate resistance to glycopeptides. Thirdly, we assessed the temporal development of methicillin resistance and its association with vancomycin MIC.

Materials and methods

Patient population and sampling

In this retrospective study, 540 bacterial isolates were evaluated that had been taken from patients or patient's caregivers of a pediatric tertiary hospital from May 2002 to July 2017. Only one isolate per patient was used for evaluation within a timeframe of six months. The isolates have been frozen after initial detection of MRSA or severe *S. aureus* infections

(blood stream infection, endocarditis, pneumonia, and osteomyelitis). The samples from adults were collected as part of an infection control program that screened the caregivers of children with MRSA or severe *S. aureus* infections. Criteria for storage had been prospectively defined. This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval was obtained from the ethics committee of the LMU Munich (ID 21-0334).

Isolates were stored using the Cryobank system (MAST Diagnostica, Reinfeld, Germany). For recultivation, the cultures were plated on Columbia agar plates with 5% sheep blood (Becton Dickinson, Heidelberg, Germany). 13 Isolates did not grow on the agar plates, and were not used for further evaluation.

Cultivation and resistance determination

The MIC was measured using the E test (Liofilchem, Roseto degli Abruzzi, Italy) method. Bacterial isolates were grown on Columbia agar plates and incubated overnight at 37 °C. Suspensions were prepared to a 0.5–1 McFarland turbidity standard and plated on Mueller—Hinton agar plates (Becton Dickinson, Heidelberg, Germany). After a rest period of 15 min at room temperature, MIC testing was performed following manufacturer's guidelines. MICs were evaluated after 24 h. The isolates were tested for vancomycin and oxacillin. For the detection of heterogeneous glycopeptide-intermediate *S. aureus* (hGISA) and glycopeptide-intermediate *S. aureus* (GISA) we used Glycopeptide Resistance Detection (GRD; Liofilchem, Roseto degli Abruzzi, Italy) strips. This test has been extensively validated [17, 18]. The following well-characterized *S. aureus* strains were used as controls for internal verification according to the manufacturer's recommendations: ATCC 29213; ATCC 700698 (Mu3; hGISA; Vancomycin MIC 3 mg/L) [19] and ATCC 700699 (Mu50; GISA; Vancomycin MIC 8 mg/L) [19]. The measured MIC values were classified using EUCAST criteria with a cutoff of 2 $\mu\text{g/mL}$ for both vancomycin and oxacillin.

Proportion of MRSA and MSSA

For methicillin resistance, antibiograms from the diagnostic routine of 15,852 patients were evaluated over a time period from 2002 to 2017. Until 2011, the microorganisms were identified using API ID test strips (bioMérieux, Marci-l'Étoile, France), Oxacillin plates (Becton Dickinson, Heidelberg, Germany) and the Mec-A test (Oxoid, Hampshire, UK). Since 2011, resistance has been determined with the Vitek system (bioMérieux, Marci-l'Étoile, France), Oxacillin plates (Becton Dickinson, Heidelberg, Germany) and the Mec-A test (ThermoFisher, Wesel, Germany).

Data source and statistical evaluation

Data were analyzed using Excel 16.0 (Microsoft, Redmond, USA). Statistical significance between two study groups was calculated using the Mann–Whitney *U* Test (Mann, Whitney 1947). Frequencies of nominally scaled variables were evaluated using the Chi-square test (χ^2 test; Pearson 1900). Both tests were calculated with the Excel 16.0 program. The significance levels were set at $p \leq 0.05$ = significant (*), $p \leq 0.01$ = very significant (**), and $p \leq 0.001$ = highly significant (***) .

Results

Susceptibility of isolates to vancomycin

A total of 540 *S. aureus* isolates obtained from 531 patients underwent susceptibility testing for vancomycin and oxacillin. The age range of the patients was between 0 and 52 years. 81% of the samples came from patients under 18 years. Of these, 34% were younger than one year at the time of sampling. 200 isolates could be assigned to an early time interval from 2002 to 2009 and 340 isolates to a late time interval from 2010 to 2017. Ninety percent of the samples were newly identified MRSA isolates, 10% of the samples came from invasive *S. aureus* infections (Supplementary Fig. 1).

All MIC values for vancomycin were within the susceptible range according to EUCAST criteria. MIC₅₀ was 1 µg/mL, MIC₉₀ 1.5 µg/mL. During the study time, we detected a decrease of the average MIC values which accounted for 0.0144 µg/mL/year (Fig. 1).

The comparison between early and late time interval also shows decreasing of MIC. Eighty percent of the samples selected since 2010 showed a MIC ≤ 1 µg/mL as compared to 67% before 2010. The mean value of the MIC values was

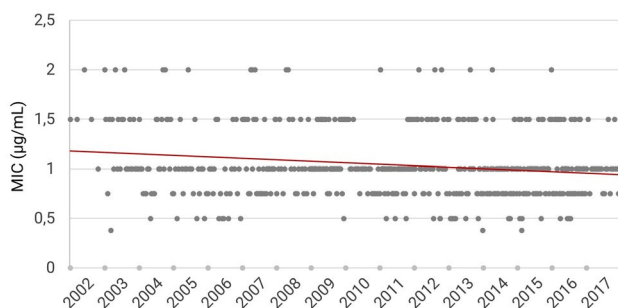


Fig. 1 Distribution of MIC values and trend line over time indicating decreasing vancomycin MIC in *S. aureus*-isolates ($R^2=0.0279$). MIC minimal inhibitory concentration

1.1 µg/mL in the early and 0.99 µg/mL in the late time interval ($p < 0.001$; *U* test; Fig. 2).

We found 83% of the samples to be resistant to methicillin and the remaining 17% were MSSA isolates. The frequency of vancomycin MIC values did not differ between MRSA and MSSA isolates; ($p = 0.79$; *U* test; Fig. 3). Mean and median MICs were also comparable (1 µg/mL in all groups). The temporal trend of MIC values in this study is independent of the patient age. The difference in MIC values between isolates of patients under 18 years of age and those of patients 18 years and older was not statistically significant (data not shown).

Heterogeneous glycopeptide-intermediate *S. aureus*

There were no samples with intermediate glycopeptide resistance. As much as 14% of all samples showed heterogeneous resistance to glycopeptides. This rate declined during the investigated period. However, there were substantial fluctuations with a share of hGISA between 0 and 40% per year. A comparison between early and late time intervals also shows a decreasing trend of hGISA strains from 28 to

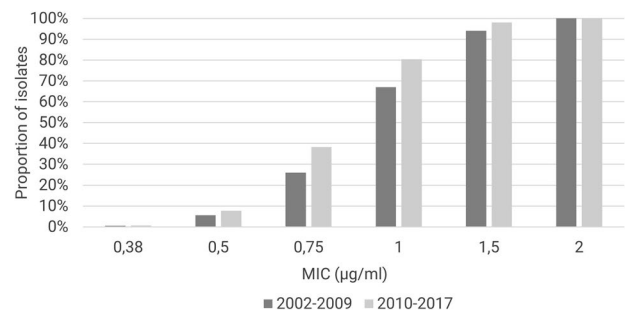


Fig. 2 Cumulative percentage of vancomycin MIC per time period showing significantly lower MICs in *S. aureus* isolates from the recent period after 2010 ($p < 0.001$). MIC minimal inhibitory concentration

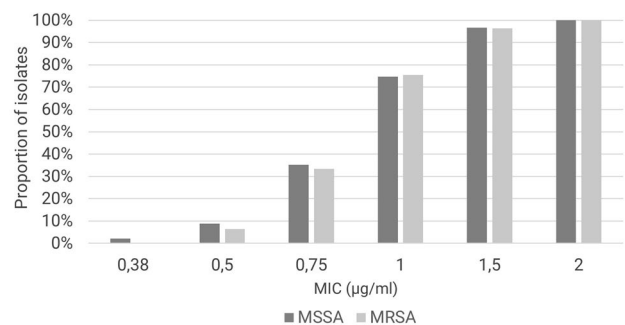


Fig. 3 Comparison of cumulative percentages of vancomycin—MIC between MSSA and MRSA samples. MIC minimal inhibitory concentration

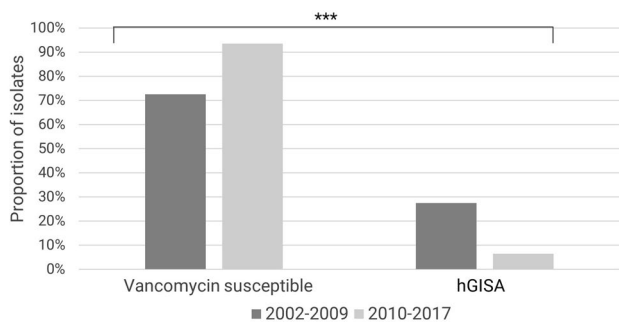


Fig. 4 Percentage of susceptible and hGISA strains per time period demonstrating decreasing heterogeneous intermediate glycopeptide resistance

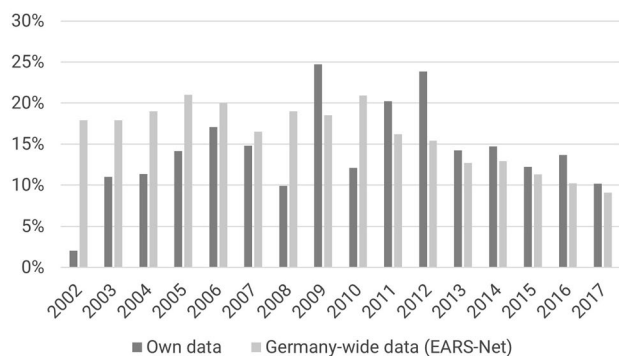


Fig. 5 Proportion of MRSA strains in the present study as compared to data from the EARS-Net (Germany only [20–22])

6% ($p < 0.001$; χ^2 ; Fig. 4). There were only minor differences in intermediate resistance of MRSA and MSSA isolates: the proportion of hGISA was 15% for MRSA isolates and 11% for MSSA isolates ($p = 0.33$; χ^2).

There was a strong correlation between vancomycin MIC values and the hGISA phenotype. High vancomycin MIC values were positively correlated with heterogeneous resistance to glycopeptides. Although the mean MIC of susceptible strains was 1 $\mu\text{g}/\text{mL}$, the mean value of hGISA was 1.2 $\mu\text{g}/\text{mL}$ ($p < 0.001$; U test).

Susceptibility of *S. aureus* to methicillin

Antibiograms of 15,852 patients from the same time period (2002–2017) were evaluated to independently assess methicillin susceptibility. In total, 14% of the *S. aureus* samples were methicillin resistant. The percentage increased until 2009 and has decreased since (Fig. 5). These were in line with the national German data. The European Antimicrobial Resistance Surveillance Network (EARS-Net) reports a mean methicillin resistance rate of 16.2% from Germany for *S. aureus* isolates from blood cultures over the same period

from 2002 to 2017 [20–22]. Similar to our study, the rate increased until 2010 and has decreased since (Fig. 5).

Discussion

We were able to show a decreasing trend for both the *S. aureus* vancomycin MIC and the presence of hGISA strains over a 15-year period in a German pediatric tertiary care center. Accordingly, we did not see a vancomycin MIC creep, but rather the opposite.

Similar to our data, a Vancomycin creep could not be confirmed in the largest study to date by SENTRY Antimicrobial Surveillance Program with isolates from 45 countries from 1997 to 2016 and in a recent metaanalysis [14, 23]. However, there are pronounced regional differences and data quality is heterogeneous. Estimates for hGISA prevalence are ranging from 1.3 to 27% [24–26]. Our hGISA rate of 14% is within this range. The marked differences are attributed to varying detection methods and origin of isolates, as well as prior exposure to vancomycin. Insufficient standardization of the respective test methods is a challenge when comparing of hGISA prevalence at different centers. Three different methods have been validated and are routinely used, namely, population analysis profile area under the curve ration (PAP-AUC), the macroversion E test, and the GRD E test which we used—none of them are commonly available in routine laboratories [17, 18, 27]. Long-term studies using the same methodology like the presented study are still important for study of trends over several years. Meta-analyses of hGISA prevalence showed a decline in most studies since 2010, albeit with regional differences [25, 26]. Namely, the world's most populous countries India and China continue to have high rates [25, 26].

There is little data regarding MRSA prevalence in pediatric populations in Germany highlighting the importance of the presented study. The decreased rate of methicillin-resistant MRSA since 2009 in our dataset and in the German EARS-Net is part of a general European trend. The European average also shows a continuously decreasing trend from 23.2% in 2009 to 16.9% in 2017, although this trend is not uniform, and there are also countries with increasing MRSA rates [20, 22, 28]. Globally, there are substantial differences. Importantly, MRSA prevalence does not seem to be improving in countries that also have high Vancomycin MICs and hGISA rates, such as India and China [29, 30].

The mechanism of hGISA is different from that of MRSA, with cell wall thickening and reduced vancomycin access to its active site being the main factors as compared to acquisition of a non-native gene encoding a penicillin-binding protein [27]. However, hGISA is not independent of MRSA. In fact, hGISA development is considered to be an indirect consequence of resistance to beta-lactam

antibiotics [6, 25, 27, 31]. Most but not all hGISA strains are also methicillin resistant [25, 31]. Suspected or confirmed MRSA infections are the most important indication for vancomycin use. In this way, higher MRSA frequencies are associated with higher Vancomycin use and subsequent evolutionary pressure favoring higher Vancomycin MICs and hGISA development [6, 14, 25, 31]. This sequence has not only been shown in epidemiological studies, but also intra-individually [32, 33]. Importantly, typing of hGISA strains have identified clonal lineages [27, 31]. These have developed independently and different clones have been associated with local and regional outbreaks, such as LIM-2 in Western Europe, USA-100 in North America, or Mu50 in Japan [27, 31, 34, 35]. Fortunately, hGISA clones seem to have lower growth rate [7]. Accordingly, higher awareness and appropriate measures against MRSA, such as routine MRSA screenings and improved infection control measures in health care institutions, have likely contributed to the observed decline in hGISA prevalence [25]. It is encouraging and should be emphasized in antibiotic stewardship programs that targeted interventions can have broader beneficial effects on antibiotic resistance. Accordingly, the positive trend seen in Germany could be replicated in large countries with higher hGISA rates such as China and India. However, a detailed evaluation of these measures is difficult and it is impossible to prove causality.

Our study has several limitations. First, it is a retrospective single-center study. Second, antibiotic exposure and clinical treatment outcomes were not correlated with antibiotic susceptibility. Third, the hGISA and GISA phenotypes are unstable and might thus drop to a lower resistance level by eliminating the selection pressure. Accordingly, storage of isolates potentially affects susceptibility testing results [16, 27, 36]. However, we do not expect this to bias our analyses since all samples were treated identically. Fourth, MIC values were only measured by one method—the E test—which can show subjective measurement inaccuracies. Fifth, strains were not genotyped. Accordingly, the impact of specific clones could not be assessed although there was no indication of any outbreak or significant nosocomial transmission. Sixth, the data on the susceptibility of *S. aureus* to methicillin over time is derived from a different patient cohort than the Vancomycin MIC and hGISA prevalence. Nevertheless, the two cohorts are from the same institution and same time period and are representative for the reported trends over time.

In summary, vancomycin remains suitable as first-line treatment for an expected severe infection with Gram-positive cocci and proven MRSA infections. However, a continuous analysis of regional conditions is essential as they may differ from global trends. The regular monitoring of vancomycin MIC is advisable—additional screens for

hGISA and GISA do not seem routinely required as they are closely correlated with vancomycin MIC.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s15010-023-02036-5>.

Author contributions UB, JH and TS designed the project. KH and MM carried out the experiments. KH and TS analysed the data, prepared the figures and wrote the manuscript. All authors reviewed the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. No external funding.

Data availability The data that support the findings of this study are available on upon reasonable request from the corresponding author with a research protocol and ethical approval.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval Ethical approval was obtained from the ethics committee of the LMU Munich (ID 21-0334).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Ikuta KS, et al. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2022. [https://doi.org/10.1016/S0140-6736\(22\)02185-7](https://doi.org/10.1016/S0140-6736(22)02185-7).
- Jones RN. Microbiological features of vancomycin in the 21st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. *Clin Infect Diseases*. 2006;42 Suppl 1:S13-24. <https://doi.org/10.1086/491710>.
- Hiramatsu K, et al. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother*. 1997;40:135–6.
- CLSI. M 100. Performance standards for antimicrobial susceptibility testing. 32nd ed. New York: Clinical and Laboratory Standards Institute; 2022.
- The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 12.0. <http://www.eucast.org> (2022). Accessed 15 Dec 2022.
- Dhand A, Sakoulas G. Reduced vancomycin susceptibility among clinical *Staphylococcus aureus* isolates ('the MIC Creep'): implications for therapy. *F1000 Med Rep*. 2012;4:4–4. <https://doi.org/10.3410/M4-4>.

7. Liu C, Chambers HF. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. *Antimicrob Agents Chemother*. 2003;47:3040–5.
8. Péricchon B, Courvalin P. VanA-type vancomycin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2009;53:4580–7. <https://doi.org/10.1128/AAC.00346-09>.
9. Soriano A, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Diseases*. 2008;46:193–200. <https://doi.org/10.1086/524667>.
10. Chang CN, Lo WT, Chan MC, Yu CM, Wang CC. An investigation of vancomycin minimum inhibitory concentration creep among methicillin-resistant *Staphylococcus aureus* strains isolated from pediatric patients and healthy children in Northern Taiwan. *J Microbiol Immunol Infect*. 2017;50:362–9. <https://doi.org/10.1016/j.jmii.2015.05.013>.
11. Yeh YC, et al. Impact of vancomycin MIC creep on patients with methicillin-resistant *Staphylococcus aureus* bacteremia. *J Microbiol Immunol Infect*. 2012;45:214–20. <https://doi.org/10.1016/j.jmii.2011.11.006>.
12. Sakoulas G, et al. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol*. 2004;42:2398–402. <https://doi.org/10.1128/JCM.42.6.2398-2402.2004>.
13. Appelbaum PC. Reduced glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). *Int J Antimicrobial Agents*. 2007;30:398–408. <https://doi.org/10.1016/j.ijantimicag.2007.07.011>.
14. Diaz R, Afreixo V, Ramalheira E, Rodrigues C, Gago B. Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis. *Clin Microbiol Infect*. 2018;24:97–104. <https://doi.org/10.1016/j.cmi.2017.06.017>.
15. Joana S, Pedro P, Elsa G, Filomena M. Is vancomycin MIC creep a worldwide phenomenon? Assessment of *S. aureus* vancomycin MIC in a tertiary university hospital. *BMC Res Notes*. 2013;6:65–65. <https://doi.org/10.1186/1756-0500-6-65>.
16. Goldman JL, Harrison CJ, Myers AL, Jackson MA, Selvarangan R. No evidence of vancomycin minimal inhibitory concentration creep or heteroresistance identified in pediatric *Staphylococcus aureus* blood isolates. *Pediatr Infect Disease J*. 2014;33:216–8. <https://doi.org/10.1097/01.inf.0000436281.18687.0c>.
17. Yusof A, et al. Evaluation of a new Etest vancomycin-teicoplanin strip for detection of glycopeptide-intermediate *Staphylococcus aureus* (GISA), in particular, heterogeneous GISA. *J Clin Microbiol*. 2008;46:3042–7. <https://doi.org/10.1128/jcm.00265-08>.
18. Leonard SN, Rossi KL, Newton KL, Rybak MJ. Evaluation of the Etest GRD for the detection of *Staphylococcus aureus* with reduced susceptibility to glycopeptides. *J Antimicrobial Chemotherapy*. 2009;63:489–92. <https://doi.org/10.1093/jac/dkn520>.
19. Hiramatsu K, et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet*. 1997;350:1670–3. [https://doi.org/10.1016/S0140-6736\(97\)07324-8](https://doi.org/10.1016/S0140-6736(97)07324-8).
20. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2010. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). (Stockholm, 2011).
21. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2014. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). (Stockholm, 2015).
22. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe—Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2017. (Stockholm, 2018).
23. Diekema DJ, Pfaller MA, Shortridge D, Zervos M, Jones RN. Twenty-year trends in antimicrobial susceptibilities among *Staphylococcus aureus* from the SENTRY Antimicrobial Surveillance Program. *Open Forum Infect Dis*. 2019;6:S47–53. <https://doi.org/10.1093/ofid/ofy270>.
24. Satola SW, Farley MM, Anderson KF, Patel JB. Comparison of detection methods for heteroresistant vancomycin-intermediate *Staphylococcus aureus*, with the population analysis profile method as the reference method. *J Clin Microbiol*. 2011;49:177–83. <https://doi.org/10.1128/JCM.01128-10>.
25. Shariati A, et al. Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate *Staphylococcus aureus* clinical isolates: a systematic review and meta-analysis. *Sci Rep*. 2020;10:12689. <https://doi.org/10.1038/s41598-020-69058-z>.
26. Zhang S, Sun X, Chang W, Dai Y, Ma X. Systematic Review and Meta-Analysis of the Epidemiology of Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* Isolates. *PLoS ONE*. 2015;10:e0136082. <https://doi.org/10.1371/journal.pone.0136082>.
27. Howden BP, Davies JK, Johnson PDR, Stinear TP, Grayson ML. 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. <https://doi.org/10.1128/CMR.00042-09>.
28. GERMAP. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Paul-Ehrlich-Gesellschaft für Chemotherapie e. V. GERMAP 2015-Bericht über den Antibiotikaverbrauch und die Verbreitung von Antibiotikaresistenzen in der Human- und Veterinärmedizin in Deutschland. (Rheinbach, 2016).
29. Wang B, et al. Methicillin-resistant *Staphylococcus aureus* in China: a multicentre longitudinal study and whole-genome sequencing. *Emerg Microbes Infect*. 2022;11:532–42. <https://doi.org/10.1080/22221751.2022.2032373>.
30. Ghia CJ, Waghela S, Rambhad G. A systemic literature review and meta-analysis reporting the prevalence and impact of methicillin-resistant *Staphylococcus aureus* infection in India. *Infect Dis (Auckl)*. 2020;13:1178633720970569. <https://doi.org/10.1177/1178633720970569>.
31. Gardete S, Tomasz A. Mechanisms of vancomycin resistance in *Staphylococcus aureus*. *J Clin Invest*. 2014;124:2836–40. <https://doi.org/10.1172/JCI68834>.
32. Chen CJ, Lin MH, Shu JC, Lu JJ. Reduced susceptibility to vancomycin in isogenic *Staphylococcus aureus* strains of sequence type 59: tracking evolution and identifying mutations by whole-genome sequencing. *J Antimicrobial Chemotherapy*. 2014;69:349–54. <https://doi.org/10.1093/jac/dkt395>.
33. Mwangi MM, et al. Tracking the in vivo evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. *Proc Natl Acad Sci USA*. 2007;104:9451–6. <https://doi.org/10.1073/pnas.0609839104>.
34. Garnier F, et al. A 1 year surveillance study of glycopeptide-intermediate *Staphylococcus aureus* strains in a French hospital. *J Antimicrobial Chemotherapy*. 2006;57:146–9. <https://doi.org/10.1093/jac/dki413>.
35. Verdier I, et al. *Staphylococcus aureus* isolates with reduced susceptibility to glycopeptides belong to accessory gene regulator group I or II. *Antimicrobial Agents Chemotherapy*. 2004;48:1024–7. <https://doi.org/10.1128/AAC.48.3.1024-1027.2004>.
36. McGuinness WA, Malachowa N, DeLeo FR. Vancomycin resistance in *Staphylococcus aureus*. *Yale J Biol Med*. 2017;90:269–81.