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Heteroresistance: a concern of increasing clinical significance?

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ABSTRACT

Recent studies have focused on issues related to heteroresistance, including its definition, methods of detection and frequency. Most such studies have reported data concerning infections caused by *Staphylococcus aureus*, but the clinical significance of heteroresistance is unclear. Six studies have described infections caused by *S. aureus* strains that were heteroresistant to vancomycin, with two suggesting an association between the emergence of heteroresistance and treatment failure or mortality, and four suggesting no such association. Further studies are required to evaluate the clinical implications of heteroresistance in an era in which rates of antimicrobial resistance are increasing alarmingly worldwide.

Keywords Antibiotic resistance, clinical implications, glycopeptides, heteroresistance, *Staphylococcus aureus*, vancomycin

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'Heteroresistance' is a widely used term, although there is no distinct and precise definition that encompasses this phenomenon in all microorganisms. In broad terms, heteroresistance is defined as resistance to certain antibiotics expressed by a subset of a microbial population that is generally considered to be susceptible to these antibiotics according to traditional in-vitro susceptibility testing. Various factors may subsequently lead to the proliferation of the resistant sub-population and the emergence of a fully resistant microbial strain.

The phenomenon of heteroresistance has been observed in a range of microbes, including *Staphylococcus aureus*, coagulase-negative staphylococci [1], *Acinetobacter baumannii* [2], *Mycobacterium tuberculosis* [3], *Streptococcus pneumoniae* [4], *Enterococcus faecium* [5] and *Cryptococcus neoformans* [6]. The frequency of heteroresistance, although this differs among species, is about one sub-clone in every 10^5–10^6 colonies, which roughly equals the normal rate of mutation. Heteroresistance could be a tool for natural evolution to drug resistance, since it provides bacteria with an opportunity to explore the possibility of growth in the presence of antibiotics before acquisition of resistance by the major proportion of the microbial population [4].

Heteroresistance is considered to be a precursor stage, which may or may not lead to the emergence of a resistant strain. Although this phenomenon has been reproduced many times in the laboratory setting, its clinical significance is largely unknown. This is because it is only possible to demonstrate the in-vitro resistance of a strain to a particular antibiotic, and the pathogenic potential of a strain for causing a clinical infection is not yet understood. Bjorkman *et al.* [7] described strains of *Salmonella* Typhimurium that were resistant to several antibiotics *in vitro*, but that were avirulent *in vivo*. These strains rapidly regained their virulence, either following compensatory mutations or by reversion to the antibiotic-susceptible virulent type, which occurs to a varying extent in different environments. Thus, the emergence of resistant strains *in vitro* may not necessarily have therapeutic implications, since acquisition of resistance may be accompanied by loss of virulence. Nevertheless, heteroresistant strains have also been deemed to be responsible for failure of prescribed treatment [8,9]. Such clinical isolates can display certain

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characteristics, e.g., structure and morphology, growth patterns and expression of genes, that may play an important role in their virulence. There are also cases in which the emergence of heteroresistance to a particular antibiotic results in increased resistance to other antimicrobial agents [10]. For example, concerns have been expressed regarding the spread of a clone of methicillin-resistant *S. aureus* (MRSA) with heteroresistance to oxacillin in Dutch hospitals [11].

Until very recently, the phenomenon of heteroresistance was ignored or underestimated. However, several studies from various parts of the world [12,13] have now provided estimates of the prevalence of heteroresistant vancomycin-intermediate *S. aureus* (hVISA), mainly among strains of MRSA. There is a relative paucity of data concerning the prevalence of heteroresistance in other organisms, apart from a few case reports, and current evidence regarding the clinical importance of hVISA/heteroresistant glycopeptide-intermediate *S. aureus* (hGISA) infections is also scarce. Six studies concerning the clinical significance of hGISA infections have been described in the literature (Table 1), most of which were retrospective and involved only a small number of patients. In the majority of these studies, the patients had bloodstream MRSA infections in different clinical settings. Only two studies [8,9] provided evidence suggesting that MRSA carriers with hVISA strains may be more prone to therapeutic failure, even after the use of vancomycin. The four remaining studies did not support this association; however, the numerous methodological issues in the design of the studies do not permit a definitive answer to this question. The small number of patients studied who yielded hVISA isolates, as well as a failure to standardise the detection methods for heteroresistance, are also important limitations.

A further consideration is that definitive conclusions regarding an association between dosage and duration of vancomycin treatment for MRSA infection and the development of heteroresistance cannot be reached from the available data. However, it is possible that prolonged exposure to antimicrobial agents exerts increased pressure for selection of microbial sub-populations that are resistant to the therapy administered. According to the mutant selection window hypothesis, there is an antibiotic concentration above the MIC (termed the mutation prevention concentration) at which selective amplification of single-step, drug-resistant mutants may occur [18]. Such mutants may proliferate readily in the absence of competition with the inhibited susceptible cells of the wild-type strain, thereby giving rise to a new resistant population with a higher MIC than that for the wild-type strain. This observation has implications for patients with infections at sites where the penetration of antibiotics is suboptimal, despite an appropriate dosage scheme, e.g., abscesses and endocardial vegetations [19].

A failure to detect MRSA isolates with reduced susceptibility to glycopeptides could already be contributing to a hidden spread of these strains within hospital facilities. Methods for the detection of such strains include determination of a simplified population analysis profile (PAP) on brain–heart infusion agar or Mueller–Hinton agar, as well as the broth microdilution, agar microdilution, Etest, PAP, agar disk-diffusion and Microscan methods [12]. However, these methods can be expensive, time-consuming or cumbersome to perform routinely. Wootton *et al.* [20] proposed that the macrodilution Etest should be performed initially as a non-labour-intensive screening method for a large number of isolates that would generate few false-positive results. If this method is considered too expensive, the screening can be restricted to dialysis patients or to patients receiving glycopeptides, who have a greater probability of developing hGISA. Microbial populations suspected of including heteroresistant sub-populations could then be subjected to modified PAP analysis for a more accurate assessment of their glycopeptide resistance status. The same strategy could also be initiated, using methods with higher sensitivity, for other organisms, e.g., *A. baumannii* and *M. tuberculosis*, in which there have been reports of the development of heteroresistant strains [2,3].

Until the clinical significance of heteroresistant strains has been investigated in greater detail, several measures should be taken to prevent the spread and/or emergence of these strains. First, rigorous infection control policies, based on agreed guidelines, should be instituted. Second, the introduction of methods for rapid laboratory detection of such strains should be considered to enable early recognition and prevention of spread. Third, antibiotics should be administered with prudence. Shorter antimicrobial regimens that are able to achieve the maximum

Table 1. Published studies that included data concerning the potential clinical significance of heteroresistant *Staphylococcus aureus* strains with intermediate resistance to glycopeptides/vancomycin (hGISA/hVISA)

Study	No. of patients	Type of infection	Method of h-GISA detection (proportion of hGISA)	Use of glycopeptides	Clinical outcome
Ariza *et al.* [8]	Retrospective (19 patients)	MRSA infection following orthopaedic surgery; 14/19 patients with orthopaedic implants	Simplified PAP (14/19 of MRSA strains were hVISA)	Vm for >2 wks (average of 30 days)	Higher therapeutic failure among the hVISA carriers
Wong *et al.* [9]	Retrospective case-control (203 strains)	Bacteraemia caused by staphylococci (112 MSSA 52 MRSA, 8 MSCNS, 31 MRCNS)	Simplified PAP on BHIA-Vm4 Broth micro dilution Etest Disk agar (18/203 strains were hrVm -not only *S. aureus*)	Vm in 7/18 patients with hrVm before or during blood culture	Mortality from bacteraemia caused by hrVm staphylococci was higher (44.4% vs. 10%)
Kim *et al.* [14]	Retrospective (4483 strains from 1709 patients)	*S. aureus* isolates from various clinical settings (3363 MRSA)	Simplified PAP on BHIA-Vm4 Broth micro dilution PAP (24/4483 strains were hVISA from 22 patients)	11/22 patients with hVISA had previously Vm or teicoplanin for >1 day, 7 patients were infected and received Vm for 9-63 days and 15 patients were colonised	All 7 hVISA infections were treated successfully with Vm with no emergence of VISA
Schwaber *et al.* [15]	Retrospective case-control, cohort (154 patients)	149 MRSA bloodstream isolates	Agar dilution PAP on BHI broth (no hVISA but isolates from 61 patients grew on screening media and 75% of them had a two-fold or four-fold increase in MIC)	Only 17% of patients in the case-control groups received Vm before blood cultures	No association with a more adverse outcome was observed among the 61 patients
Bert *et al.* [16]	Retrospective (48 patients)	Liver transplant patients infected or colonised with MRSA	Etest Simplified PAP (13/48 were hGISA (+), 35/48 were GSSA)	Only 2/13 of patients with hGISA had previously received Gp, but 70% had received β-lactams	The time of infection, the rates of bacteraemia and the mortality had NS difference between the hGISA and the GSSA group
Khosrovaneh *et al.* [17]	Prospective (22 patients)	Persistent or recurrent MRSA bacteraemia	Etest Modified PAP (3/22 were heteroresistant)	The majority received Vm for >1 month	Most patients did not have hrVm isolates, and those who did had other reasons for relapse or persistence

hVISA, heteroresistant vancomycin-intermediate *S. aureus*; hGISA, heteroresistant glycopeptide-intermediate *S. aureus*; GSSA, glycopeptide-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; Vm, vancomycin; PAP, population analysis profile; MSSA, methicillin-susceptible *S. aureus*; MSCNS, methicillin-susceptible coagulase-negative staphylococcus; MRCNS, methicillin-resistant coagulase-negative staphylococcus; BHIA, brain–heart infusion agar; hrVm, heteroresistant to vancomycin; Gp, glycopeptides; NS, not significant.

effectiveness with the minimum selective pressure should be further investigated. In-vitro studies have suggested that a combination of antimicrobial agents may delay the emergence of resistance [21]; however, meta-analyses of data from randomised controlled studies have proved inconclusive [22,23].

In conclusion, heteroresistance is increasingly recognised, but is not a new phenomenon. Detection of heteroresistance is difficult, and there are few laboratory or clinical data concerning this subject. The clinical significance and therapeutic implications of heteroresistance remain to be determined. More relevant in-vitro, experimental and clinical research data are urgently required.

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