Abstract of the PhD thesis

"*Staphylococcus aureus* with decreased susceptibility to glycopeptides – clinical and microbiological importance"

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Glycopeptide antibiotics are reserved for the treatment of severe infections due to methicillin-resistant S*taphylococcus aureus* strains (MRSA). Since the emergence of *S. aureus* strains with varying degrees of resistance to glycopeptides the efficacy of vancomycin and teicoplanin treatment became debatable, especially when life threatening infections are concerned. Although *Staphylococcus aureus* strains with decreased susceptibility to glycopeptides were characterised from a genotypic and phenotypic point of view, there are still several open questions regarding strains of *S. aureus* with heterointermediate susceptibility to glycopeptides (hGISA). There is a lack of data in the field about the clinical and microbiological impact of these strains.

Data from the literature regarding *S. aureus* strains with decreased susceptibility to glycopeptides were summarized in ten chapters of the **general part** of the thesis. Definition of terminology, review of epidemiological data, description of resistance mechanisms and detection methods, as well as the current state of knowledge with regard to the clinical importance of these strains (low virulence, therapeutic failure with vancomycin) were presented.

The second part of the thesis, which consists of the **personal contribution,** treats the problem of the *S. aureus* strain with heterointermediate susceptibility to glycopeptides in two studies. It augments the actual stage of knowledge with new data of local and national interest, as well as with data about the clinical importance of the hGISA strains.

In the first study the occurrence of methicillin-resistant *Staphylococcus aureus* strains with decreased susceptibility to glycopeptides was studied in Târgu-Mureş Clinical Emergency Hospital.

Introduction and objectives: In Romania the high prevalence of MRSA strains involved in invasive infections implies frequent use of antibiotics from the class of the glycopeptides. Development of resistance to vancomycin and teicoplanin is linked to previous treatment with glicopeptides. Methods used in routine microbiological diagnosis have limited utility in identifying *S. aureus* strains with decreased susceptibility to glycopeptides. Their detection requires the use of special methods which are laborious and time consuming. Our aim was to evaluate the frequency of MRSA strains with decreased susceptibility to glycopeptides.

Materials and methods: We performed a retrospective study assessing a batch of one hundred and twenty-two non-duplicate MRSA strains, characterized previously using molecular methods. The screening for decreased susceptibility to glicopeptide was carried out using Etest GRD (Glycopeptide Resistance Detection)

method. The selected strains were confirmed with population analysis profile (PAP) method, which is considered to be the "gold standard" for the identification of hGISA strains.

Results: Out of the 24 (19.5%) strains with positive screening, only two (1,63%) were confirmed to have the hGISA phenotype by (vagy using) population analysis.

Conclusions: In Târgu-Mureş Clinical Emergency Hospital the prevalence rate of the hGISA strains was low during the assessed period. However, it is recommended to pursuit their incidence, especially because glycopeptides are often used in the treatment of serious infections with MRSA and treatment failure may occure due to the heteroresistant phenotype of the bacteria. Therefore it is necessary to develop a standardized method for the detection of *Staphylococcus aureus* strains with decreased susceptibility to glycopeptides, which would be accessible to clinical laboratories and helps in testing isolates especially involved in severe infections.

The second study was designed to evaluate the clinical importance of S*. aureus* strains with heterointermediate susceptibility to glycopeptides.

Introduction and objectives: Although *S. aureus* strains with varying degrees of resistance to glycopeptides were studied *in vitro*, the need of *in vivo* assessment of the clinical relevance of hGISA strains is often suggested. We conducted an experimental study to analyse *in vivo* the behavior of *S. aureus* isolates with heterointermediate susceptibility to glicopeptides in response to teicoplanin treatment.

Materials and methods: In an experimental model of infective endocarditis on rabbits, three groups of animals were infected with high load bacterial inoculum of three MRSA strains: one clinical hGISA strain, one clinical *S. aureus* strain susceptible to glycopeptides (GSSA) and the reference hGISA strain, respectively. Teicoplanin was injected intramuscularly in a single dose per day for the animals in the treated groups. The animals have been euthanized at the completion of the three day treatment, while those in the control groups the day after inoculation. The endocardial vegetations were collected to determine the number of colony forming units per gram of tissue using the successive dilution method. For the determination of teicoplanin serum levels a method of liquid chromatography-mass spectrometry was used.

Results: After completion of the three days of treatment the bacterial load found in vegetations decreased more in the group of rabbits infected with GSSA strain compared to the group infected with clinical hGISA strain ($P = 0.039$).

Conclusions: Treatment with teicoplanin was less effective in reducing the number of bacteria in high load bacterial infection caused by hGISA strain compared with GSSA strain. Although hGISA strains are characterized by lower virulence compared to fully susceptible strains, their clinical importance should not be ignored. It is necessary to perform new studies to optimize the treatment of infections caused by hGISA strains.

Key words: resistance to glycopeptides, *Staphylococcus aureus*, bacterial endocarditis, Etest GRD, teicoplanin, *in vivo*