

PROGNOSTIC PARAMETERS OF THE SUSCEPTIBILITY OF *STAPHYLOCOCCUS SPP.* TO AMINOGLYCOSIDES AND DOXYCYCLINE

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ABSTRACT

The aim: To perform microbiological investigation and analytic mathematic prediction of clinical isolates of *S. aureus* to aminoglycosides in patients with severe burns.

Materials and methods: We analyzed resistance of 199 *S. aureus* strains to aminoglycosides (gentamicin, tobramycin and amikacin) and doxycycline from 435 patients treated in the regional hospital due to burns for the period from 2011-2017.

Results: We created predictive curves for the prediction of susceptibility of *S. aureus* strains to aminoglycosides and doxycycline based on the changes in *S. aureus* resistance during the years of observation and expressed in mathematic equations. Susceptibility of *S. aureus* to gentamicin was 42.86 % at the end of observation and will decline in future. Despite tobramycin was efficient against 72.86 % of strains in 2017, mathematic modeling indicates rapid decline in its efficacy in future. Efficacy of amikacin was dropping during the last years, but according to the equation it efficacy will increase over 60 % in 2018. *S. aureus* susceptibility to doxycycline was 65.38 % in 2017 and mathematic modeling indicates its gradual decline in the nearest future.

Conclusions: Predictive values of *S. aureus* susceptibility indicates not sufficient efficacy of these drugs in patients with infectious complications of burns. Tendency of the slight decline of *S. aureus* susceptibility to doxycycline still indicates sufficient levels of its efficacy in the nearest future. This justifies its use as a second-line therapy with the causative agent in patients with burns.

KEY WORDS: Antibiotic resistance, gentamicin, tobramycin, amikacin, doxycycline, burns

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INTRODUCTION

Isolates of *Staphylococcus aureus* according to the data of multiple observations have leading positions among causative agents of the infectious complications around the globe during the medical care. This causative agent plays an important role in wound infection and respiratory complications in critically ill patients [1-3]. *Staphylococcus aureus* is referred to the 12 “high-priority” bacterial species, which are most dangerous for the human health, according to the list of resistant bacteria published by the World Health Organization (WHO). Methicillin-resistant (MRSA) and vancomycin intermediate-resistant (VISA) or vancomycin resistant *Staphylococcus aureus* (VRSA) are represented in the WHO list as the high-priority causative agent according to the necessity in new antibiotics against it. [4]. These microorganisms developed resistance to the wide range antibiotics including beta-lactames. They have resistance to methicillin and vancomycin [3, 5-8].

MRSA and VRS is a source of concern nowadays due to the resistance to many other chemotherapeutical and antimicrobial drugs. Investigation of new antimicrobial drugs for the treatment of infectious complications caused by *S. aureus* is relevant nowadays. Investigation of the prognostic

parameters of the susceptibility to different antibiotics of different clinical isolates of *Staphylococcus aureus*, that are determined in patients with severe burns, is very important step in the improvement of antimicrobial means against resistant strains of this causative agent, because resistance of MRSA to aminoglycosides and tetracyclines is known [7-14].

THE AIM

To perform microbiological investigation and analytic mathematic prediction of clinical isolates of *S. aureus* to aminoglycosides in patients with severe burns.

MATERIALS AND METHODS

Patients (n=435) that were hospitalized to the burns department of the Vinnytsia regional clinical hospital named after M.I. Pirogov due to the severe burns during the period of 2011-2017 were included in the study. Clinical strains of *S. aureus* were obtained from patients with stage II-III burns (20.0 – 85.0 of total body surface area) before the initiation of antibacterial therapy. Treatment of the patients with severe burns was performed according

to the existing guidelines. All patients undergone surgical treatment (early necrectomy in first three days, alloplastic skin substitute). Patients also received a complex of intensive care procedures for the stabilization of their condition, which consisted of balanced infusion-transfusion therapy, antibacterial and symptomatic therapy.

Microbiological investigation of the biological material was performed in the scientific bacteriological laboratory of the Department of Microbiology of Vinntsy National Pirogov Memorial Medical University, certified by Ministry of Health (certificate of attestation № 049/15 from 02.02.2015). Material for the microbiological investigation was obtained from the wound surface before the treatment initiation with the following isolation of the pure culture of *S. aureus*. Identification of its morphological, cultural and biochemical features. 199 strains of *S. aureus* in patients with burns during the period of 2011-2017 (2011 – n 37; 2012 – n=25; 2013 – n=27; 2014 – n=24; 2015 – n=35; 2016 – n=25; 2017 – n=26).

Susceptibility of all *S. aureus* strains (n=199) to antimicrobial drugs was determined. Study and analysis of susceptibility was performed using disk-diffusion test (Mueller-Hinton medium) and qualitative methods of two-fold serial dilutions according to the recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST Expert rules) [15].

During our study we performed mathematical analysis of the data on susceptibility of clinical strains of *S. aureus* to the following aminoglycoside antibiotics: gentamicin, tobramycin, amikacin and also doxycycline. Statistical methods were used during the analysis of susceptibility of *S. aureus* clinical isolates to the mentioned antibiotics, which allowed determining a relation between qualitative values of changeable features and the probability of realization of these values in the mass of conducted observations [16].

Performed mathematical analytical forecasting provided for the determination of real susceptibility of *S. aureus* clinical strains with the extrapolation of results on the investigated system by the creation of hypothetical mathematical model of the predicted susceptibility of *S. aureus* to aminoglycosids using methods of the normal analysis with the specification of values of absolute and relative optimum. Reliability and accuracy of each created mathematical model of the susceptibility prediction to gentamycin, tobramycin, amikacin and doxycycline was assessed using the determination coefficient (r^2). Obtained data was proceeded using license packs of original computer programs «STATISTICA 10.0»; «Matlab 7.11» [16, 17].

Arithmetic average (M), standard deviation (m), mean squared deviation (σ) were determined for each sample of *S. Aureus* isolates with the interval of 1 year for the objective assessment of confidence of the obtained data. Approximation and interpolation of data was performed using mathematical analysis, which allow to obtain analytical dependence of predicted values of susceptibility changes of *S. aureus* isolates, which have etiological values in the causation of infectious complications in patients with severe burns.

RESULTS

Low susceptibility to gentamicin (40.54 %) of *S. aureus* was established at the beginning of observation (2011). We observed a gradual increase in the number of susceptible staphylococci from 52.94 % (2012) to 70.59 % (2013). Susceptibility of *S. aureus* was the highest (89.47 %) in 2014 with the following decrease to 42.86 % in 2015-2017.

Mathematic formula was created on the basis of values of the *S. aureus* susceptibility to gentamicin (1).

Gentamicin

$$y = (a + cx + ex^2) / (1 + bx + dx^2) \quad (1),$$

where

a=19.8615; b=-0.00099; c=-0.0197; d=2.4646*10⁻⁷; e=4.8953*10⁻⁶; x – years

This mathematic equation describes the parabolic predictive curve, that indicates the temporary increase of the *S. aureus* susceptibility to gentamicin with its further exponential decline (Fig. 1).

Critically low values of the *S. aureus* susceptibility to tobramycin was established at the beginning of microbiological investigation. The number of susceptible strains was only 45.95 % – in 2011, and 52.94 % – in 2012. The dynamic increase of the *S. aureus* susceptibility to tobramycin was established in 2011-2016 according to the obtained data, but only 73.08 % of strains were susceptible to this drug in 2017. Creation of the mathematical model, which is described by the following equation (formula 2), showed predicted decrease of the *S. aureus* susceptibility to tobramycin (Fig. 2).

Tobramycin

$$y = (a + cx) / (1 + bx + dx^2) \quad (2),$$

where

a=0,0106857; b=-0,0009928; c=-5,182878*10⁻⁶; d=2,4642*10⁻⁷; x – years

Microbiological analysis of the *S. aureus* strains to amikacin has shown low efficacy (lower than 50 %) of this drug in 2011-2012. Despite the obtained data, the curve of the *S. aureus* susceptibility change to amikacin, which is described by formula 3, showed ambiguous susceptibility in dynamics (Fig. 3).

Amikacin

$$y = a + bx + cx^2 \ln x + d / \ln x + e / x \quad (3),$$

where

a=4.7075*10¹⁰; b=-10773; c=198.6721; d=-2.0041*10¹¹; e=-1.0406*10¹³; x – years.

Further microbiological investigations defined significant increase of the *S. aureus* strains susceptibility increase in dynamics. The number of staphylococci susceptible to amikacin increased to 88.24-89.47 % (2013-

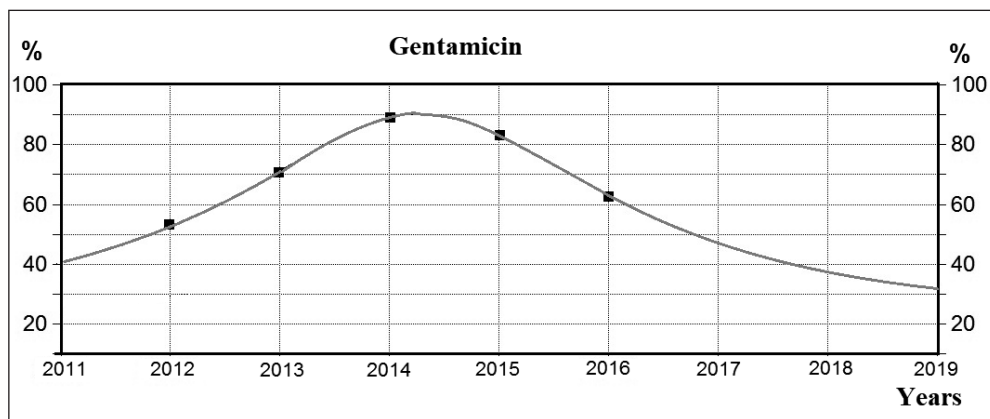


Fig 1. The temporary increase of the *S. aureus* susceptibility to gentamicin.

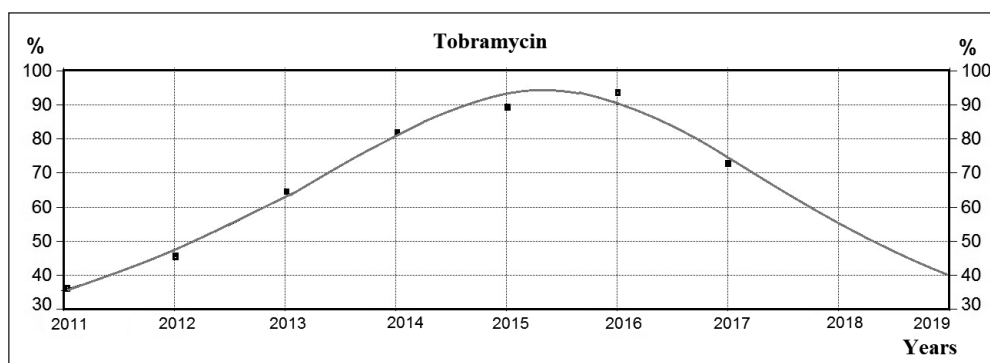


Fig 2. Decrease of the *S. aureus* susceptibility to tobramycin.

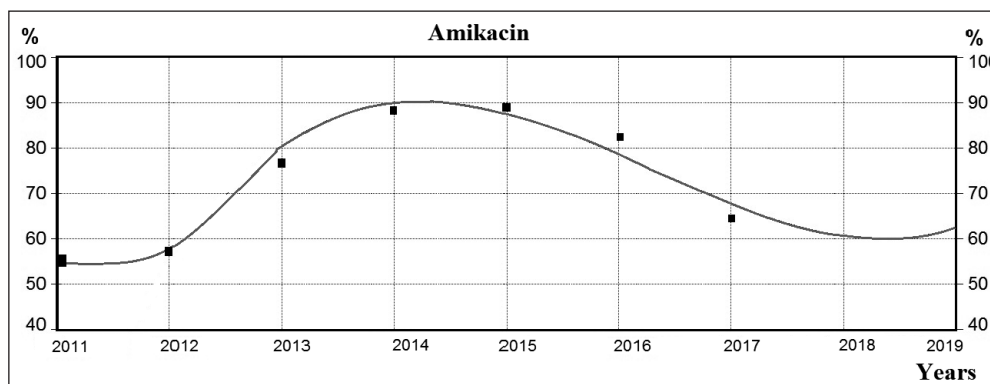


Fig 3. The curve of the *S. aureus* susceptibility change to amikacin.

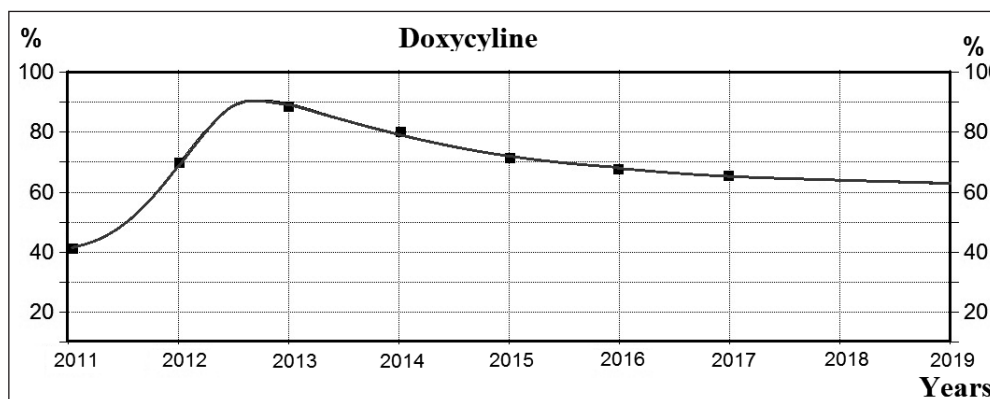


Fig 4. Efficacy of doxycycline against *S. aureus*.

2015). But gradual decrease of *S. aureus* susceptibility to amikacin to 64.69 % was determined in 2016-2017. Predicted increase of *S. aureus* susceptibility to amikacin (>60 %) from 2018 year was determined after the creation of mathematical model, despite the previous tendency if its gradual decrease.

It was established that efficacy of doxycycline against *S. aureus* was also low in 2011 (40.54 %). Gradual improvement of its efficacy was determined during the investigation of *S. aureus* susceptibility in 2012-2015. Susceptibility of *S. aureus* was the highest in 2013 and reach 88.24 % (Fig. 4).

Doxycycline

$$y = (a + cx + ex^2) / (1 + bx + dx^2) \quad (4),$$

where

$a=55,8202$; $b=-0,00099$; $c=-0,0554$; $d=2,4699 \cdot 10^{-7}$;
 $e=1,3792 \cdot 10^{-5}$; x – years

Further investigations revealed gradual slight decrease of *S. aureus* susceptibility to doxycycline. So, the number of susceptible strains decreased on 22.86 % and consisted 65.38 % (2017). Mathematic model, constructed based on the data of gradual increase of susceptibility with its gradual decrease, describes exponential type of curve and predicts further gradual decrease (formula 4; Fig. 4).

DISCUSSION

Therapeutic approaches in patients with severe burns requires use of antibacterial drugs [18-20]. Opportunistic microorganisms such as *S. aureus*, which are the leading causative agents of opportunistic infections, are an important problem nowadays as these microorganisms developed resistance to methicillin [21] and some other antibiotics such as aminoglycosides and tetracyclines due to their uncontrolled used in medicine [1, 2, 4, 12, 13]. In such conditions analytical mathematical prediction of the susceptibility level based on the data from dynamic microbiological investigation of the *S. aureus* susceptibility to staphylococci and doxycycline is considered important in the effective assessment of the feasibility of alternative use of these drugs in patients with severe burns [14].

We used modern methods of the mathematical prediction, which is defined as the process of investigation of the real system and transfer of the obtained data on the investigation system. Model can be defined as object, which is similar in some aspects with prototype and is a tool for description, explanation and prediction of its behavior. Mathematic modeling of the real process is a sum of interrelations (formulas), which define characteristics of processes depending on their parameters, external and initial conditions and time. Ambiguous susceptibility of *S. aureus* to aminoglycosides of different generations was determined during the microbiological investigation in patients with severe burns.

Analysis of the observation results for the period of 2011-2017 became a basis for the predictive model of *S. aureus* susceptibility to gentamicin and showed a decrease of this antibiotic efficacy in patients with severe burns. Obtained mathematical predictive model and its graphical curve of the susceptibility to gentamicin describes the following pattern in the form of parabolic curve and indicates the high probability of the loss of the *S. aureus* susceptibility loss in patients with severe burns in the nearest future.

Predictive model, which described parabolic type of the curve was created according to the results of *S. aureus* susceptibility to tobramycin. The tendency to the susceptibility increase during the period of 2011-2016 was observed,

which was similar to gentamicin. Predictive curve of the *S. aureus* susceptibility had similar character and indicated the declining tendency of the tobramycin efficacy in the nearest future.

Obtained predictive models of the *S. aureus* susceptibility to gentamicin and tobramycin indicate that *S. aureus* has dynamic properties to lose susceptibility to aminoglycosides of the first and second generation, that can be conditioned with rapid adaptive properties of this microorganism [22, 23].

Susceptibility to amikacin was ambiguous, because graphic function had atypical parabolic character. Despite exponential decrease of the *S. aureus* susceptibility in 2014-2017 predictive curve indicated an increase of susceptibility in the nearest future. Determined differences in the predicted tendencies of *S. aureus* susceptibility to various aminoglycosides can be in the directly dependent on the genetically predetermined mechanisms of different resistance genes expression by the microorganism to the drugs of this group [12, 23-25].

Use of tetracyclines in the modern clinical practice is rather limited. But in the conditions of growing resistance of *S. aureus* to beta-lactame antibiotics, glycopeptides and aminoglycosids, increased number of purulent complications caused by MRSA, doxycycline should be considered as one of the first-line oral antibiotic [26-28]. So, determination of the susceptibility to doxycycline is an important issue. Activity of doxycycline against staphylococci increased during two years of observation is the following slight gradual decrease. Moderate decrease of the *S. aureus* susceptibility to doxycycline was determined by the mathematic analysis. But despite the tendency to the gradual decline in susceptibility, which had linear character, predictive model determined sufficient efficacy of doxycycline against *S. aureus* in the nearest future. Efficacy of doxycycline is conditioned by the absence of cross-resistance with other antibiotics, as this drug acts at the early stages of translation and inhibits protein synthesis on the bacterial ribosomes.

CONCLUSIONS

1. This seven-year monitoring allows to develop analytic formula for the prediction of *S. aureus* susceptibility to aminoglycosids (gentamicin, tobramycin and amikacin) and doxycycline.
2. Predictive mathematic values indicates an unfavorable dynamics of the *S. aureus* susceptibility decline to gentamicin (42.86 %) and tobramycin (73.08 %). *S. aureus* susceptibility to amikacin is decreasing (64.69 %) and indicates its possible improvement in the nearest future (above 60 %).
3. Predictive values of *S. aureus* susceptibility indicates not sufficient efficacy of these drugs in patients with infectious complications of burns. Tendency of the slight decline of *S. aureus* susceptibility to doxycycline still indicates sufficient levels of its efficacy in the nearest future. This justify its use as a second-line therapy with the causative agent in patients with burns.

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Conflict of interest:

The Authors declare no conflict of interest.

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