SUMMARY

Staphylococcus are one of the most important groups of microorganisms that play a role in the pathology of human infections. Some species are often responsible for various infections, including those that are severe and life-threatening, in both inpatients and outpatients.

The aim of the study was to evaluate the presence of selected genes determining resistance to macrolides, lincosamides, streptogramins B and pleuromutilins.

The material for research and further analyzes were bacterial strains belonging to the genus *Staphylococcus*. Representatives of the three species *S. aureus*, *S. epidermidis* and *S. hominis* were used according to grouping based on the presence or absence of the methicillin-resistance mechanism (methicillin-resistant: *S. aureus* – MRSA, *S. epidermidis* – MRSE, *S. hominis* – MRSH; methicillin-susceptible: *S. aureus* – MSSA, *S. epidermidis* – MSSE, *S. hominis* – MSSH). Strains were originally identified using biochemical methods and their species affiliation was confirmed using the multiplexPCR technique. The presence of appropriate genes (*Staphylococcus* – gene *16SrRNA*, *S. aureus* – gene *nuc*, *S. epidermidis* – gene *rdr*, *S. hominis* – gene *nuc*) was determined utilizing the specific primer sets. PCR products were analyzed using the 1.5% agarose gel horizontal electrophoresis technique and then archived using the ChemiDoc XRS gel documentation system.

The mechanism of methicillin-resistance was determined by the phenotypic method using a cefoxitin disc on Mueller-Hinton medium, and the results were interpreted according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). A PCR method with specific primers was also used to detect the *mecA* and *mecC* genes, which are the basis of this resistance mechanism. The amplified gene fragments were similarly separated by the technique of horizontal electrophoresis.

Resistance phenotypes to macrolides, lincosamides and group B streptogramins in *Staphylococcus* were determined using the disc diffusion method with erythromycin and clindamycin discs according to the EUCAST methodology. In the interpretation of the results, the currently applicable criteria and features of individual variants were used, which allowed for the determination of K_R and K_{HD} constitutive phenotypes, I_D and I_{D+} inductive phenotypes as well as the MS_B and L phenotype. To define

the genotypes of *Staphylococcus* strains associated with resistance to antibiotics belonging to the group of macrolides, lincosamides and group B streptogramins, the following genes were selected: *erm*(A), *erm*(B), *erm*(C), *mef*(A), *msr*(A), *msr*(D) and *cfr*. A classic PCR or multiplexPCR reaction was performed using the appropriate primer sequences. Reaction products were analyzed using the 1.5% agarose gel horizontal electrophoresis technique and then archived using the ChemiDoc XRS gel documentation system.

The clinical evaluation of drug susceptibility of *Staphylococcus* strains was based on a study performed with the use of GP cards and the Vitek 2 automatic system (bioMerieux), which was carried out in accordance with the manufacturer's instructions and recommendations. The following antibiotics were included in the analysis: cefoxitin, penicillin, tetracycline, gentamicin, erythromycin, clindamycin, ciprofloxacin, moxifloxacin, vancomycin, teicoplanin, linezolid, tigecycline, trimethoprim/sulfamethoxazole, and fusidic acid.

The activity of selected antibiotics from the group of macrolides and derivatives (erythromycin, clarithromycin, azithromycin), lincosamides (clindamycin), group B streptogramins (quinupristin/dalfopristin), pleuromutilins (tiamulin) and glycopeptides (vancomycin) against *Staphylococcus* strains was assessed. The minimum inhibitory concentrations were established using the diffusion method with antimicrobial gradient stripes. The obtained results were interpreted based on the currently applicable criteria recommended by EUCAST.

The statistical analysis used the non-parametric ANOVA Kruskal-Wallis rank test and the post-hoc test of multiple comparisons of rank mean for all trials. The Chi-square test of independence was also performed. The results were statistically significant at the level of $p < 0.05$. Statistica 13 package by StatSoft was used.

In the *S. aureus* group, almost half of the strains did not show the resistance phenotype associated with MLS_B antibiotics. The constitutive phenotype of K_R was noted in 21.31% of the strains, K_{HD} in 18.03% of the strains, the inductive phenotype I_D in 3.28%, and I_{D+} in 6.56%, and the L phenotype in 1.64%. None of them showed MS_B-type resistance. MRSA strains had MLS_B resistance only in the constitutive variant – 40.00% K_R type and 36.67% K_{HD} type. The most common MSSA phenotype was I_{D+} (12.91% strains), but almost 75% showed no resistance associated with MLS_B antibiotics. Among *S. epidermidis*, the dominant pattern of resistance was the constitutive MLS_B phenotype of K_R type (27.45%) and the MS_B phenotype (19.61%). Both variants

of the inductive phenotypes I_D and I_{D+} were found with the frequency of 5.88% and 7.84%, respectively, and the phenotype L with the frequency of 3.92%. In the MRSE group the K_R variant was the most common (45.17%) and the MS_B type (40.00%) in the MSSE group. The most prevalent pattern of resistance in *S. hominis* turned out to be I_{D+} (45.83%), followed by K_R (12.50%). The I_D and MS_B phenotype was present in the same number of strains, which corresponded to 4.17% of all isolates. In the MRSH group, I_{D+} resistance dominated (66.66%), while in the MSSH group only one pattern of resistance was found in 11.11% of the strains, i.e. I_{D+}.

The prevalence of genes in MLS_B-positive *S. aureus* strains was: *erm*(A) in 32.26% of strains, *erm*(B) in 61.29% of strains, and *erm*(C) in 3.23%. In MRSA, the presence of the *erm*(A) gene was found in 26.09% of the strains and *erm*(B) in 73.91% of the strains. The *erm*(B) determinant was associated with both the K_R and K_{HD} phenotype, and the *erm*(A) determinant only with the K_R phenotype. In 8 phenotype-positive MSSA strains, the *erm*(A) gene was present in 1 out of 2 I_D strains, in 3 out of 4 I_{D+} strains, the *erm*(B) gene was present in two strains (single I_{D+} and K_R) and the *erm*(C) gene in one strain. In total, the frequency of genes in *S. epidermidis* was as follows: *msr*(D) – 84.85%, *erm*(C) – 51.52%, *msr*(A) – 33.33%, *erm*(A) – 30.30% and *erm*(B) – 9.09% strains. In MRSE, the most common gene turned out to be *msr*(D) (81.82% of strains) and *erm*(C) (59.09%), followed by *erm*(A) (40.91%). The *msr*(D) gene was present in all strains with inductive MLS_B phenotypes. In MSSE, the most common gene was *msr*(D), which was found in 90.91% of strains with the MLS_B resistance phenotype. The predominant gene in the MRSH group was *erm*(C), which occurred in all selected strains with any MLS_B resistance phenotype (1 I_D, 10 I_{D+}, 3 K_R and 1 MS_B). A single MSSH strain with the I_{D+} resistance phenotype had only the *erm*(C) gene. In conclusion, in the studied *Staphylococcus* strains, it was found that the *erm*(C) gene was present in 42.50% of the strains, followed by *msr*(D) (36.25%), *erm*(B) (27.50%), *erm*(A) (25.00%) and *msr*(A) (15.00%). The most common in the methicillin-resistant *Staphylococcus* group was the *erm*(C) gene (46.67% of the strains), and in the methicillin-susceptible *Staphylococcus* group, the *msr*(D) gene (50.00% of the strains). The *mef*(A) and *cfr* genes were not detected in any of the *Staphylococcus* strains.