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MIKROBIOLOGIJOS GRUPĖ

PREVALENCE OF PENA GENE MODIFICATIONS AMONG CLINICAL STRAINS OF NEISSERIA MENINGITIDIS

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Introduction. *Neisseria meningitidis (N. meningitidis)* is a Gram–negative bacterium that is a major cause of septicemia and meningitis and contributes to morbidity and death due to its high fatality rate. Reduced susceptibility to penicillin has been detected over the last few years. The most prevalent resistance mechanism to penicillin appears to involve modifications to the *penA* gene. *penA* gene in *N. meningitidis* encodes penicillin–binding protein 2 (PBP2) and is important in cell wall synthesis. Many mosaic alleles in *penA* gene have been documented, reflecting both distinct recombination events and additional mutations, which promote reduced susceptibility to penicillin, as they are thought to create a reduction of affinity of PBP2.

Goals. To determine the prevalence of the *penA* gene modifications among strains of *N. meningitidis* and their relationship with penicillin susceptibility in strains isolated from hospitalized patients in Lithuania.

Materials and methods. *N. meningitidis* isolates were collected from blood and cerebrospinal fluid samples from patients at Lithuanian hospitals between 2016 to 2019. The pure *N. meningitidis* cultures were stored at -70°C in the Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University. The frozen strains were refreshed on 5 % Sheep Blood Agar (*Bio–Rad*, France) to prepare them for subsequent analysis. DNA extraction from the collected *N. meningitidis* strains involved combining deionized sterile water and a colony from the inoculated Blood agar, followed by heating and centrifugation. The method used to determine the prevalence of the modified *penA* gene was polymerase chain reaction (PCR) for amplification of *penA* gene in combination with restriction fragment length polymorphism (RFLP) analysis for detection of different mosaic allele profiles. 1 µl of the sample was used in PCR amplification of DNA fragments. To amplify the *penA* gene in the samples, two oligonucleotides, with the following sequences, were used: *AA–1 (5–ATCGAACAGGCGACGATGTC–3; nucleotides 1237 to 1256) and 99–2 (5–GATTAAGACGGTGTTTTGACGG–3; nucleotides 1728*

to 1748). After PCR amplification and cleavage with restriction enzyme *TaqI*, different profiles of the strains were detected via agarose gel electrophoresis of PCR-amplified DNA fragment of the *penA* gene. The results of this were assigned random numbers. To test if these RFLP profiles vary in penicillin resistance, the gradient method of minimal inhibitory concentration (MIC) was performed using Liofilchem® MIC gradient strips (Liofilchem, Italy). The results of MIC were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST Version 13.3, valid from 2023–06–29) breakpoint tables.

Results. A total of 100 *N. meningitidis* strains were tested. The analysis found 5 different RFLP types (RFLP-1 to -5). *penA* RFLP-1 was found in 64 % (n = 64) of all tested strains. *penA* RFLP-2 type mutations were found in 26 % (n = 26), RFLP-3 were detected in 4% (n = 4), RFLP-4 were analyzed in 5% (n = 5), and RFLP-5 mutations were observed in 1 % (n = 1) of tested strains. Most diverse were the samples of strains from 2016 in which all modification types were found. 93 % (n = 93) of all strains showed susceptibility to penicillin. Resistant to penicillin were 7 % (n = 7) of the strains. Penicillin resistance was found in 7.8 % (n = 5) of all *penA* RFLP-1 strains and 50% (n = 2) of RFLP-3 profile strains. *penA* RFLP-2, -4, and -5 strains of *N. meningitidis* were susceptible to penicillin.

Summary. Most commonly *penA* RFLP-1 was found in the tested *N. meningitidis* strains. 7 % of tested strains of *N. meningitidis* were resistant to penicillin. 7.8 % of the *penA* RFLP-1 modification showed penicillin resistance. Of the variations of *penA* gene modifications, only RFLP-3 profile showed decreased susceptibility to penicillin. All other variations in *penA* alleles presented with 100% susceptibility to penicillin.

Keywords. *Neisseria meningitidis; penA*; Penicillin susceptibility.