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Short communication

The *aac(6`)Ib* gene in *Proteus mirabilis* strains resistant to aminoglycosides

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Abstract: The aim of this study was to evaluate the presence of *aac(6')-Ib* gene conferring resistance to aminoglycosides in *Proteus mirabilis* strains. Five isolates had *aac(6')-Ib* gene. In one case the gene was no-expressed. Three isolates were resistant to all aminoglycosides and minimum inhibitory concentrations were ≥ 256 μg/ml. Additionally, all positive strains were resistant to tetracycline and ciprofloxacin.

Keywords: *Proteus mirabilis, aac(6')-Ib,* aminoglycosides resistance, PCR

Introduction

Aminoglycosides (Amgs) are highly potent, broad-spectrum antibiotics with many desirable properties for the treatment of human infections. Among resistance mechanisms to Amgs inactivation by aminoglycoside-modifying enzymes (AMEs) is the most important both in terms of level and frequency of resistance conferred to the bacterium [1]. There are three family of AMEs including aminoglycoside acetyltransferases (AACs), phosphotransferases (APHs), and nucleotidyltransferases (ANTs). Each group of enzymes consists of different isozymic forms that differ in substrate regiospecificity for their reactions [1]. Amgs modification has emerged in all clinically relevant bacteria of both Gram-positive (*Staphylococcus* spp., *Enterococcus* spp.) and Gram-negative, including *Proteus* spp. strains [1-4]. These enzymes are often plasmid-encoded but may also be associated with transposable elements facilitating the rapid dissemination of the genes in various bacterial populations [1,5].

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In this study we present occurrence of *aac(6`)-Ib* gene encoding AAC(6`)-I enzyme in clinical strains of P*roteus mirabilis*.

Materials and methods

Bacterial strains. *Proteus mirabilis* strains were obtained from patients admitted to University Hospital in Bialystok. The samples were streaked onto MacConkey agar. Gram-negative bacteria were identified using API 32E (bioMerieux, France), a standardized biochemical identification system for *Enterobacteriaceae*.

Susceptibility testing. The disk diffusion method was performed to detection of Amgs resistance (patterns of resistance) and other agents according to CLSI standards [6], using the following antibiotics: gentamicin, netilmicin, tobramycin, amikacin, ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, ceftazidim, cefotaxime, cefepime, aztreonam, tetracycline, ciprofloxacin, imipenem, trimethoprim-sulfamethozaxole. The bacterial suspensions (0.5 McFarland) had been inoculated on the surface of Mueller-Hinton Agar, disks (Becton Dickinson) were added and the suspensions were left to incubate in an incubator at 35°C. Analysis was performed after 16-18 h of incubation by measuring the zone diameters.

Minimal inhibitory concentrations (MICs) of gentamicin, netilmicin, tobramycin and amikacin (range 0.016 to 256 μg/ml) were determined by E-tests on Mueller-Hinton Agar according to CLSI [6]. *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used as standard strains.

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PCR experiments. Plasmid DNA was extracted and purified from overnight cultures by using the Plasmid Mini kit (A&A Biotechnology, Gdynia, Poland).The PCR method proposed by Noppe-Leclercq et al. [7] was adopted in this study. Oligonucleotide primers AMG-F1 (5`-TATGAGTGGCTAAATCGAT-3`) and AMG-F2 (5`-CCCGCTTTCTCGTAGCA-3`) were employed and early described by Ploy [8] and were used to amplify a 395 bp fragment of gene encoding the AAC(6`)-I enzyme. PCR amplification was carried out in a 25 µl volume with the Cyclone 96 (PEQLAB Biotechnology, GmbH) thermal cycler. The PCR mixtures contained: 25 pmol of each primers, 1x reaction buffer, 2 mM $MgCl_2$, 1 µl of dNTPs, 0.5U of Delta2 DNA polymerase (DNA-Gdansk II, Gdansk, Poland), 2 µl of template DNA and ultra pure H2O to final volume. The cycling parameters of amplification were: initial denaturation at 94°C for 2 min 20 s; denaturation at 94°C for 30 s, annealing at 54°C for 40 s, and extension at 72°C for 80 s repeated for 45 cycles. The final elongation step was at 72°C for 4 min. Products of PCR were analyzed by electrophoresis at 5V/cm for 90 min in 1.8% agarose gel containing 0.5 µg/ml ethidium bromide in TBE buffer and photographed on a UV transilluminator. The sizes of the fragments produced in the amplifications were calculated from their positions relative to the positions of the molecular weight marker.

Results and discussion

Among examined strains of *Proteus mirabilis* (14) we present seven isolates coded as Pm1 – Pm7 (Fig. 1). The aac(6')-Ib gene was detected in five isolates (Pm1-2, Pm5-7). The minimum inhibitory concentration value of gentamicin, tobramycin, amikacin, and netilmicin in three positive isolates (Pm1, Pm2, and Pm6) were ≥256 µg/ml. The enzymes produced by various bacteria can often be predicted from the antibiogram data [4], but hidden genes could be present. One *Proteus mirabilis* strain (Pm5) had non-acitve gene aac(6')-Ib which MIC values of Amgs were located in a criterion of the susceptibility. However, certain strain (Pm4) did not have the aac(6')-Ib gene, but MICs of aminoglycosides were ≥256 µg/ml. Pm3 strain (Fig. 1) represents a group of isolates susceptible to four main aminoglycosides. The AAC(6`)-I enzyme confers resistance to tobramycin, amikacin, netylmicin, and kanamycin. Only one component (gentamicin) can remains active but in vivo use best avoided [1,4]. Of all Amgs amikacin is the most resistant to the action of AMEs. AAC(6')-I type enzymes can utilize amikacin as substrate and confer resistance to this antibiotic in addition to other Amgs [5]. Additionally, all positive strains were resistant to tetracycline and ciprofloxacin.

Using a phenotypic approach the resistance pattern according to activity of aac(6')-Ib gene can be probably found in 6.4% of *Proteus* spp. strains [9]. Of all *Enterobacteriaceae* family the aac(6') genes were the most frequently found genes and the isolated *aac(6')-Ib* gene represented the most frequently encountered resistance mechanism [10,11].

In general, aminoglycoside resistance rates between different European countries were generally higher in Italy, Portugal, Spain, Greece, France, the UK, and Poland [12], but resistance of *Proteus mirabilis* to aminoglycosides still remains low [12,13].

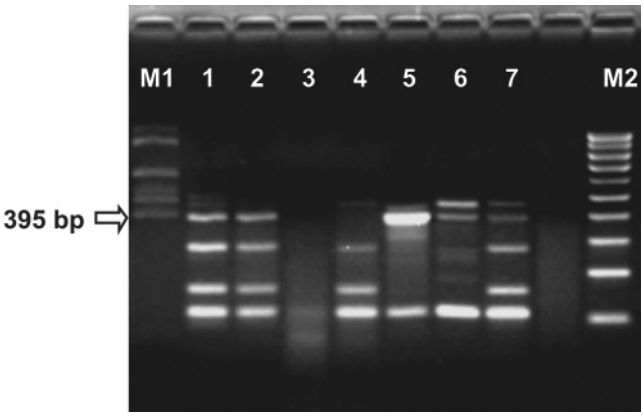

Fig. 1. Demonstration of aac(6`)Ib gene with PCR in agarose gel. M1, molecular size marker M2 (pKO3/HinfI) (DNA-Gdansk II, Gdansk, Poland); M2, molecular size marker M100-1000 bp (DNA-Gdansk II, Gdansk, Poland), 395 bp band, *aac(6`)Ib* gene; Lanes 1-7, numbers of clinical *Proteus mirabilis* strains Pm1-Pm7.

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