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AP-PCR TYPING OF CARBAPENEM SENSITIVE *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CLINICAL SAMPLES

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ABSTRACT

In this study the antibiotic susceptibility of 51 *P.aeruginosa* strains isolated from clinical samples were detected by the disc diffusion test. The susceptibility of *P. aeruginosa* strains were found as respectively 55% amicacin, 43% aztreonam, 75% netilmycin, 68% sefepim, 73% ceftazidim, 76% ciproflaxacin, 37% gentamicin, 84% meropenem, 76% piperasillin/tazobactam, 47% tobramycin and 84% imipenem. These results show that carbapenems are the most effective antibiotics for *P. aeruginosa* strains and the efficacy of meropenem and imipenem are high for *P. aeruginosa* strains Molecular typing profiles of 43 P. aeruginosa strains which are sensitive to meropenem and imipenem antibiotics with AP 1 primary were determined in AP-PCR. As a result of AP-PCR molecular typing study of this 43 *P. aeruginosa* isolate, no correlation was found out between antibiotic sensitivities and molecular types. This situation once again reveals that reasonable antibiotic usage in absolutely

INTRODUCTION

As well as most of the *Pseudomonases* (pyocyanic) infect people, some among them form temporizer pathogen which is important especially for people having weak immunity system. In particular, *P. aeruginosa* known as temporizer pathogen has such an important place among *Pseudomonases*.It is realized that infections caused by *P. aeruginosa* in humans increase in hospital ambient lately. It is also observed that these bacterias shelter very easily and their resistance increases in hospital ambient. Generally, treatment of *Pseudomonas* infections is ratherly slow and difficult because of this bacteria is resistant to frequently used antibiotics. (1, 2, 3) Therefore, multiple antibiotic usage is recommended for treatment of *P. aeruginosa* (4,5). Particularly, in *P. aeruginosa* infections, the carbapenems are frequently used antibiotics in treatment. The reason why carbapenems are preferred in treatment is that they have wider influence spectrums than beta-lactam antibiotics. Furthermore, carbapenems are enounced as stabile against plasmid and also chromosomal betalactamases in different researches (6,7). Important infections caused by *Pseudomonases* are lung infections, respiratory infections, bacteriemia, articulation infections, urinary tract infections, gastrointestinal tract infections, burn and trauma infections, epidermic and soft tissue infections (8)

In study, AP-PCR technic was used to determine molecular typing profiles of 43 *P. aeruginosa* isolate that is sensitive to carbapenems. AP-PCR technic is frequently used in molecular typing of *P. aeruginosa* isolates which are isolated from clinical samples. (9).

MATERIALS AND METHODS

Obtaining samples: In study, 51 *P. aeruginosa* isolate, which was isolated from different samples belong to impatient in several clinics, was used.

Bacteria identification: API 20 NE (BioMeriux, Marcy l'Etoile, France) kit was used in identification of bacterias (10).

Antibiotic sensitivity test: In accordance with NCCSL standards, antibiotic sensitivity test was studied in Mueller-Hinton Agar with the method of standard disc diffusion. In disc diffusion test, amicasin 30 µg, aztreonam 30 µg, netilmicin 30 µg, sephepim 30 µg, ceftasidime 30 µg, ciproflaxacin 5 µg, gentamicin 10 µg, meropenem 10 µg, piperacillin/ tazobactam 110 µg, tobramycine 10 µg and imipenem 10 µg (Oxoid-England) antibiotics were used. In research, *P. aeruginosa* ATCC 27853 was used as control element (11). As a result of this test, sensitive *P. aeruginosa* isolates were chosen.

DNA isolation: Isolation of 51 *P. aeruginosa* isolate was performed by phenol-chloroform technic (12, 13)

AP-PCR Amplification: For AP-PCR reaction, API "5 – GTT GCG ATC -3" (Bio Thesis, Germany) primary was used. Each, including 100 µl reaction compound, was respectively calculated and put into 0,5 ml ependorph tubes.10 x Tampon (NH4)2 SO4) (Promega,USA) 10 µl, dATP, dCTP, dGTP, dTTP (Fermantes,Lithuania) 2 mM, Primary Ap 1 (Bio Thesis, Germany) 50 pmol, $MgCl_2$ (Fermantes,Lithuania) 25 mM, Taq DNA polymerase (Promega, USA) 1U and deionized water were added to compound to fullfil volume. AP-PCR reaction was performed in automatic PCR device (Techne,USA) under circumstances like:following denaturation for 5 minutes at 94°C, denaturation for 1 minute at 94°C making 40 cycles, adhesion for 2 minutes at 72°C and following last elongation for 6 minutes at 72°C, PCR products were kept at +4°C until extrapolation. Obtained PCR products were conducted in % 12 polyacrilamid gel electrophoresis (PAGE) (20 cm x 20 cm) (Owl Scientific, USA), 120 volts, 80 mA and 10 watts. PAG was painted by silver painting technic later on.

Distributions of 51 *P. aeruginosa* isolates utilized in study as per the clinical samples are shown in Table 1.

Table 1. The distribution of isolators obtained from patient materials with respect to clinical samples

Clinical samples	*P. aeruginosa*
Blood	15
Sputum	6
Aspirate	10
Urine	12
Wound	8
Total	51

Sensitivities of *P. aeruginosa* isolates to different antibiotics by the method of disc diffusion are shown in Table 2.

Table 2. Sensitivity proportions of isolated *P.aeruginosa* to tested antibiotics with disc diffusion methods

ANTIBIOTICS	SENSITIVE	(%)	MEDIAL SENSITIVE	(%)	RESISTANT	(%)
Amicasin	28	55	11	21	12	24
Aztreonam	22	43	9	18	20	39
Netilmycin	38	75	-	-	13	25
Sefepim	35	68	5	10	11	22
Ceftazidime	37	73	-	-	14	27
Ciprofloxacin	39	76	4	8	8	16
Gentamicin	19	37	14	28	18	35
Meropenem	43	84	1	2	7	14
Piperasillin/tazobaktam	39	76	3	6	9	18
Tobramycin	24	47	8	16	19	37
Ýmipenem	43	84	3	6	5	10

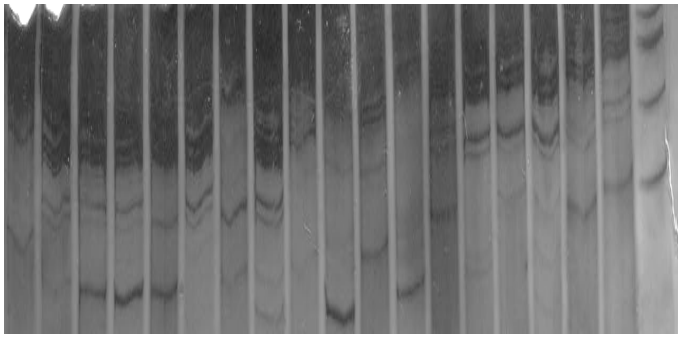
In study, 43 of 51 *P. aeruginosa* isolate were found out sensitive to carbapenems. Sensitivity to imipenem and meropenem from these antibiotics was determined as % 84. Distributions of isolates as per the samples are shown in Table 3. Molecular typing profiles of 43. *P. aeruginosa* which is sensitive to meropenem and imipenem antibiotics with AP 1 primary were determined in AP-PCR (picture 1a and picture 1b). In study, genotypic proximity was determined in number 3,4 and 5 *P. aeruginosa* isolates (picture 1a). In study, no common genotypic profile was found out among left 40 *P. aeruginosa* isolates sensitive to meropenem and imipenem (picture 1a and picture 1b). Besides, a dominant genotypic pattern was not determined among sensitive isolates.

Table 3. 43 *P. aeruginosa* isolates sensitive proportions of meropenem and imipenem antibiotics

Clinical samples	Imipenem			Meropenem		
	D	AD	R	D	AD	R
Blood	13	1	2	12	1	3
Sputum	5	-	-	5	-	-
Aspirate	9	1	-	10	-	-
Urine	10	1	1	10	-	2
Wound	6	-	2	6	-	2
TOTAL	43	3	5	43	1	8

Figure 1a. *P. aeruginosa* isolates sensitive to meropenem and imipenem with AP1 primer were determined in AP-PCR

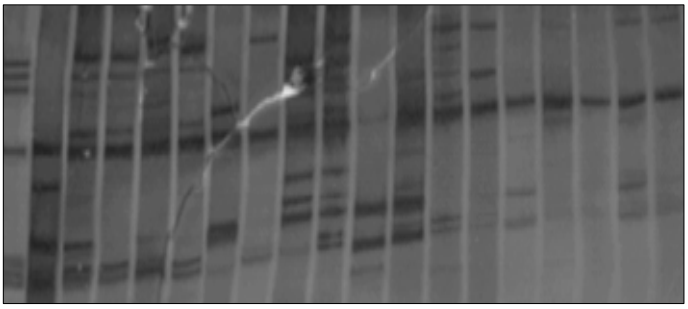
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M



M: molecular size indicator [ÖX174 *HaeIII* cut (Fermentas, Lituanya) genotypic proximity was determined in number 3,4 and 5 *P. aeruginosa* isolates

Figure 1b. *P. aeruginosa* isolates sensitive to meropenem and imipenem with AP1 primer were determined in AP-PCR

M 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36



M: molecular size indicator [ÖX174 *HaeIII* cut (Fermentas, Lituanya)]

DISCUSSION

More than one antibiotic are used in treatment of infections coused by *P. aeruginosa* Especially in treatment, aminoglycosides, cephalosporins, carbapenems and betalactam/betalactamases inhibitored antipseudomonal penicillin combinations are preferred for the reason of ease-of-use (14). For this reason, in accordance with antibiotic sensitivity results, 43 *P. aeruginosa* isolate determined as sensitive to carbapenems was chosen in research. Sensitivities to imipenem and meropenem from carbapenem antibiotics are determined as % 84. As a result of AP-PCR molecular typing study of this 43 *P. aeruginosa* isolate, no correlation was found out between antibiotic sensitivities and molecular types. In the result of the study, imipenem and meropenem usage can be recommended in *P.aeruginosa* infection treatment. Because, carbapenems used in treatment are separated than other betalactam antibiotics with their wider influence spectrum and strong antibacterial influences. Furthermore, carbapenems are more stabile to plasmid and also chromosomal beta lactamases.

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