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Sensitivity to Antimicrobial Drugs of *Pseudomonas Aeruginosa* Extreme-Resistant Strains Isolated in the Major Hospitals of Central Kazakhstan

Ilya S. Azizov[1], Alyona V. Lavrinenko[2]*, Ilya A. Belyaev[2], Dmitry B. Babenko[2], Natalya A. Shambilova[1], Nelya M. Bissenova[2]

[1]Institute of Antimicrobial Hemotherapy, Smolensk, Russia; [2]Karaganda State Medical University, National Scientific Medical Center, Karaganda, Kazakhstan

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***Correspondence:** Alyona V. Lavrinenko. Karaganda State Medical University, National Scientific Medical Center, Karaganda, Kazakhstan. E-mail: lavrinenko.alena@gmail.com

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Abstract

AIM: The article presents the current data on the sensitivity of the main 37 strains of eXtremaly Drugs Resistance (XDR) category to anti-pseudomonas drugs.

MATERIAL AND METHODS: The strains were collected during the prospective multicenter study in large multidisciplinary hospitals of Central Kazakhstan. Susceptibility to antimicrobial drugs was carried out by disk method and the serial dilution method with the interpretation of the results according to EUCAST criteria. Detection of carbapenemases gene of VIM, IMP, NDM and GES classes was carried out by PCR method using the commercial kits.

RESULTS: All identified carbapenemases were sorted to VIM class and accounted for 63.64%. Resistance to aminoglycoside drugs exceeded 80%. All the strains were susceptible to polymyxin.

CONCLUSION: Thus, at the present stage the circulation of P. aeruginosa strains of XDR category continues in major hospitals in Kazakhstan. The strains remain sensitiveness only to polymyxin.

Introduction

Antibiotic resistance as a phenomenon of microorganisms' insensibility to achievable concentrations of antibiotics in clinical conditions has become in the last 20 years the pattern of the global problem, migrated from the level of individual departments and hospitals to the level of a global epidemic process, threatening the future of humanity.

Every year more than 20 thousand patients die in the USA in the result of infectious processes caused by multidrug-resistant microorganisms. The USA government spends more than 20 billion dollars a year for the control of antibiotic-resistant strains spreading [1].

The EU countries annually spend more than 9 billion euros for the solution of the problem. At the same time in the European Union more than 25 thousand patients a year die because of ineffective antimicrobial chemotherapy, and more than half of the cases caused by Gram-negative microorganisms [2]. The last in most cases have multidrug resistance mechanisms, leading to a significant narrowing of the list of choice drugs and in the cases of pan-resistance – to almost no alternative solutions [3].

Till recently carbapenems were regarded as the drugs of extreme selection. However wide spreading of carbapenemases genes significantly expanded the list of problematic strains in which of *Pseudomonas aeruginosa* is regarded as a classic representative [4]. Modern strains of *P. aeruginosa* in addition to many natural mechanisms and

antimicrobial resistance mechanisms due to the high genetic lability constantly replenish its arsenal of acquired resistance mechanisms [5] and have the predilection to the clonal global spreading [6]. From this perspective, the continuous supervision for the local spread of multidrug-resistant strains is important for practical health care as well as for fundamental science. The strains of XDR (eXtremaly Drugs Resistance) category [7] are the particular problem for medicine because of extreme multi-resistance to a wide range of antimicrobial agents.

Our study focuses on the description of the sensitivity of antimicrobial agents and detection of genes that determine resistance to carbapenems in of *P. aeruginosa* strains of XDR category, isolated in large hospitals of Central Kazakhstan.

Materials and Methods

The study included strains collected in the period from 2015 to 2016 during the prospective multicenter microbiological research covering large multidisciplinary hospitals of Central Kazakhstan (Karaganda and Astana).

Isolation of strains was conducted in local bacteriological laboratories of participating centres, and after the strains were forwarded to the microbiology laboratory of the Scientific-Research Center of Karaganda State Medical University, where it was conducted the re-identification methods of time-of-flight mass-spectrometry (MALDI-TOF) using MALDI-Biotyper software (Bruker). Determination of sensitivity to antimicrobial agents was conducted by disk- diffusion method and by the method of serial microdilution in a liquid medium according to EUCAST recommendations [8].

The primary test for detection of Metallo-beta-lactamase activity was carried out with 100 mM EDTA by the recommendations [9]. Additional screening CIM test for detection of carbapenemases activity was carried out by the recommendations [10].

The presence of carbapenemases genes of VIM and IMP classes were performed by Real-Time PCR methods using the commercial kit «AmpliSens MDR MBL-FL» produced by Interlab Service (Russia).

Statistical processing was performed by determining the average values, the definition of rank correlation coefficient by Spearman and determining the 95% confidence interval for the mean values by Klopper-Pearson with the use of MS Excel and Whonet 6.5 [11].

Results

As a result of screening, it was selected 37 strains with XDR phenotypic profile among 270 strains collected in large multidisciplinary hospitals of Central Kazakhstan.

Data on antimicrobial resistance is shown in Figure 1.

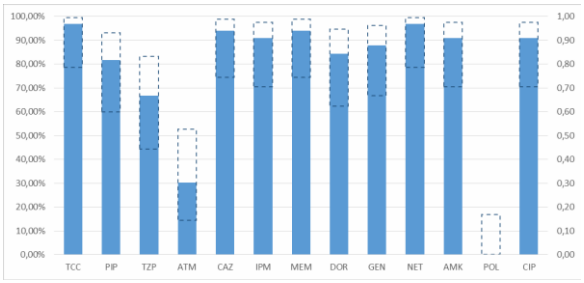

Figure 1: The share of non-sensitive (%R+%I) hospital P. aeruginosa strains to antimicrobial drugs. The dotted squares represent 95% confidence intervals

The studied strains were characterised by resistance to the absolute majority of drugs with anti-pseudomonas activity. The exception was polymyxin, to which we did not reveal any resistant strain. Taking into account the trend towards the emergence of resistant strains of *P. aeruginosa* to colistin [12] we carried out a quantitative evaluation of the sensitivity of the studied strains to polymyxin.

MIC50 for studied strains was 0.5 μg/ml but MIC range was 0.5-2 μg/ml. This pattern suggests polymyxin as the only available anti-pseudomonas drug with high activity, and it actualizes the questions on the development of technologies increasing the bioavailability of the drug [13]. Sensitivity to aztreonam was observed in more than half of the cases (51.43%; 95% CI, 31.25-71.15). A similar pattern is due to the high frequency of occurrence of strains producing Metallo-beta-lactamase (B class) hydrolyzing all beta-lactams except aztreonam [14], which proportion was 68.75%.

Genetic typing of the mechanisms of resistance to carbapenems identified the carbapenemases genes of VIM class, the proportion of strains producing carbapenemases of VIM class was 63.64% (95% CI 39.63-81.17). Meanwhile, the test with chelating agent EDTA showed inhibitory activity in 31 strains that in combination with MIC values corresponding to the expected moderate stability permit to expect the low-affinity carbapenemases of GES class. However, conducted research has not revealed GES carbapenemases genes. Ecoff analysis of distributions on imipenem (Fig. 2) allows surmising sampling heterogeneity.

According to that 75% isolates have MIC higher 32 μg/ml we expected an equal number of

producers of carbapenemases. At the same time, PCR detection of VIM producers was positive in 63.64% which is clearly linked to other mechanisms of resistance. Strains with moderate resistance imipenem were moderately resistant to meropenem and also to aminoglycosides that can be connected with other on- enzymatic resistance mechanisms [15].

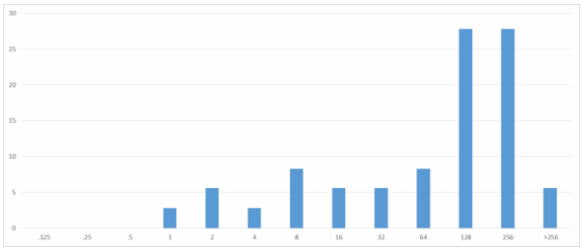

Figure 2: Ecoff distribution of MIC imipenem for studied P. aeruginosa strains (boundary values 4-16 μg/ml), S – sensitive population, I – moderately resistant, R – resistant population

Resistance to fluorinated quinolones was detected in more than 90%, the average MIC values were extremely high too (>32 μg/ml). Resistance to ciprofloxacin had a strong positive correlation with resistance to levofloxacin, which is obviously connected with the general resistance mechanisms and can approximate these results to the whole group of fluorinated quinolones.

Discussion

At present the only drug with a high-clinically significant activity against studied pan-resistant *P. aeruginosa* strains is polymyxin. We did not reveal any cases of resistance; all the studied strains had MIC less than 1 μg/ml. The average values of the MIC of polymyxin totalled 0.49 μg/ml.

The resulting picture clearly shows that there is no alternative situation on the choice of drugs for the causal treatment of infections caused by extremely resistant *P. aeruginosa* strains.

Thus, at the present stage, the circulation of *P. aeruginosa* strains of XDR category continues in major hospitals in Kazakhstan, which have been shown previously [16]. The strains remain sensitiveness only to polymyxin.

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