ECA 2021

sciforum

Conference Proceedings Paper

Mechanisms of resistance in the *Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii* strains isolated from blood and cerebrospinal fluid of children.

Sadeeva Zulfirya *, Novikova Irina, Lazareva Anna and Nikolaeva Anna

Antibiotic resistance and mechanisms of resistance of *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from blood and cerebrospinal fluid of children in intensive care units.

Klebsiella pneumoniae, Pseudomonas aeruginosa and *Acinetobacter baumannii* are the most common and problematic causative agents of nosocomial infections [1]. These microorganisms have the ability to form secondary resistance to antibiotics of different classes and therefore belong to the group of the most problematic bacterial causative agents of nosocomial infections - ESKAPE [2-4].

This article presents the results of assessing the sensitivity, presence of resistance genes and determination of the phenotypic groups of 63 *K. pneumoniae* isolates, 23 *P. aeruginosa* strains, and 14 *A. baumannii* strains isolated from blood and cerebrospinal fluid of children in intensive care units from 2014 to 2020. Enterobacteralis are the leading bacterial infectious agents in intensive care units (ICU). Of particular concern is the progressive resistance of gram-negative bacteria to carbapenem antibiotics [5, 6]. The main mechanism of resistance to carbapenems is the production of carbapenemases, enzymes that destroy antibiotics [7, 8]. The non-fermenting glucose gram-negative bacteria (NGOB) *P. aeruginosa* and *A. baumannii* play an important role in the spread of resistance to carbapenems [9, 10]. They have significantly higher natural antibiotic resistance. This significantly complicates the treatment of infections caused by these pathogens [11, 12]. Currently, the emergence and spread of resistance to carbapenems in nosocomial pathogens is a real threat and determines the need for regular monitoring of sensitivity.

Materials and research methods

For the study, we selected strains of *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* isolated from blood and cerebrospinal fluid of children in intensive care units in Moscow from 2014 to 2020. All blood samples were incubated in a BACTEC 9050 blood culture analyzer (Becton Dickinson, USA) until microbial growth was recorded, then inoculated onto solid nutrient media to isolate a pure culture of the pathogen by classical microbiological methods. The biological material was inoculated on nutrient media: blood agar and Uri-select agar (BioRad, USA), then incubated in a thermostat at 37 ° C for 24 - 48 h. Identification of microorganisms to species was carried out by the method of matrix-associated laser desorption / ionization mass spectrometry MALDI-TOF (Bruker Daltonics, Germany). The recommended score ≥ 2, 0 was used as a criterion for reliable identification. Minimum inhibitory concentrations of antibiotics were determined using the serial microdilution method in Mueller-Hinton broth (BioMerieux, France) SensititreTM (ThermoScinetific, UK). The results were interpreted according to the evaluation criteria of the European Committee for Antibiotic Susceptibility Testing (EUCAST) version 10.0 [13].

To isolate DNA, a daily culture obtained by plating on solid nutrient media, indicated above, was used. Bacterial DNA was isolated using commercial kits "GK-express" (Central Research Institute of Epidemiology of Rospotrebnadzor) according to the manufacturer's instructions. The obtained samples were stored until use at t - 20 ° C. The identification of genes encoding the production of carbapenemases was carried out using kits with hybridization-fluorescence detection "AmpliSens®

MDR MBL-FL" (IMP, NDM, VIM), "AmpliSens® MDR KPC / OXA-48-FL" (KPC, OXA-48), "AmpliSens MDR Ab-OXA-FL" (OXA-23, OXA-40, OXA-58), produced by TsNIIE Rospotrebnadzor.

RT-PCR included the following steps: DNA isolation, amplification with hybridization-fluorescence detection in "real time", analysis and interpretation of the results. The components of the reaction mixture were mixed immediately before carrying out the amplification. The amplification reaction was carried out according to the scheme specified in the manufacturer's instructions.

Results and discussion

In the period 2014 - 2020. 621 samples of blood cultures and cerebrospinal fluid obtained from children with symptoms of bacterial infection and for diagnostic purposes (monitoring) were analyzed.

Coagulase-negative staphylococci (SCN) took the leading place in terms of the frequency of isolation: *Staphylococcus hominis* and *Staphylococcus haemolyticus* -189 (30.4%) and 109 (17.6%), respectively. *K. pneumoniae* was the second most important microorganism - 10.3% (63) of the microbial spectrum. *P. aeruginosa* was isolated with a frequency of 3.5% (22), *Staphylococcus aureus* was detected in 3.4%. *A. baumannii* accounted for 2.3%.

In total, 63 strains of *K. pneumoniae*, 23-*P. aeruginosa* and 14-*A. baumannii* isolated from blood and cerebrospinal fluid of children were studied. Monitoring of antibiotic resistance, determination of resistance genes and phenotypic groups were carried out for gram-negative microorganisms *K. pneumoniae, P. aeruginosa* and *A. baumannii*, which are the most dangerous in bloodstream infections. All microorganisms were divided into three categories for each antibiotic susceptible, susceptible to increased exposure and resistant strains.

The results of determining the sensitivity of *K. pneumoniae* strains revealed a rather low in vitro activity in aztreonam, ticarcillin / clavulanate, and piperacillin / tazobactam, the resistance to which was 94%, 96% and 81%, respectively (Table 1).

Table 1. Antibiotic sensitivity of *K. pneumoniae* isolated from blood and cerebrospinal fluid of children in intensive care units from 2014-2020 (n = 63).

Antibiotic	*Sensitive*		*Sensitive at increased exposure*		*Resistant*	
	n	**%**	**n**	**%**	**n**	**%**
Meropenem	33	52	9	14	21	33
Imipenem	35	55	5	8	23	37
Colistin	42	67	-	-	21	33
Polymyxin	48	76	-	-	15	24
Tobramycin	6	10	-	-	57	90
Amikacin	22	35	-	-	41	65
Gentamicin	12	19	-	-	51	81
Fosfomycin	33	52	-	-	30	48
Aztreons	4	6	-	-	59	94
Cefepime	2	3	6	10	55	87
Ceftazidime	3	4	1	2	59	94
Ticarcillin / clavulanate	1	2	1	2	61	96
Piperacillin / tazobactam	9	14	3	5	51	81
Biseptol	16	25	-	-	47	75
Ciprofloxacin	11	17	6	10	46	73
Levofloxacin	-	-	18	29	45	71

Resistance to aminoglycosides reached 90%. The proportion of strains resistant to ciprofloxacin was 73%, all studied isolates of *K. pneumoniae* were resistant to levofloxacin. Fosfomycin was resistant to 48% of the isolates. Resistance to colistin and polymyxin in *K. pneumoniae* isolates was 33% and 24%, respectively. The resistance of *K. pneumoniae* strains to cephalosporins reached 94%. Resistance to carbapenem antibiotics - meropenem and imipenem, was shown by 37% and 37%, respectively. The determination of carbapenemases was carried out in carbapenem-resistant strains. The production of

carbapenemases belonging to the OXA-48 group was detected in 25 (89%) isolates. The presence of NDM, VIM, KPC carbapenemases was not detected.

When comparing the results of different years, an increase in resistance to carbapenems was observed. In accordance with internationally accepted criteria, 23 (37%) isolates had the phenotype of multidrug resistance (MDR-Multy Drug Resistance - that is, resistance to at least one drug of three or more classes of antibiotics). Among them, only three isolates were found to have OXA-48 carbapenemase. The phenotype of extreme resistance (XDR-extremely resistant strains-resistance to at least one drug from all classes of antibiotics, except for two or less classes) - 31 (50%) isolates. Of these, 18 (58%) possessed OXA-48. The phenotype of pan-resistance (PDR - pan-resistant strains - resistance to all classes of antimicrobial drugs) was exhibited by five isolates, and four of them possessed OXA-48 carbapenemase.

Non-fermenting gram-negative microorganisms are no less significant microorganisms in bacteremia. *P. aeruginosa* was the second most common in our study. The results of assessing the sensitivity of *P. aeruginosa* are presented in Table 2.

Table 2. Antibiotic sensitivity *P. aeruginosa* isolated from blood and cerebrospinal fluid of children in intensive care units from 2014-2020 (n = 23).

Antibiotic	*Sensitive*		*Sensitive at increased exposure*		*Resistant*	
	n	%	n	%	n	%
Meropenem	6	26	2	9	15	65
Imipenem	-	-	6	26	17	74
Colistin	23	100	-	-	-	-
Tobramycin	8	35	-	-	15	65
Amikacin	14	61	-	-	9	39
Ciprofloxacin	-	-	7	30	16	70
Piperacillin / tazobactam	13	57	-	-	10	43
Cefthalosan / tazobactam	-	-	10	43	13	57
Ceftazidime / avibactam	9	39	-	-	14	61
Aztreons	-	-	18	78	5	22
Ceftazidime	-	-	6	26	17	74

43% of the isolates were resistant to piperacillin / tazobactam. 74% of isolates were resistant to ceftazidime. Resistance to aminoglycosides (tobramycin, amikacin) was detected in 65% and 39% of the strains, respectively. In relation to ciprofloxacin, 70% of the isolates were resistant. The combined drugs ceftazidime / avibactam and cefthalosan / tazobactam were resistant to 61% and 57%, respectively. The highest in vitro activity was exhibited by polymyxins. No strains that were insensitive to colistin were identified.

The proportion of strains resistant to carbapenems for the period from 2016 to 2021 was 65% for meropenem, and 74% for imipenem. The detection rate of metallo-β-lactamases (MBL) in *P.*

aeruginosa strains was 48%. Only VIM-type MBLs were identified. No other types of MBL have been found. Five isolates had a multiple resistance (MDR) phenotype. Extreme resistance phenotype (XDR) - 15 (65%) isolates. Moreover, all VIM producers were included in the XDR group of 11/15 strains. No strains with a pan-resistance (PDR) phenotype were identified.

A. baumannii also occupies an important place among non-fermenting bacteria in bloodstream infections in our study. The results of assessing the sensitivity of *A. baumannii* are presented in Table 3.

Table 3. Antibiotic sensitivity of *A. baumannii* isolated from blood and cerebrospinal fluid of children in intensive care units from 2014-2020 (n = 14).

Antibiotic	*Sensitive*		*Sensitive at increased exposure*		*Resistant*	
	n	%	n	%	n	%
Meropenem	4	29	1	7	9	64
Imipenem	4	29	-	-	10	71
Colistin	10	71	-	-	4	29
Polymyxin	14	100	-	-	-	-
Tobramycin	5	36	-	-	9	64
Amikacin	2	14	-	-	12	86
Gentamicin	2	14	-	-	12	86
Biseptol	4	29	1	7	10	64
Ciprofloxacin	-	-	2	14	12	86
Levofloxacin	-	-	2	14	12	86

64% of isolates were resistant to biseptol. High resistance values were noted for aminoglycosides: amikacin and gentamicin 86%. The proportion of strains resistant to tobramycin was 64%. Four isolates were found to be resistant to colistin. There were no strains resistant to polymyxin. 86% of isolates were resistant to ciprofloxacin and levofloxacin. Of all the strains studied, 64% were resistant to meropenem and 71% of the isolates to imipenem. In the period from 2014 to 2021 the level of resistance of *A. baumannii* to carbapenems increased. The presence of carbapenemases was investigated in carbapenem-resistant strains. The OXA-40 gene was found in four strains, the OXA-23 gene was found in three strains.

One isolate with a molecular class D OXA-40 carbapenemase was found to have a multiple resistance (MDR) phenotype. Five isolates had an extreme drug resistance (XDR) phenotype. Of these, three were found to have the OXA-40 gene. Four isolates showed a pan-resistance (PDR) phenotype. All of them possessed genes for carbapenemases. Only one strain had OXA-40 and three isolates had OXA-23.

Conclusion

The results of this study indicate a widespread resistance to most antibacterial drugs among the strains of *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* isolated from blood and cerebrospinal fluid.

The increasing resistance to carbapenems and polymyxins is especially alarming. Thus, the proportion of carbapenem-resistant *K. pneumoniae* strains to meropenem and imipenem was 33% and 37%, respectively, of all isolates. Resistance to colistin and polymyxin in *K. pneumoniae* isolates was

33% and 24%, respectively. Among *P. aeruginosa*, 65% were resistant to meropenem, and 74% to imipenem. The highest activity against *P. aeruginosa* in vitro was exhibited by polymyxins. No strains that were insensitive to colistin were identified. *A. baumannii* was 71% resistant to carbapenems. The highest activity against *A. baumannii* in vitro was shown by polymyxin; there was not a single resistant strain to it. Colistin was resistant to 29% of the isolates.

Mandatory control of resistance to carbapenems and reduction of the incidence of inappropriate use of these antibiotics are necessary to prevent further growth of resistance.

References

1. *Klebsiella pneumoniae*: microbiological characteristics, antibiotic resistance and virulence Shamina O.V., Samoilova E.A., Novikova I.E., Lazareva A.V. Russian Pediatric Journal. 2020.Vol. 23.No. 3.S. 191-197.
2. Santajit S., Indrawattana N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. Biomed Res Int. 2016; DOI: 10.1155/2016/2475067
3. Jack N Pendleton, Sean P Gorman & Brendan F Gilmore (2013) Clinical relevance of the ESKAPE pathogens, Expert Review of Anti-infective Therapy, 11:3, 297-308, DOI: 10.1586/eri.13.12.
4. Boucher H.W., Talbot G.H., Bradley J.S., Edwards J.E., Gilbert D., Rice L.B., Scheld M., Spellberg B., Bartlett J. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin. Infect. Dis. 2009;
5. Bassetti M., Vena A., Croxatto A., et al. How to manage *Pseudomonas aeruginosa* infections. Drugs Context. 2018;7: 1-18. DOI: 10.7573/dic.212527
6. Edelstein, M. Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals / M. Edelstein, I. Pimkin, I. Palagin, I. Edelstein, L. Stratchounski // Antimicrobial Agents and Chemotherapy. – 2003. – Vol. 47. – P. 3724-32
7. GENOTYPES AND CARBON OF CARBAPENEMASES IN CARBAPENEMORESISTENT *KLEBSIELLA PNEUMONIAE* ISOLATED IN CHILDREN IN MOSCOW Kryzhanovskaya O.A., Shamina O.V., Lazareva A.V., Alyabyeva N.M., Mayansky N.A. Clinical microbiology and antimicrobial chemotherapy. 2019.Vol. 21. No. S1. P. 38.
8. Pfeifer, Y. Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens / Y. Pfeifer, A. Cullik, W. Witte // International Journal of Medical Microbiology. – 2010. – Vol. 300, № 6. – P. 371-9
9. Russo T.A., Luke N.R., Beanan J.M., Olson R., Sauberan S.L., MacDonald U., Schultz L.W., Umland T.C., Campagnari A.A. The K1 capsular polysaccharide of *Acinetobacter baumannii* strain 307-0294 is a major virulence factor. Infect. Immun. 2010;
10. Lazareva A.V., Chebotar I.V., Kryzhanovskaya O.A., Chebotar V.I., Mayansky N.A. *Pseudomonas aeruginosa*: pathogenicity, pathogenesis and pathology. Clinical microbiology and antimicrobial chemotherapy. 2015; 17 (3): 170-86.
11. Zarrilli R., Pournaras S., Giannouli M., Tsakris A. Global evolution of multidragresistant *Acinetobacter baumannii* clonal lineages. Int J Antimicrob Agents. 2013;41:11-9.
12. Potron, A. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Mechanisms and epidemiology / A. Potron, L. Poirel, P. Nordmann // International Journal of Antimicrobial Agents. – 2015. – Vol. 45, № 6. – P.568-85
13. EUCAST: European Committee on Antimicrobial Susceptibility Testing, version 10.0, valid from 2020-01-01. p. 10-20

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2019 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).