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RESEARCH ARTICLE

ASPECTS OF ANTIBIOTIC RESISTANCE FOR *KLEBSIELLA PNEUMONIAE* IN A CHILDREN HOSPITAL

COSMIN-RĂDUCU RĂILEANU[1,2], ELENA-ROXANA MATACHE[1,2*], LUCIAN-DANIEL PEPTINE[1,2], LARISA GOROFTEI[1,2], ANDREI NEAGU[1,3], ELENA LĂCRĂMIOARA LISĂ[1], NICOLETA-MARICICA MAFTEI[1,2], CIORTEA DIANA-ANDREEA[1,2], AUREL NECHITA[1,2]

[1]*Faculty of Medicine and Pharmacy, "Dunărea de Jos" University of Galati, Romania;*

[2] *Emergency Clinical Hospital for Children, "St. Ioan", Galati, Romania;*

[3]*Emergency County Hospital, "Sf. Andrei", Galati, Romania;*

*Corresponding author: elena.matache@ugal.ro (Matache Elena-Roxana)

Abstract: This study showed the resistance profile of *Klebsiella pneumoniae* strains which were isolated between January 2018 - November 2021, from various samples at Emergency Clinical Hospital for Children "St. Ioan" in Galati, Romania. Identification of *K. pneumoniae* isolates was achieved by analyzing colony morphology, microscopic examination, and by performing biochemical tests and automatic methods using Vitek 2 system. Testing of antibiotics susceptibility was done by using the disk diffusion method and by determining the minimum inhibitory concentration (MIC). Further, 133 strains were identified, the most of them were isolated from the respiratory samples (74 strains representing 57.31%). Additional, 80 strains (60.15%) were isolated from patients under one year of age, 68 isolates (51.13%) from female patients, 72 isolates (54.14%) from patients living in urban areas. More than 80% of *K. pneumoniae* strains were susceptible to carbapenems, fosfomycin and amikacin while over 70% were susceptible to ciprofloxacin, nitrofurantoin and gentamicin. Over 42% of the isolates were multidrug resistant (MDR). Antimicrobial resistance in *K. pneumoniae* is a real threat that requires vigilant monitorization. This study revealed a low rate of resistance to carbapenems, fosfomycin and amikacin.

Keywords: *Klebsiella pneumoniae*, antimicrobial resistance, extended spectrum beta-lactamase

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Introduction

Klebsiella pneumoniae belongs to the *Enterobacteriaceae* family and was described as a Gram-negative, encapsulated and non-motile bacteria. A wide range of factors ensured the bacterium's virulence, factors that lead to infections and antibiotic resistance (Effah, *et al*., 2020). The most important virulence factor is the polysaccharide capsule of the microorganism that allows the bacteria to avoid opsonophagocytosis and serum killing by the host organism.

K. pneumoniae cause a wide range of infections in humans (mainly urinary and respiratory, but also systemic and digestive). Typically, *K. pneumoniae* colonizes the oropharynx and the gastrointestinal tract in humans, where it can easily spread to the circulatory system and other tissues (Guo, *et al*., 2016). As a result, patients may develop infections such as bacteraemia, septicaemia, surgical site infections, urinary tract infections, hospital acquired pneumonia, and ventilator-associated pneumonia (Podschun & Ullmann, 1998). At patients with immunosuppressed conditions, such as diabetes mellitus or bladder neuropathy, it also contributes to the high prevalence of opportunistic infections.

Antimicrobial resistance (AMR) is a rapidly expanding problem in today's healthcare institutions all over the world (Nirwati, *et al*., 2019). *K. pneumoniae* is rapidly evolving in multidrug resistant (MDR) strains, and these strains frequently pose a major hazard to the patients due to an elevated mortality rate as a result of the diminished efficiency of available therapeutic alternatives. Although it has lately become frequently noted as a major cause of hospital acquired pathogens, *K. pneumoniae* is recognized to be the cause of community acquired infections. By producing enzymes like extended spectrum β-lactamase (ESBL), *K. pneumoniae* strains develop antibiotic resistance more quickly than most of bacteria (Parveen, *et al*., 2011). Antibiotic exposure is the main risk factor for AMR. The intense and prolonged use of antibiotics in the hospital setting is one of the key factors in the emergence and spread of highly resistant bacteria for healthcare linked illnesses.

Thus, the aim of this study was to identify the antibiotic resistance patterns of *K. pneumoniae* strains isolated from various pathological products and to determine the antimicrobial resistance of these strains against the antibiotics prescribed for treatment within the Emergency Clinical Hospital for Children “St. Ioan” in Galati, Romania.

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Materials and methods

Bacterial strains

The study was a retrospective investigation over four years, from January 2018 to November 2021, regarding the isolation of one hundred thirty-three isolates of *K. pneumoniae*, collected from child patients, aged between 0-18 years, hospitalized at the Emergency Clinical Hospital for Children "St. Ioan" in Galati. Among these, 31 strains were isolated from urine samples, 74 isolated from respiratory samples (2 sputum, 61 laryngotracheal secretion, and 11 nasal secretion), 8 strains were obtained from blood and 20 from different samples (7 otic secretion, 5 conjunctival secretions, 5 from pus, 2 gastric juice, 1 pucture fluid).

The isolation of bacteria from these samples was performed according to the classical methodology of microbiological diagnosis. Gram-stained smears were made for the majority of the samples and studied in order to determine the inflammatory character of the products and to observe the morphotinctorial characteristics of the microorganisms (Gurău, *et al*., 2014).

Inoculation on culture media was differentiated, depending on the type of pathological product. Urine was inoculated on Blood agar (Thermo Fisher Scientific, Germany) and Bromothymol blue lactose agar (Sanimed, Romania), using the quantitative urine culture technique to evaluate the number of microorganisms by the "calibrated loop" method (Karah, *et al*., 2020). For blood, the blood culture technique was practiced using the automated rapid microbial detection system BACT/ALERT 3D (bioMérieux, France). Laryngo-tracheal secretions, purulent secretions and sputum were cultured on Blood agar, Chocolate agar (Thermo Fisher Scientific, Germany) and Bromothymol blue lactose agar.

The inoculated media were incubated at 37ºC, for 24-48 h, under aerobic conditions. From the suspicious colonies on the Bromothymol blue lactose agar, inoculations were made on T.S.I medium (Triple Sugar Iron) (Sanimed, Romania), M.I.U medium (Sanimed, Romania) (Mobility, Indol, Urease), M.I.L.F medium (Sanimed, Romania) (Mobility, Indole, Lysine decarboxylase, Phenylalanine deaminase) and on Simmons citrate medium (Sanimed, Romania). The identification of the *Klebsiella* genus was made based on the biochemical characters observed on these media, and the species identification was made with the help of the automatic system Vitek 2 COMPACT 15 (bioMérieux, France).

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Determination of the antimicrobial resistance

The determination of the spectrum of susceptibility to antibiotics and chemotherapeutics was assessed by Kirby-Bauer disk diffusion method on Mueller Hinton agar (Oxoid, UK) (Patel, *et al*., 2011) and the disks were placed manually and using Oxoid antimicrobial susceptibility disc dispenser with the following antimicrobial disks (Oxoid, UK): ampicillin (10 µg), amoxicillin-clavulanate (20/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), amikacin (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tetracycline (30 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), imipenem (10 µg), meropenem (10 µg), piperacillin-tazobactam (100/10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg). Also, with the help of automatic analyzer VITEK 2 COMPACT 15 was determined the production of extended spectrum β-lactamase and susceptibility to antibiotics of bacteria using the antibiogram card, which is designed according to the method of consecutive dilutions and the determination of the minimum inhibitory concentration (MIC). The interpretation of the results regarding the MIC values was done following Clinical and Laboratory Standards Institute (CLSI) guidelines (Ballén, *et al*., 2021).

The results were collected in hospital databases on regularly monitoring of local antibiotic resistance and were statistically processed by Microsoft Excel software 2019.

Results and discussion

Characteristics of clinical samples

A number of 133 of *K. pneumoniae* isolates were examined from January 2018 to November 2021 at Emergency Clinical Hospital for Children “St. Ioan” Galati, Romania. Variables used to analyse the patients were the following: sex, age range, environment and the type of pathological product from which *K. pneumoniae was* isolated (Man, *et al*., 2018). *K. pneumoniae* samples were isolated from 65 male (48.87%) and 68 female (51.13%) patients. Most of *K. pneumoniae* were isolated from patients under one year of age (60.15%) (Table 1). *K. pneumoniae* samples were mostly isolated from respiratory specimens (55.64%) (Sorlozano, *et al*., 2014) (Table 1).

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Table 1. Distribution of patients from whom samples of *K. pneumoniae* was isolated according to the analyzed variables

Characteristics		Total n	%
Sex	Male	65	48.87%
	Female	68	51.13%
Environment	Urban	72	54.14%
	Rural	61	45.86%
Age	<1 year	80	60.15%
	1-4	21	15.79%
	5-9	11	8.27%
	10-14	9	6.77%
	15-18	12	9.02%
Clinical Specimens	Pucture fluid	1	0.75%
	Pus	5	3.76%
	Laryngotracheal secretion	61	45.86%
	Blood	8	6.02%
	Conjunctival secretion	5	3.76%
	Nasal secretion	11	8.27%
	Otic secretion	7	5.26%
	Sputum	2	1.50%
	Gastric juice	2	1.50%
	Urine	31	23.31%

n – total number

Antimicrobial resistance

According to the antimicrobial resistance profile, the strains were considered as susceptible, resistant to one or two antimicrobial categories, multidrug-resistant (MDR), extensively drug-resistant (XDR), or pandrug-resistant (PDR). The definitions for MDR, XDR, and PDR were suggested by the European Centre for Disease Prevention and Control (ECDC) (Magiorakos, *et al*., 2011). An isolate is classified as PDR if it is non-susceptible to all listed antimicrobial agents, XDR if it is non-susceptible to at least one agent in all but two or fewer antimicrobial categories, and MDR if it is non-susceptible to at least one agent in at least three antimicrobial categories.

Overall, 45 isolates (33.83%) were susceptible to all antimicrobial agents tested, 30 isolates (22.56%) were non-susceptible to 1 or 2 categories of antibiotics, 56 isolates (42.11%) were considered multidrug-resistant (MDR), and 2 strains (1.50%) were considered extensively drug-

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resistant (XDR). None was classified as pandrug-resistant. The percentages of resistance for each antibiotic tested are shown in Table 2.

Most of the collected samples positive for extended spectrum β-lactamase producing *K. pneumoniae* was corresponding for children between 5-9 years old (36.36%). The age group of <1 year (16.25%) and that of 15-18 years (16.67%) had relatively close percentages in terms of value. In the other age categories, 1-4 years and 10-14 years we also had a close percentage, 9.52%, respectively 11.11% (Figure 1).

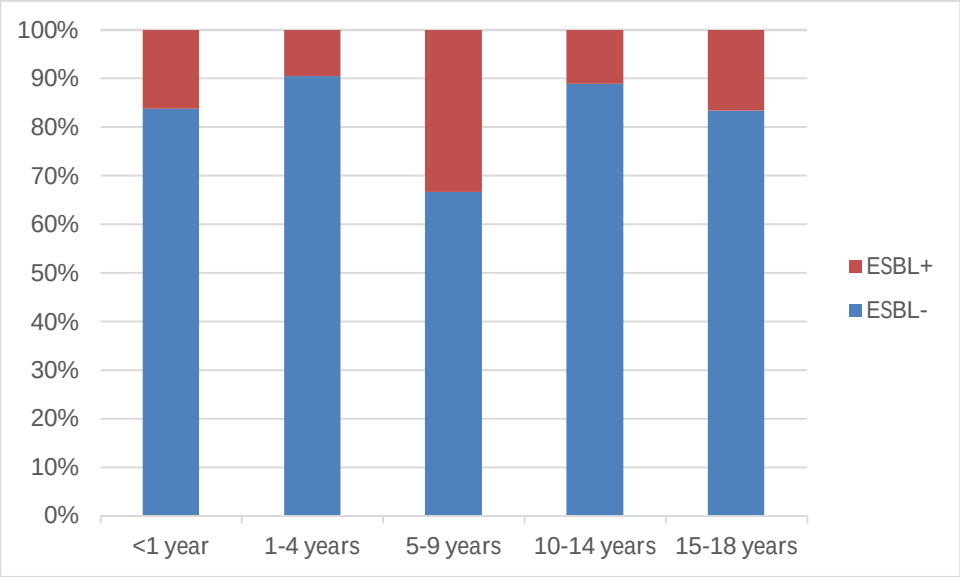

Figure 1. Distribution of extended spectrum β-lactamase producing *K. pneumoniae* strains

Antibiotic susceptibility pattern

Most of *K. pneumoniae were* resistant to a wide range of antibiotics (Gurău, *et al*., 2014). It had a good susceptibility to carbapenems: ertapenem (93.14%), imipenem (96,47%) and meropenem (95.24%). Also, *K. pneumoniae* showed good susceptibility to fosfomycin, amikacin, ciprofloxacin, nitrofurantoin and gentamicin with 87.72%; 81.74%; 78.23%; 76.92%, and 71.31% of susceptibility respectively (Table 2).

A 2020 study with data collected by the European Antibiotic Resistance Surveillance Network (EARS-Net), which was coordinated by the European Center for Disease Prevention and Control (ECDC) (ECDC, 2020) showed that the highest EU/EEA population-weighted mean AMR percentage was reported for third-generation cephalosporins like cefotaxime and ceftazidime

(33.9%), in contradiction with our study because these two cephalosporins had a prevalence higher than 39%.

Table 2. Antibiotic susceptibility pattern of *K. pneumoniae*

No	Antibiotics	Number tested	Susceptible		Intermediate		Resistant	
			n	%	n	%	n	%
1	Amikacin	115	94	81.74%	11	9.57%	10	8.70%
2	Ampicillin-Sulbactam	41	23	56.10%	0	0.00%	18	43.90%
3	Amoxicillin/Clavulanic acid	62	33	53.23%	2	3.23%	27	43.55%
4	Cefaclor	32	14	43.75%	2	6.25%	16	50.00%
5	Cefepime	94	53	56.38%	12	12.77%	29	30.85%
6	Cefotaxime	128	76	59.38%	1	0.78%	51	39.84%
7	Ceftazidime	111	65	58.56%	2	1.80%	44	39.64%
8	Cefuroxime	27	15	55.56%	3	11.11%	9	33.33%
9	Ciprofloxacin	124	97	78.23%	4	3.23%	23	18.55%
10	Trimethoprim-Sulfamethoxazole	117	70	59.83%	0	0.00%	47	40.17%
11	Ertapenem	102	95	93.14%	0	0.00%	7	6.86%
12	Fosfomycin	57	50	87.72%	1	1.75%	6	10.53%
13	Gentamicin	122	87	71.31%	1	0.82%	34	27.87%
14	Imipenem	85	82	96.47%	0	0.00%	3	3.53%
15	Meropenem	126	120	95.24%	1	0.79%	5	3.97%
16	Nitrofurantoin	26	20	76.92%	2	7.69%	4	15.38%
17	Norfloxacin	81	35	43.21%	1	1.23%	45	55.56%
18	Piperacillin-Tazobactam	120	80	66.67%	17	14.17%	23	19.17%

n – total number

Many diseases are brought on by *K. pneumoniae*, which also colonizes and spreads in the human body by using a variety of virulence factors. The scientific community is extremely concerned about the rising antibiotic resistance of this bacteria in recent years (Giubelan, *et al*., 2021).

In this study, a collection of 133 clinical *K. pneumoniae* strains isolated from urine, the respiratory tract, blood and different samples and compared these strains according to antimicrobial resistance was characterized. Most of *K. pneumoniae was* resistant to various antibiotics, with norfloxacin, ampicillin-sulbactam, amoxicillin/clavulanic acid, trimethoprim-sulfamethoxazole and third-generation cephalosporins being the least effective for *K. pneumoniae* while carbapenems, fosfomycin and amikacin had the most favourable profile (Kidd, *et al*., 2017).

The overall proportion of MDR isolates in this study was 42.11%. Because of the MDR pattern that some microorganisms are showing, controlling infections is becoming increasingly difficult (Hennequin & Robin, 2016). As a result, it is crucial to track and manage antibiotic use

through antibiotic stewardship programs. As therapy failures are frequently observed in patients who only receive single antibiotic therapy, numerous studies have demonstrated that treatment with a combination of antibiotics can assist to avoid the establishment of new resistant strains. Lack of knowledge regarding infections and antibiotic use is the main reason why improper antibiotic prescriptions are written (Paterson, 2006). Collaboration between clinicians and microbiologists is crucial to ensuring efficient infection management.

Conclusions

Antimicrobial resistance in *K. pneumoniae* is a real threat that requires vigilant monitorization. Although this research found a low rate of resistance to carbapenems (ertapenem, imipenem, meropenem), fosfomycin and amikacin more cautious attempts should be undertaken to develop a new line of antimicrobials, as resistance to these medications is on the rise. Monitoring and reporting changes in isolates that are resistant to antibiotics is crucial for public health departments. In order to stop the spread of multi-drug resistant germs and eradicate the hospital born microorganisms that are sharply increasing mortality, global efforts should be stepped up.

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