

# Antibiotic resistance profile of *Klebsiella pneumoniae* strains isolated in an infectious diseases clinic

Lucian Giubelan, Livia Dragonu, Andreea Cristina Stoian,  
Florentina Dumitrescu

Infectious Diseases Clinic, University of Medicine and Pharmacy, Craiova, Romania

## ABSTRACT

**Objective.** To establish the resistance profile of *Klebsiella pneumoniae* (KP) strains isolated in the Craiova Infectious Diseases Clinic.

**Material and method.** Retrospective study (January 2017-December 2018); KPs were identified using the automated Vitek 2 system, which subsequently established their susceptibility to antimicrobials (usual testing for 17 antibiotics, extended testing for another 9); for each strain the multiple antibiotic resistance index (MAR) was calculated (limits: 0-1); the information was entered into an Excel database. 45 strains were tested in 2017 and 290 in 2018. The percentage of MDR strains was calculated based on internationally accepted definitions.

**Results.** 335 strains were identified, the vast majority isolated by sputum culture (192 strains - 57.31%). Demographic data: 330 strains (99%) were isolated in adult subjects, 190 (57%) in male patients, 185 (55%) in patients living in urban areas. The overall value of MAR was 0.37 (compared to 0.32 for all strains of isolated Gram-negative germs). Over 80% of KP strains were susceptible to Amikacin or Polymyxin E; susceptibilities between 60 and 79% were observed for Meropenem, Gentamicin, Tobramycin, Ciprofloxacin, Ertapenem, Trimethoprim-Sulfamethoxazole, Levofloxacin, Cefepima, and between 40 and 59% for Ceftriaxone, Cefoxitin, Ceftazidime, Minocycline and Im. Less than 39% of strains are susceptible to Ampicillin ( $\pm$  Sulbactam), Piperacillin ( $\pm$  Tazobactam), Ticarcillin ( $\pm$  Clavulanic acid), Aztreonam, Pefloxacin, Cefazolin, Nitrofurantoin. Resistance to the main classes of antibiotics shows lower values than national and european data for 3rd generation cephalosporins, fluoroquinolones and aminoglycosides, but a much higher percentage of resistance to carbapenems. Over 68% of isolates are multidrug-resistant (MDR); over 60% of the strains come from sources where there is a significant contact with antimicrobials.

**Conclusions.** Over 80% of the isolated strains are sensitive to Amikacin or Polymyxin E; a percentage of over 29% of the strains demonstrates resistance to the carbapenem class in 2018; over 68% of isolates are multidrug-resistant; over 60% of the strains come from sources where there is significant contact with antimicrobials.

**Keywords:** *Klebsiella pneumoniae*, antimicrobial resistance, MAR

## BACKGROUND

*Klebsiella pneumoniae* (KP) is a Gram-negative bacillus (GNB) important in human pathology, generating a wide range of infections (mainly urinary and respiratory, but also systemic, digestive or meningial infections).

Antimicrobial resistance is a major global public health problem, recognized as such by the World Health Organization (WHO) [1], which published in

2017 a list of 12 bacteria that urgently need new antimicrobials development; among these, priority 1 (critical need) is *Enterobacteriaceae* (of which KP is a part) producing broad spectrum beta-lactamases and, in particular, carbapenemases [2]. The latest report of the European Center for Disease Prevention and Control (ECDC) [3], but also the data from our country (see the CARMIN-ROM study [4]) show worrying data regarding the KP resistance to antibi-

Corresponding author:

Lucian Giubelan

E-mail: ligiubelan@yahoo.com

Article History:

Received: 28 December 2020

Accepted: 6 January 2021

otics. The bacillus has been a “problem” germ since 1971, when a number of cases have been described with Gentamicin-resistant strains [5], after that, in the 90s, cephalosporin-resistant strains became an impediment to the treatment of patients [6], and now the spread of beta-lactamase-producing strains take on the character of a true epidemic [7-9]. It can be said that KP is one of the most important bacteria involved in nosocomial infections.

It is important for clinicians to know the general antibiotic resistance of KP strains in the area in which they operate, for a rapid and appropriate therapeutic response.

## OBJECTIVE

Establishing the resistance profile of the *Klebsiella pneumoniae* (KP) strains isolated in the Infectious Diseases Clinic from Craiova (within the “Victor Babeş” Infectious Diseases and Pneumoftiziologie Hospital).

## MATERIAL AND METHOD

Retrospective study (January 2017-December 2018) based on data from the hospital laboratory register; GNB and, in particular, KP were identified using the Vitek 2 automated system, which subsequently established their susceptibility to antimicrobials; most strains (317, 94.62%) were tested to 17 antibiotics, the exact situation being shown in Figure 1; for each

strain the multiple antibiotic resistance index (MAR) was calculated (limits: 0–1). 45 strains were tested in 2017 and 290 in 2018. An Excel database was compiled for analysis.

## RESULTS

335 strains were identified (out of a total of 1,358 GNB strains, KP being the second most important bacteria in this group (24.66%), after *Escherichia coli*).

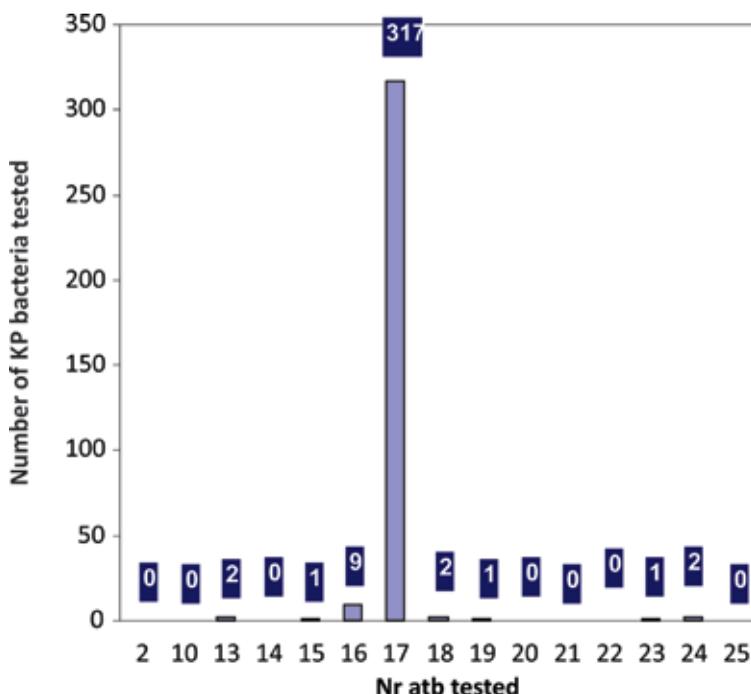
### Demographic data

The median age of the patients was 64 years (with limits between <1 year - 88 years); 330 strains (98.50%) were isolated in adult subjects, 190 (56.71%) in male patients, 185 (55.22%) in patients living in urban areas.

**TABLE 1.** Samples and corresponding number of KP isolates

Sample	No. of isolates
Sputum	192
Urine culture	123
Blood culture	6
Wound pus	6
Vaginal discharge	5
Bronchial aspirate	3

The vast majority of KP were isolated from sputum (192 strains, 57.31%) or urine culture (123 strains - 36.71%). An exact situation of the patho-



**FIGURE 1.** Distribution of strains based on the number of antimicrobials for which KP have been tested

logical products from which the bacillus was isolated is presented in Table 1. It should be noted that, in pediatric patients, KP was only isolated from urine samples.

The KP resistance profile for a particular antibiotic test is shown in Table 2.

Over 80% of KP strains were susceptible to Amikacin and Polymyxin E; Susceptibilities between 60% and 79% were observed for Meropenem, Gentamicin, Tobramycin, Ciprofloxacin, Ertapenem, Trimethoprim-Sulfamethoxazole, Levofloxacin, Cefepime, and between 40% and 59% for Ceftriaxone, Cefoxitin, Ceftazidime, Minocycline and Imipenem-cilastatin. Less than 39% of the strains are susceptible to Ampicillin ( $\pm$  Sulbactam), Piperacillin ( $\pm$  Tazobactam), Ticarcillin ( $\pm$  Clavulanate), Aztreonam, Pefloxacin, Cefazolin, Nitrofurantoin. Figure 2 graphically shows the susceptibility profile of KP strains.

The overall MAR value was 0.37 (compared to 0.32 for all strains of isolated Gram-negative germs). Figure 3 graphically shows the distribution of KP strains according to the values of MAR fractions.

Table 3 shows the variation of the KP resistant strains percentage to different classes of antibiotics in two consecutive years (2017 and 2018).

**TABLE 3.** Percentage of KP strains resistant to different classes of antimicrobials, 2017 vs. 2018

	AminoP	CEF 3	FQ	AG	CarbaP
	2017				
<b>total KP</b>	45	45	45	45	45
<b>r</b>	45	27	13	15	17
<b>%</b>	100	60	28.89	33.33	37.78
	2018				
<b>total KP</b>	290	290	290	290	290
<b>r</b>	289	134	88	78	85
<b>%</b>	99.66	46.21	30.34	26.90	29.31

AminoP = aminopenicillins, CEF 3 = 3rd generation cephalosporins, FQ = fluoroquinolones, AG = aminoglycosides, CarbaP = carbapenems, EC = *Escherichia coli*, r = (no. of strains) resistant (to antimicrobials)

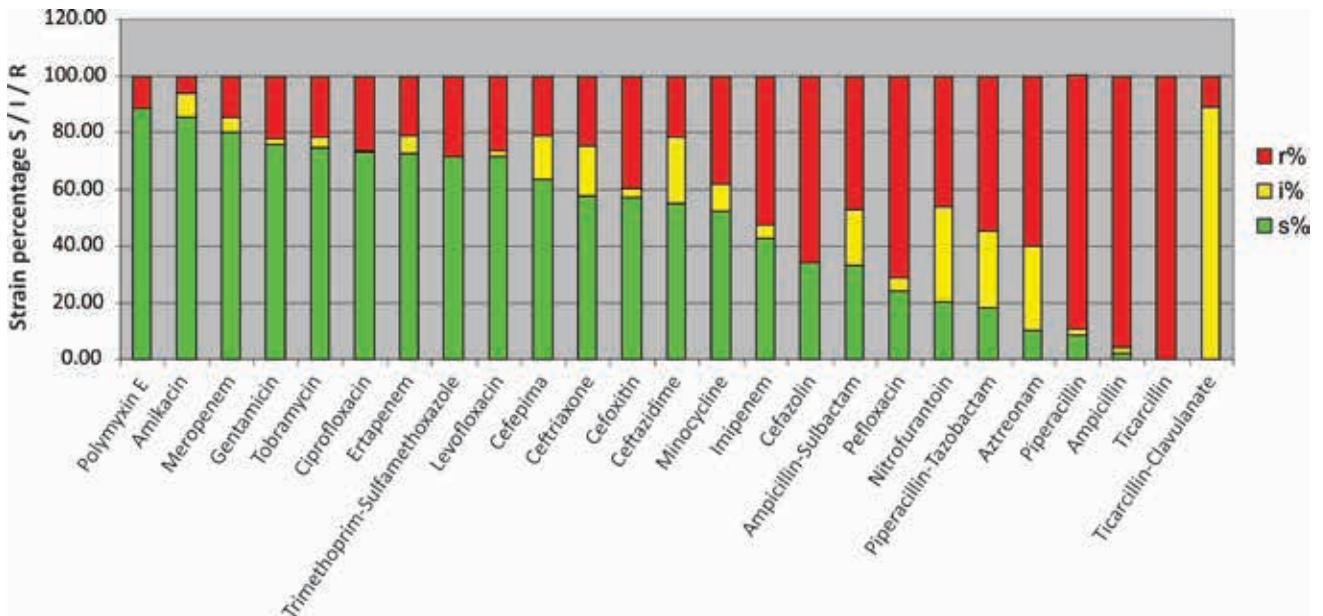
## DISCUSSIONS

The resistance to aminopenicillin of KP strains in our clinic was on average 99.7% (100% in 2017 and 99.66% in 2018); resistance to this class of antimicrobials is practically no longer mentioned either by

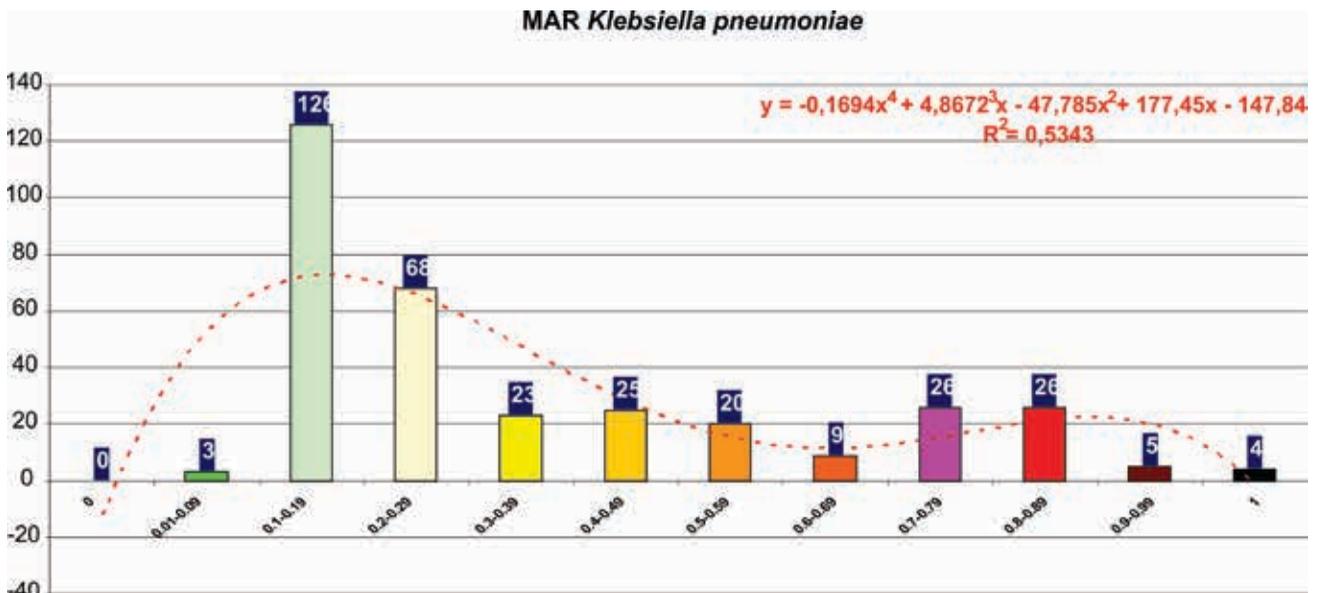
**TABLE 2.** Resistance profile of KP strains according to the tested antibiotic

Antibiotic	s	i	r	s%	i%	r%
Amikacin	286	29	20	85.37	8.66	5.97
Ampicillin	6	8	302	1.90	2.53	95.57
Ampicillin-Sulbactam	103	62	148	32.91	19.81	47.28
Aztreonam*	2	6	12	10.00	30.00	60.00
Cefazolin	108	0	208	34.18	0.00	65.82
Cefepime	210	52	70	63.25	15.66	21.08
Cefoxitin	178	10	125	56.87	3.19	39.94
Ceftazidime	183	77	72	55.12	23.19	21.69
Ceftriaxone	182	56	79	57.41	17.67	24.92
Ciprofloxacin	244	2	89	72.84	0.60	26.57
Ertapenem	227	21	66	72.29	6.69	21.02
Gentamicin	254	7	74	75.82	2.09	22.09
Imipenem-cilastatin*	9	1	11	42.86	4.76	52.38
Levofloxacin	239	7	88	71.56	2.10	26.35
Meropenem	267	18	49	79.94	5.39	14.67
Minocycline*	11	2	8	52.38	9.52	38.10
Nitrofurantoin	63	105	145	20.13	33.55	46.33
Pefloxacin*	5	1	15	23.81	4.76	71.43
Piperacillin	29	6	298	8.71	1.80	89.49
Piperacillin-Tazobactam*	4	6	12	18.18	27.27	54.55
Polymyxin E*	23	0	3	88.46	0.00	11.54
Ticarcillin*	0	0	20	0.00	0.00	100.00
Ticarcillin-Clavulanate*	0	8	1	0.00	88.89	11.11
Tobramicin	250	13	72	74.63	3.88	21.49
Trimethoprim-Sulphamethoxazole	238	0	94	71.69	0.00	28.31

s = susceptible, i = dose-dependent susceptibility, r = resistant; \* = extended testing



**FIGURE 2.** Antibiotic susceptibility profile of isolated KP strains. Legend: S = susceptible (green) I = dose-dependent (yellow), R = resistant strain (red)



**FIGURE 3.** Distribution of EC strains according to MAR fraction values

the ECDC report, or by the CARMIN-ROM study [3,4]. Most likely, we will give up this type of testing in the Craiova clinic as well.

Resistance to 3rd generation cephalosporins in the EU averaged 31.2-31.7% in the EU (2017 and 2018); in 2018 Romania was on the 4th place at European level, with an average percentage value of 61.4%, slightly below the value registered in 2017 (62.5% - ECDC [3], respectively 63.4% – CARMIN [4]). Our data show a resistance of 60% in 2017, respectively 46.21% in 2018 (significantly lower than the values presented previously).

Fluoroquinolone resistance of KP isolates in Romania is 1.5-2 times above the European average values (31.5-31.6% in 2017 and 2018 [3]), being 64.1% in 2017 (66.5% according to CARMIN-ROM [4]), slightly decreasing in 2018, to the percentage value of 57.4. In our clinic, however, the situation is much different, the resistance to Ciprofloxacin and Levofloxacin being on average 30.15% (28.89 in 2017, respectively 30.34% in 2018, when a significantly higher number of strains were tested).

Regarding the resistance to aminoglycosides, the European average level was 22.7-24.1% in two con-

secutive years, 2017 and 2018, Romania occupying the 5th place with a percentage value of 58.6 in 2017, decreasing to 50.9 for 2018; the percentage in 2017 is also confirmed by the Romanian multicenter study [3,4]. The data obtained by us show a percentage value of 33.3% in 2017, decreasing to 26.9% in 2018 (but based on a number of tests over 6 times higher), regional values much different from those recorded at the national or european level. The lowest KP resistance was recorded for Amikacin (almost 4 times lower than for Gentamicin or Tobramycin). A similar situation was recorded for *Escherichia coli* strains [10].

For 2018, the percentage of KP resistance to carbapenems in the EU is 7.5%, slightly increasing compared to 2017 – 7.2% [3]; Romania ranks 3rd, with a percentage almost 4 times higher than European data (29.5% in 2018), also increasing compared to 2017 – 22.5%. The CARMIN-ROM study confirms the data for 2017, identifying a percentage of 22.5% carbapenem-resistant KP strains [4]. Our data show, similar to what we found for EC strains [10], a percentage of resistance to Meropenem, Ertapenem or both of 37.79 in 2017, respectively 29.31 in 2018. Due to the small number of strains tested not we considered the data for Imipenem-Cilastatin.

Polymyxin-resistant strains have been described, mostly in systemic infections or in special hosts, with a significant impact on case mortality [11-14]. The CARMIN-ROM study identified 55.8 percent Colistin-resistant KP strains among the already carbapen-

*Conflict of interest:* none declared

*Financial support:* none declared

## REFERENCES

1. WHO. 2015 Global action plan on antimicrobial resistance, available at: <https://www.who.int/antimicrobial-resistance/publications/global-action-plan/en/>.
2. WHO. 2017 WHO publishes list of bacteria for which new antibiotics are urgently needed, available at: <https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed>.
3. ECDC. 2019 Surveillance of antimicrobial resistance in Europe 2018, available at: <https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2018.pdf>.
4. Popescu GA., Șerban R., Niculcea A. 2019 CARMIN-ROM 2017 (Consumul de antibiotice, rezistența microbiană și infecțiile asociate asistenței medicale (nosocomiale) în România – 2017):44-49. Available at: <https://www.cnscbt.ro/index.php/analiza-date-supraveghere/infectii-nosocomiale-1/1309-consumul-de-antibiotice-rezistența-microbiană-si-infectii-asociate-asistenței-medicele-nosocomiale-in-romania-2017/file>.
5. Martin CM., Ikari NS., Zimmerman J., Naitz JA. A virulent nosocomial *Klebsiella* with a transferable R factor for Gentamicin: emergence and suppression, *J Infect Dis.* 1971;124(suppl):s24-s29.
6. Pena C, Pujol M, Ardanuy C et al. Epidemiology and successful control of a large outbreak due to *Klebsiella pneumoniae* producing extended-spectrum betalactamases. *Antimicrob Agents Chemother.* 1998;42(1):53-58.
7. David S, Reuter S, Harris SR et al. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nat Microbiol.* 2019;4:1919-1929.
8. Jin C, Shi R, Jiang X et al. Epidemic characteristics of carbapenem-resistant *Klebsiella pneumoniae* in the pediatric intensive care unit of Yanbian University Hospital, China. *Infect Drug Resist.* 2020; 13:1439-1446.
9. Rojas LJ, Weinstack GM, De La Cadena E et al. An analysis of the epidemic of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*: convergence of two evolutionary mechanisms creates the "perfect storm". *J Infect Dis.* 2018; 217(1):82-92.
10. Giubelan L, Diaconescu I, Dragonu L et al. Profilul de rezistență la antimicrobiene al tulpinilor de *Escherichia coli* într-o clinică de boli infecțioase. *Ro J Infect Dis.* 2020;23(3):224-228.
11. Capone A, Gianella M, Fortini D et al. High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. *Clin Microbiol Inf.* 2013;19(1):e23-e30.

em-resistant isolates; In contrast, of the carbapenem-sensitive strains, only 5 of 104 strains (4.8%) also demonstrated resistance to polymyxins [4]. In our clinic, three strains were resistant to Colistin (0.89% of the total strains), out of a number of 23 isolates tested.

According to the definition criteria for multidrug resistance (MDR) and extended antimicrobial resistance (XDR) [15] we identified 68.86% of KP strains as MDR, but we did not register any XDR strains.

Based on Krumperman's criteria [16], we identified 206 KP strains (61.49%) with a MAR index of over 0.2, suggesting that most of the analyzed strains came from sources with significant exposure to antibiotics (most likely nosocomial).

## CONCLUSIONS

Over 80% of the isolated strains are susceptible to Amikacin or Polymyxin E; compared to the national or european data (with reference to our country), the data from the Infectious Diseases Clinic from Craiova show lower percentages of resistance to the class of 3rd generation cephalosporins, fluoroquinolones or aminoglycosides, but much higher for carbapenems; a percentage of over 29% of the strains demonstrates resistance to the carbapenem class in 2018; over 68% of isolates are multidrug-resistant; over 60% of the strains come from sources where there is significant contact with antimicrobials.

12. Emergence of colistin resistance in multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli* strains isolates from cancer patients. *Ann Clin Microbiol Antimicrob.* 2019;18:40.
13. Menekese S, Cag Y, Isik ME et al. The effect of colistin resistance and other predictors on fatality among patients with bloodstream infection due to *Klebsiella pneumoniae* in an OXA-48 dominant region. *Int J Inf Dis.* 2019;86:208-211.
14. El-Sayed Ahmed MAEG, Zhang LL, Shen C et al. Colistin and its role in the era of antibiotic resistance: an extended review (2000-2019). *Emerg Microbes Infect.* 2020;9(1):868-885.
15. Magiorakos AP, Srinivasan A, Carey RB et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012;18:268-281.
16. Krupperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl Environ Microbiol.* 1983;46(1):165-170.