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Article

In vitro Activity of "last chance" Antimicrobials against *Klebsiella pneumoniae* Complex

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Abstract: *Klebsiella pneumoniae* complex is one of the most commonly isolated bacteria within human infections. These microorganisms are opportunistic pathogens that pose a serious threat to public health due to possibility of transmission in the human population. Resistance to carbapenems is one of the most important antimicrobials resistance mechanisms amongst them. In this situation therapeutic options are limited. Therefore, the aim of this study was to evaluate fosfomycin, colistin, ceftazidime-avibactam and meropenem-vaborbactam *in vitro* activity against multidrug-resistant *K. pneumoniae* complex strains. The study involved 160 strains of Gram-negative rods: *K. pneumoniae* – 138 and *K. variicola* - 22. Minimal inhibitory concentration of fosfomycin was estimated by agar dilution method and for colistin by microdilution method. The susceptibility to ceftazidime-avibactam and meropenem-vaborbactam was determined by gradient strip method. All of the analysed *K. pneumoniae* complex isolates produced extended-spectrum beta-lactamases and 60.0% carbapenemases. The most of the analysed strains were susceptible to fosfomycin and colistin (62.5%). Among pandrug-resistant *K. pneumoniae* complex isolates the most were susceptible to colistin (43.9%). Fosfomycin has good activity against ESBL- and VIM-positive isolates from this complex. In turn, colistin has satisfactory *in vitro* activity against KPC- and VIM-positive isolates from *K. pneumoniae* complex. Ceftazidime-avibactam has good activity against *K. pneumoniae* complex strains producing ESBL, KPC and OXA enzymes. In turn, meropenem-vaborbactam has satisfactory *in vitro* activity against ESBL- and KPC-positive isolates from this complex.

Keywords: ceftazidime-avibactam; colistin; fosfomycin; *K. pneumoniae; K. variicola*; meropenem-vaborbactam

1. Introduction

Klebsiella pneumoniae complex is one of the common bacteria causing community- and hospital-acquired infections. The most important species from this complex is *K. pneumoniae*, but in the last years the other species are more often isolated from this complex, like *Klebsiella variicola*. Both species cause severe infections with high mortality in immunocompromised patients [1,2]. Additionally, these bacteria often produce various mechanisms of resistance to antimicrobials. For many years the most common and important mechanism of antimicrobial resistance in *K. pneumoniae* complex was the production of extended-spectrum beta-lactamases [ESBLs], but in the last ten years carbapenemase-producing strains have been increasingly identified [3]. Strains producing ESBLs and carbapenemases can colonize gastrointestinal tract of healthy people and then cause serious infections. *K. pneumoniae* isolates can produce three classes of carbapenemases; class A (*Klebsiella pneumoniae* carbapenemases, KPCs), class B (metallo-beta-lactamases, MBLs) and class D (oxacillinases, OXA) [4]. Strains producing KPC enzymes are frequently isolated in the United States and Israel [5]. In Poland the first KPC-positive *K. pneumoniae* strain was isolated in 2008 and in 2021 KPC accounted for 18%, an increase of about 200% compared to 2019 [6,7]. In Poland the first MBL-positive *K. pneumoniae* strain was isolated in 2006 and it was VIM-1 enzyme [8]. Since the isolation of

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the first strain in Poland, VIM enzymes have been identified in various species, but the two dominant ones were *Enterobacter hormaechei* and *Klebsiella oxytoca*. Currently, the situation has changed and in recent years there is an increase in the frequency of isolation of *K. pneumoniae* and *Escherichia coli* strains [7]. VIM-positive strains were the only carbapenemase-producing isolates for which no increase was recorded in Poland in 2020-2021. The first NDM-positive strain appeared in Poland in 2012 and quickly spread to other hospitals, causing local epidemics. In 2021, NDM-positive strains accounted for 73% of all carbapenemase-producing Enterobacterales strains in Poland, and compared to 2019, an increase of 99% was observed [7]. In Poland the first OXA-48-positive isolate was *E. hormaechei* subsp. *steigerwaltii* identified in 2012. OXA-48-positive strains were relatively rarely isolated in Poland until 2017, but in 2021, an increase in the frequency of isolation of strains with this phenotype by approximately 70% was observed compared to 2019. OXA-48 like is a group of carbapenemases, which includes: OXA-48, OXA-181, OXA-232, OXA-204, OXA-162 and OXA-244 [9]. Although carbapenemases can be produced by all Enterobacterales, the main species in which they are identified is still *K. pneumoniae*. According to the data of the European Antimicrobial Resistance Surveillance System, in Poland the share of *K. pneumoniae* strains resistant to carbapenems isolated from invasive infections increased from 0.8% in 2012 to 6.4% in 2017 and to 16.8% in 2022 [3]. The prevalence of carbapenem-resistant *K. pneumoniae* strains in Europe varies. According to EARSS data, the percentage of these strains isolated from invasive infections in 2022 ranges from 0.0% in Finland and Iceland to 72.0% in Greece [3]. Additionally, strains producing more than one beta-lactamase from different groups are increasingly being identified, including: ESBLs and carbapenemases or two different carbapenemases. In this situation, therapeutic options are very limited. There is not much information about resistance to antimicrobials of *K. variicola* strains and usually they are case reports. Fosfomycin, colistin, ceftazidime-avibactam and meropenem-vaborbactam have registration for severe infections caused by strains with limited therapeutic options.

Therefore, the purpose of the study was to compare the *in vitro* activity of fosfomycin, colistin, ceftazidime-avibactam and meropenem-vaborbactam against multidrug-resistant (MDR) *K. pneumoniae* complex, including isolates producing ESBLs and various carbapenemases.

2. Materials and Methods

2.1. Isolates Collection

The study included 160 *K. pneumoniae* complex non-replicated strains isolated from different clinical samples derived from the patients of the Dr. A. Jurasz University Hospital No. 1 in Bydgoszcz. *K. pneumoniae* complex were isolated from specimens collected from 146 cases of infections and from 14 cases of gastrointestinal colonization (stool and rectal swab). The collected strains were obtained during a standard diagnostic procedures at the Microbiology Department of Dr. A. Jurasz University Hospital No. 1 in Bydgoszcz, Poland.

2.2. Identification of Strains and Antimicrobial Susceptibility Testing

The isolates were identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) using MALDI TOF Biotyper Microflex LT/SH system (Bruker, Germany) with version 7.0.0.1 software. For strains with value ≥ 2.300 identification was done one time. For isolates with value between 2.000 and 2.299 identification was done three times.

Antimicrobial susceptibility tests for all the analysed strains were performed in Phoenix M50 system (Becton Dickinson, USA) using NMIC-408 panels. The obtained results were automatically interpreted according to EUCAST 2023 Recommendations (v 12.0) [10].

2.3. Detection of Extended-Spectrum Beta-Lactamases and Carbapenemases

For *K. pneumoniae* strains the ESBLs enzymes were detected *via* the Phoenix M50 System (Becton-Dickinson, USA) with the NMIC-408 panels. For *K. variicola* isolates ESBLs enzymes activities were detected by the disc diffusion method – double disc synergy test with ceftazidime (30 μg), cefotaxime (30 μg), cefepim (30 μg) and amoxicillin with clavulanic acid (30 μg) (Liofilchem, Italy). *E. coli* ATCC

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25922 (ESBL-negative) and *K. pneumoniae* ATCC 700603 (ESBL-positive) were used as the control strains. For strains resistant to carbapenems Carbapenem Inactivation Method [11] and eazyplex® SuperBug CRE test (Amplex Diagnostics, Germany) were used. *K. pneumoniae* NCTC 13442 (OXA-positive), *K. pneumoniae* NCTC 13438 (KPC-positive), *K. pneumoniae* BAA-2146 (NDM-positive), *K. pneumoniae* NCTC 13440 (VIM-positive) and *K. pneumoniae* ATCC 700603 (ESBL-positive) were used as reference strains.

2.4. MIC Determination

Minimal inhibitory concentration (MIC) of fosfomycin was determined by dilution in agar using AD Fosfomycin (Liofilchem, Italy). Fosfomycin MICs ranged from 0.25 mg/L to 256 mg/L. Minimal inhibitory concentration (MIC) of colistin was determined by microdilution method using MIC COL test (Inc. Diagnostics, Slovakia). Colistin MICs ranged from 0.25 mg/L to 16 mg/L. Susceptibility breakpoints for ceftazidime-avibactam and meropenem-vaborbactam were determined by gradient strip method (Liofilchem, Italy). Ceftazidime-avibactam and meropenem-vaborbactam MICs ranged from 0.016 mg/L to 256 mg/L. MIC values for all antimicrobials were interpreted according to the EUCAST recommendations (EUCAST). *E. coli* ATCC 25922 and *E. coli* NCTC 13846 were used as reference strains.

3. Results

3.1. Isolates Collection

The study included 160 *K. pneumoniae* complex strains: 138 (86.2%) *K. pneumoniae* and 22 (13.8%) *K. variicola*. One houndred and forty six (91.2%) *K. pneumoniae* complex strains were isolated from clinical specimens (125 *K. pneumoniae* and 21 *K. variicola*) and 14 (8.8%) from gastrointestinal colonization (13 *K. pneumoniae* and 1 *K. variicola*). Seventy two (45.0%) of analysed strains were isolated as monoculture (63 *K. pneumoniae* and 9 *K. variicola*).

3.2. Identification of Strains and Antimicrobial Susceptibility Testing

In the mass spectrometry method, the obtained identification value for all *K. pneumoniae* complex strains was over 2.300 which means reliable identification to the genus and species level.

All of the analysed *K. pneumoniae* complex strains were multidrug-resistant. Among analysed isolates 41 (25.6%) were pandrug-resistant (PDR), and all belonged to *K. pneumoniae* species. In the group of PDR strains 39 (95.1%) produced different carbapenemases and 2 (4.9%) only ESBLs enzymes. The detailed data of the susceptibility *K. pneumoniae* complex strains to antimicrobials are presented in Supplementary Material Table S1.

3.3. Detection of Extended-Spectrum Beta-Lactamases and Carbapenemases

In the automated method for all *K. pneumoniae* strains (n = 138) and in double disc test for all *K. variicola* (n = 22) strains ESBLs production was confirmed. In the eazyplex® SuperBug CRE test 140 of analysed isolates produced ESBLs, 139 strains produced ESBLs from CTX-M1 group and 1 strain produced two ESBLs from groups CTX-M1 and CTX-M9. For 115 of analysed isolates (113 *K. pneumoniae* and 2 *K. variicola*) resistant to minimum one carbapenem CIM test was performed. Ninety six (60.0%) of *K. pneumoniae* strains gave positive result in CIM test and produced carbapenemases: NDM - 40 strains, KPC – 17, VIM – 15, OXA-181 – 7, OXA-48 - 2, strains producing more than one carbapenemase (multi-carbapenemase strains) – 15. None of the *K. variicola* isolates produced carbapenemases. The detailed data of the *K. pneumoniae* complex strains producing beta-lactamases are presented in Table 1.

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Table 1. Beta-lactamases produced by *K. pneumoniae* complex strains (*n* = 160).

Enzymes Producing by *Klebsiella pneumoniae* Complex Strains (*n* = 160)
OXA-48-, CTX-M1-positive *K. pneumoniae* strains (*n* = 2)
OXA-181-, CTX-M1-positive *K. pneumoniae* strains (*n* = 7)
KPC-, CTX-M1-positive *K. pneumoniae* strains (*n* = 12)
KPC-positive *K. pneumoniae* strains (*n* = 5)
NDM-, CTX-M1-positive *K. pneumoniae* strains (*n* = 33)
NDM-positive *K. pneumoniae* strains (*n* = 7)
VIM-, CTX-M1 positive *K. pneumoniae* strains (*n* = 7)
VIM-positive *K. pneumoniae* strains (*n* = 8)
NDM-, OXA-181-, CTX-M1-positive *K. pneumoniae* strains (*n* = 13)
NDM-, OXA-181-, CTX-M1-, CTX-M9-positive *K. pneumoniae* strains (*n* = 1)
VIM-, NDM-, CTX-M1-positive *K. pneumoniae* strains (*n* = 1)
ESBL-positive *K. pneumoniae* strains (*n* = 42)
ESBL-positive *K. variicola* strains (*n* = 22)

3.4. MIC Determination

Among 160 *K. pneumoniae* complex strains, 100 (62.5%) were susceptible to fosfomycin and to colistin, 99 (58.1%) to ceftazidime-avibactam and 88 (55.0%) to meropenem-vaborbactam. The detailed susceptibility profiles for the particular groups of the analysed *K. pneumoniae* complex strains are presented in Figure 1.

In the group of the pandrug-resistant *K. pneumoniae* isolates (41 isolates) the most were susceptible to colistin 18 (43.9%), next to fosfomycin 10 (24.4%) and ceftazidime-avibactam 10 (24.4%) and then meropenem-vaborbactam 6 (14.6%).

Among the carbapenemase-positive strains (96 isolates) the most were susceptible to colistin 60 (62.5%), next to fosfomycin 47 (49.0%), then 35 (36.5%) to ceftazidime-avibactam and 29 (30.2%) to meropenem-vaborbactam.

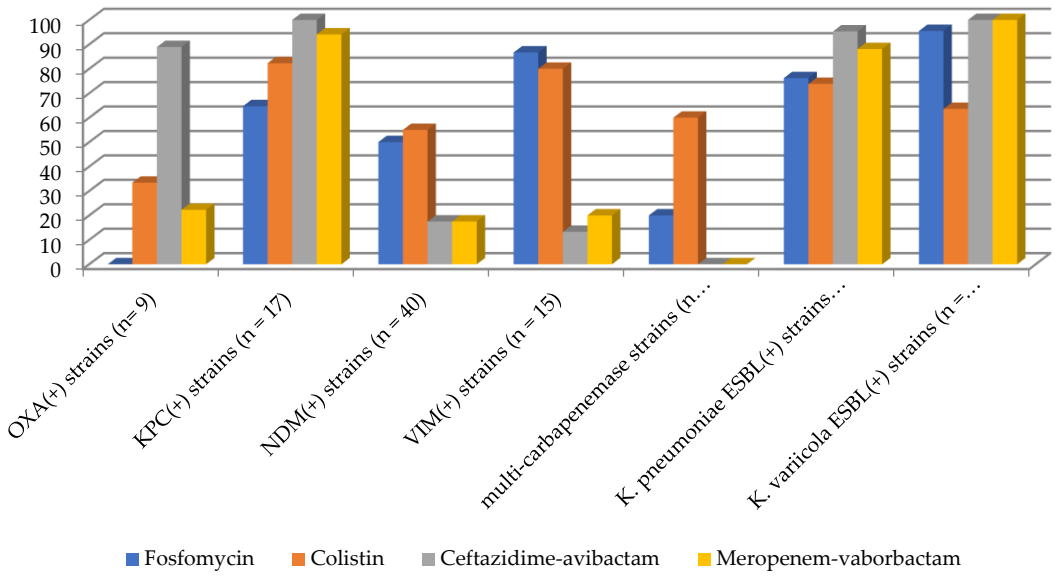

Figure 1. Susceptibility to selected antimicrobials of *K. pneumoniae* complex (*n* = 160).

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The lowest MIC_{50} and MIC_{90} values were observed for *K. variicola* ESBL-positive strains for all of antimicrobials tested. The exception was MIC_{50} for colistin. It had the highest value from all of the analysed groups. The detailed data about MIC_{50} and MIC_{90} values were presented in Table 2.

Table 2. MIC_{50} and MIC_{90} (mg/L) values for selected antimicrobials.

Antimicrobial	*K. pneumoniae* Carbapenemase-Positive Strains (*n* = 96)		*K. pneumoniae* ESBL-Positive Strains (*n* = 42)		*K. variicola* ESBL-Positive Strains (*n* = 22)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Fosfomycin	24	48	12	32	4	16
Colistin	0.75	8	0.5	4	1.5	4
Ceftazidime-avibactam	16	128	0.75	2	0.5	0.5
Meropenem-vaborbactam	24	128	0.25	1.5	0.25	0.5

Fosfomycin, colistin, ceftazidime-avibactam and meropenem-vaborbactam MICs distribution for the particular groups of strains are shown in Tables 3, 4 and 5.

The MICs of fosfomycin ranged from 4 to >256 mg/L for PDR, carbapenemase-positive and ESBLs-positive *K. pneumoniae* isolates and from 4 to 128 mg/L for *K. variicola* ESBLs-positive isolates.

Table 3. MIC distribution for fosfomycin.

MIC Value (mg/L)	*K. pneumoniae* Carbapenemase-Positive strains (*n* = 96)	*K. pneumoniae* ESBL-Positive Strains (*n* = 42)	*K. variicola* ESBL-Positive Strains (*n* = 22)
4	**3**	**4**	**5**
8	**5**	**6**	**4**
16	**16**	**12**	**6**
32	**24**	**10**	**6**
48	1	0	0
64	17	2	0
128	18	4	1
256	7	1	0
>256	5	3	0

Bold: susceptible according to EUCAST breakpoints.

The MICs of colistin ranged from 0.25 to >16 mg/L for PDR and carbapenemase-positive *K. pneumoniae* isolates, from 0.25 to 16 mg/L for ESBLs-positive *K. pneumoniae* isolates and from 0.25 to 8 mg/L for *K. variicola* ESBLs-positive isolates.

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Table 4. MIC distribution for colistin.

MIC Value (mg/L)	*K. pneumoniae* Carbapenemase-Positive Strains (*n* = 96)	*K. pneumoniae* ESBL-Positive Strains (*n* = 42)	*K. variicola* ESBL-Positive Strains (*n* = 22)
0.25	**2**	**1**	**4**
0.5	**29**	**20**	**5**
1	**25**	**8**	**2**
2	**3**	**2**	**3**
8	11	6	8
16	13	5	0
>16	12	0	0

Bold: susceptible according to EUCAST breakpoints.

The MICs of ceftazidime-avibactam ranged from 0.25 to 256 mg/L for PDR isolates, from 0.38 to 256 mg/L for carbapenemase-positive *K. pneumoniae* isolates, from 0.047 to 64 mg/L for ESBLs-positive *K. pneumoniae* isolates and from 0.047 to 2 mg/L for *K. variicola* ESBLs-positive isolates. The MICs of meropenem-vaborbactam ranged from 0.064 to 256 mg/L for PDR strains, from 0.032 to >256 mg/L for carbapenemase-positive strains, from 0.032 to >256 mg/L for ESBLs-positive *K. pneumoniae* strains and from 0.032 to 2 mg/L for *K. variicola* ESBLs-positive strains.

Table 5. MICs distribution for ceftazidime-avibactam and meropenem-vaborbactam.

MIC Value (mg/L)	*K. pneumoniae* Carbapenemase-Positive Strains (*n* = 96)		*K. pneumoniae* ESBL-positive Strains (*n* = 42)		*K. variicola* ESBL-Positive Strains (*n* = 22)	
	CZA	MV	CZA	MV	CZA	MV
0.032	**0**	**1**	**0**	**1**	**0**	**1**
0.047	**0**	**1**	**1**	**2**	**2**	**0**
0.064	**0**	**1**	**0**	**1**	**1**	**2**
0.094	**0**	**1**	**0**	**1**	**0**	**3**
0.125	**0**	**0**	**0**	**6**	**0**	**0**
0.19	**0**	**0**	**0**	**2**	**0**	**0**
0.25	**0**	**1**	**5**	**0**	**0**	**4**
0.38	**1**	**0**	**1**	**3**	**4**	**2**
0.5	**2**	**1**	**3**	**6**	**5**	**4**
0.75	**3**	**1**	**3**	**1**	**4**	**3**
1	**4**	**4**	**12**	**3**	**3**	**1**
1.5	**11**	**6**	**7**	**0**	**0**	**1**
2	**8**	**6**	**6**	**6**	**3**	**1**
3	**1**	**1**	**0**	**2**	**0**	**0**
4	**4**	**2**	**2**	**3**	**0**	**0**
8	**1**	**3**	**1**	**2**	**0**	**0**
12	0	0	0	1	0	0

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16	2	2	0	0	0	0
32	4	2	0	0	0	0
48	1	8	0	0	0	0
64	3	5	1	0	0	0
96	1	2	0	0	0	0
128	6	3	0	0	0	0
256	16	18				
>256	28	27	0	2	0	0

Bold: susceptible according to EUCAST breakpoints, CZA: ceftazidime-avibactam, MV: meropenem-vaborbactam.

4. Discussion

The problem of increasing bacterial resistance to antimicrobials has led to search for new drugs, but also to return of well known old drugs. Fosfomycin and colistin are antimicrobials that were discovered several decades ago. The introduction of the intravenous form of fosfomycin in 2019 in some European countries, including Poland has expanded the possibility of using it in the treatment of infections caused by MDR strains. In turn, colistin has been used for many years, but its use has been limited after 1970 due to its toxicity. The advantage of both drugs is a wide range of activity including strains producing all classes of beta-lactamases from A to D. There are available articles concerning activity to fosfomycin and colistin, but most of them refer to isolates derived from Western and South Europe and Americas. The results of this study showed that over 62% of analysed *K. pneumoniae* complex strains were susceptible to fosfomycin. The only report considering the isolates from Poland was conducted by Kowalska-Krochmal et al. [12] on a relatively big group of the *Klebsiella* spp. derived from patients with invasive infections. Among the 250 of analysed strains 66.0% were susceptible to fosfomycin with MIC_{50} 32 mg/L and MIC_{90} 512 mg/L. In the analysed group 86 isolates were ESBL-positive, 26 ESBL- and carbapenemase-positive and 58 carbapenemase-positive. The results of this study showed that fosfomycin was highly potent (> 86% susceptibility) against *K. pneumoniae* VIM-positive isolates and *K. variicola* ESBL-positive isolates.

According to research conducted in Turkey [13], 53.2% of *Klebsiella* spp. isolates were susceptible to fosfomycin. The highest value was obtained for OXA-48- and NDM-positive strains - 73.3% susceptible and the lowest for strains producing only NDM enzymes – 33.3%. In this study, none of the 9 analysed OXA-positive isolates were susceptible to fosfomycin, but only 2 isolates produced OXA-48 and 7 strains – OXA-181. In opposite to this fact, in the study of Demirci-Duarte et al. [13] 50.5% of OXA-48-positive *Klebsiella* spp. isolates (n = 104) were susceptible to fosfomycin. In turn, Aprile et al. [14] obtained 76% of KPC-positive *K. pneumoniae* strains susceptible to fosfomycin and none NDM- and OXA-48-positive strains. In this study, value for KPC-positive strains was lower around 64%, but the number of strains was smaller. Another study from Latin America [15] reported that susceptibility of *K. pneumoniae* strains to fosfomycin varied from 90.9% for strains isolated in Chile to 100% for strains isolated in Mexico. The study included 601 *K. pneumoniae* isolates. For carbapenem non-susceptible *K. pneumoniae* strains (183) the values were 71.4% and 100% respectively. In turn, Zarakolu et al. [16] reported 90.7% of carbapenem-susceptible *K. pneumoniae* isolates and 69.4% of carbapenem-resistant isolates were susceptible to fosfomycin.

The results of this study showed that over 62% of analysed *K. pneumoniae* complex strains were susceptible to colistin. Similar results were found in Greece [17], in which antimicrobial activity of colistin was demonstrated on 392 carbapenem-resistant *K. pneumoniae* strains, with a susceptibility rate of 64%. The only report considering the isolates from Poland was conducted by Pruss et al. [18] on a group of 200 the *K. pneumoniae* isolated from clinical samples. Among the analysed ESBL-positive strains all were susceptible to colistin. In the group of carbapenemase-positive isolates value varied and depended on the phenotype. The highest value of colistin-susceptible strains was obtained for KPC-positive isolates (100%), next for NDM-positive – 82.3% and only 50.0% for OXA-48-positive

strains. The results of this study showed that colistin was highly potent (≥ 80% susceptibility) against KPC-positive and VIM-positive *K. pneumoniae* isolates. The lowest value colistin-susceptible strains was noted for OXA-positive strains - only 33.4%, including OXA-48 and OXA-181 strains. According to research conducted in European countries including Poland [19], 95.6% of *K. pneumoniae* among 4201 isolates were susceptible to colistin with $MIC_{50/90}$ 1 mg/L and ranged from ≤ 0.12 mg/L to > 4 mg/L. Another study from Spain [20] found that susceptibility of carbapenemase-producing *K. pneumoniae* strains to colistin decreased from 86.5% to 68.3% over 3-years. Interestingly, in the cited articles and in this study, a huge difference in colistin MIC_{90} values was observed between carbapenemase-producing *K. pneumoniae* isolates in Greece, India and Poland, 64 mg/L, 32 mg/L and 8 mg/L, respectively. Meanwhile, colistin MIC_{50} values were similar - 1 mg/L, 0.5 mg/L and 0.75 mg/L, respectively.

There are no data in the literature about the frequency of occurrence of colistin-susceptible *K. variicola* isolates. However, there are a few articles describing *K. variicola* strains resistant to this antimicrobial [21,22]. In previous study, among 13 *K. variicola* strains isolated from clinical samples 46.1% were resistant to colistin [unpublished data].

Ceftazidime-avibactam and meropenem-vaborbactam are combinations of known beta-lactams with new beta-lactamase inhibitors. These antimicrobials were approved by Food and Drug Administration and European Medicines Agency several years ago. Ceftazidime-avibactam and meropenem-vaborbactam are active against strains producing class A and C beta-lactamases. Additionally, ceftazidime-avibactam is active against strains producing class D beta-lactamases.

The results of this study showed that ceftazidime-avibactam and meropenem-vaborbactam were highly potential (> 88% susceptibility) against *K. pneumoniae* KPC-positive isolates, *K. variicola* and *K. pneumoniae* ESBL-positive isolates. Earlier studies from this medical center [23] confirmed high *in vitro* activity of ceftazidime-avibactam against *K. pneumoniae* strains producing ESBL enzymes. In the cited study only 4 strains were producing carbapenemases (2 OXA-48 and 2 KPC) and three of them were susceptible to ceftazidime-avibactam. Study from Latin America [15] found that susceptibility of *K. pneumoniae* strains to ceftazidime-avibactam varied from 87.0% for strains isolated in Brazil to 100% for strains isolated in Mexico. For carbapenem non-susceptible *K. pneumoniae* strains (n = 183) the values were 83.3% and 100% respectively, but the lowest value for these strains was obtained in Colombia 68.6%. According to research conducted in European countries including Poland [19], 98.9% of *K. pneumoniae* among 4201 isolates were susceptible to ceftazidime-avibactam with MIC_{50} 0.12 mg/L and MIC_{90} 1 mg/L and ranged from ≤ 0.015 mg/L to > 128 mg/L. Another study from Pittsburgh [24] noted that susceptibility of *K. pneumoniae* strains to ceftazidime-avibactam achieved 79.0%. All of analysed isolates were carbapenem-resistant and 93% of them produced KPC enzymes. In this study, above 35% of carbapenemase-positive *K. pneumoniae* strains were susceptible to ceftazidime-avibactam, but among KPC-positive strains all were susceptible to this drug. Additionally, high value OXA-positive *K. pneumoniae* strains susceptible to ceftazidime-avibactam was noted – almost 89%. In turn, Bianco et al. [25] analysed susceptibility to selected antimicrobials 7 multi-carbapenemase *K. pneumoniae* isolates. None of the strains were susceptible to ceftazidime-avibactam. Similar results were obtained in this study including 15 multi-carbapenemase *K. pneumoniae* strains.

According to research conducted in US medical centers between 2018-2022 [26], among 7153 of *K. pneumoniae* isolates 97.1% were susceptible to meropenem-vaborbactam. Another study from Pittsburgh [24] noted that susceptibility of carbapenem-resistant *K. pneumoniae* strains to meropenem-vaborbactam achieved 99.0% and MIC ranged from ≤ 0.015 mg/L to > 32 mg/L. Most of the strains (39%) were KPC-positive. In turn, Gaibani et al. [27] noted that 87% of KPC-positive *K. pneumoniae* strains were susceptible to meropenem-vaborbactam. These strains were isolated from bloodstream infections from patients from one hospital in Bologna. In this study, over 30% of carbapenemase-positive *K. pneumoniae* strains were susceptible to meropenem-vaborbactam, but among KPC-positive strains all were susceptible to this drug. In turn, Bianco et al. [25] analysed susceptibility to selected antimicrobials multi-carbapenemase *K. pneumoniae* isolates. Two out of seven strains were susceptible to meropenem-vaborbactam. In this study, including 15 multi-

carbapenemase isolates none of the analysed *K. pneumoniae* strains were susceptible to meropenem-vaborbactam.

None of the analysed *K. variicola* strains produced carbapenemases, but in available literature there are articles about carbapenemase-positive *K. variicola* strains producing NDM, KPC, IMP or OXA enzymes [28–31]. There are a few reports in the literature assessing the susceptibility of *K. variicola* isolates to antimicrobials, but they did not included fosfomycin or new combinations of beta-lactams with beta-lactamase inhibitors. Small number of articles about susceptibility *K. variicola* to antimicrobials may be related to the difficulties in identifying this species using automatic methods. Also, in the mass spectrometry method, the identification result depends on the number and type of spectra collected in the virtual library, so misidentification to other species from the complex is possible.

In the era of increasing bacterial resistance to antimicrobials, rapid identification of carbapenemase-positive strains is microbiological, clinical and epidemiological issue. The emergence of multidrug-resistant isolates often leads to difficult choices, such as the use of drugs with reduced susceptibility or increasing the dose of the drug. Therefore, it is necessary to monitor the emergence of resistant strains and quickly detect resistance to last chance antimicrobials.

5. Conclusions

Probably this is the first report about susceptibility to fosfomycin, colistin, ceftazidime-avibactam and meropenem-vaborbactam of multidrug-resistant *K. pneumoniae* complex from Poland.

The results obtained in this study indicate how important is identification of carabapenemase. Taking into account known drugs, fosfomycin may be the option of choice in the treatment of infections caused by VIM-positive *K. pneumoniae* and ESBL-positive *K. variicola* strains. In turn, colistin may be an effective option in the treatment of infections caused by KPC-positive strains. New combinations of beta-lactams with beta-lactamase inhibitors may be the drugs of choice in the treatment of infections caused by multidrug-resistant *K. pneumoniae* complex strains producing ESBL or KPC enzymes. Currently, strains producing more than one carbapenemase are a big problem. The results of this study indicate that only colistin showed good, but unsatisfactory *in vitro* activity against *K. pneumoniae* complex strains. Therefore, the level of susceptibility to any antimicrobial agents is not constant over time and should be monitored on an ongoing basis.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, TableS1: The susceptibility to antimicrobials *K. pneumoniae* complex strains.

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References

1. Karampatakis, T.; Tsergouli, K.; Behzadi, P. Carbapenem-Resistant Klebsiella Pneumoniae: Virulence Factors, Molecular Epidemiology and Latest Updates in Treatment Options. *Antibiotics (Basel)* **2023**, *12*, 234, doi:10.3390/antibiotics12020234.

2. Rodríguez-Medina, N.; Barrios-Camacho, H.; Duran-Bedolla, J.; Garza-Ramos, U. *Klebsiella Variicola*: An Emerging Pathogen in Humans. *Emerging Microbes & Infections* **2019**, *8*, 973–988, doi:10.1080/22221751.2019.1634981.
3. European Antimicrobial Resistance Surveillance Network (EARS-Net) Available online: https://www.ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/ears-net (accessed on 9 October 2022).
4. Cantón, R.; Akóva, M.; Carmeli, Y.; Giske, C.G.; Glupczynski, Y.; Gniadkowski, M.; Livermore, D.M.; Miriagou, V.; Naas, T.; Rossolini, G.M.; et al. Rapid Evolution and Spread of Carbapenemases among Enterobacteriaceae in Europe. *Clinical Microbiology and Infection* **2012**, *18*, 413–431, doi:10.1111/j.1469-0691.2012.03821.x.
5. Oelschlaeger, P.; Ai, N.; DuPrez, K.T.; Welsh, W.J.; Toney, J.H. Evolving Carbapenemases: Can Medicinal Chemists Advance One Step Ahead Of The Coming Storm? (Perspective). *J Med Chem* **2010**, *53*, 3013–3027, doi:10.1021/jm9012938.
6. Baraniak, A.; Izdebski, R.; Herda, M.; Fiett, J.; Hryniewicz, W.; Gniadkowski, M.; Kern-Zdanowicz, I.; Filczak, K.; Łopaciuk, U. Emergence of Klebsiella Pneumoniae ST258 with KPC-2 in Poland. *Antimicrob Agents Chemother* **2009**, *53*, 4565–4567, doi:10.1128/AAC.00436-09.
7. Rekomendacje: Pałeczki Enterobacterales wytwarzające karbapenemazy (CPE) – Epidemiologia, diagnostyka, leczenie i profilaktyka zakażeń, 2022 – Narodowy program ochrony antybiotyków.
8. Sekowska, A.; Gospodarek, E.; Kruszyńska, E.; Hryniewicz, W.; Gniadkowski, M.; Duljasz, W.; Kusza, K.; Wawrzyniak, K. [First isolation of metallo-beta-lactamase producing Klebsiella pneumoniae strain in Poland]. *Anestezjol Intens Ter* **2010**, *42*, 27–30.
9. Pitout, J.D.D.; Peirano, G.; Kock, M.M.; Strydom, K.-A.; Matsumura, Y. The Global Ascendency of OXA-48-Type Carbapenemases. *Clin Microbiol Rev* **2019**, *33*, e00102-19, doi:10.1128/CMR.00102-19.
10. Eucast: EUCAST Available online: https://www.eucast.org/ (accessed on 3 October 2023).
11. van der Zwaluw, K.; de Haan, A.; Pluister, G.N.; Bootsma, H.J.; de Neeling, A.J.; Schouls, L.M. The Carbapenem Inactivation Method (CIM), a Simple and Low-Cost Alternative for the Carba NP Test to Assess Phenotypic Carbapenemase Activity in Gram-Negative Rods. *PLoS One* **2015**, *10*, e0123690, doi:10.1371/journal.pone.0123690.
12. Kowalska-Krochmal, B.; Mączyńska, B.; Rurańska-Smutnicka, D.; Secewicz, A.; Krochmal, G.; Bartelak, M.; Górzyńska, A.; Laufer, K.; Woronowicz, K.; Łubniewska, J.; et al. Assessment of the Susceptibility of Clinical Gram-Negative and Gram-Positive Bacterial Strains to Fosfomycin and Significance of This Antibiotic in Infection Treatment. *Pathogens* **2022**, *11*, 1441, doi:10.3390/pathogens11121441.
13. Demirci-Duarte, S.; Unalan-Altintop, T.; Gulay, Z.; Sari Kaygisiz, A.N.; Cakar, A.; Gur, D. In Vitro Susceptibility of OXA-48, NDM, VIM and IMP Enzyme- Producing Klebsiella Spp. and Escherichia Coli to Fosfomycin. *J Infect Dev Ctries* **2020**, *14*, 394–397, doi:10.3855/jidc.12456.
14. Aprile, A.; Scalia, G.; Stefani, S.; Mezzatesta, M.L. In Vitro Fosfomycin Study on Concordance of Susceptibility Testing Methods against ESBL and Carbapenem-Resistant Enterobacteriaceae. *J Glob Antimicrob Resist* **2020**, *23*, 286–289, doi:10.1016/j.jgar.2020.09.022.
15. Appel, T.M.; Mojica, M.F.; De La Cadena, E.; Pallares, C.J.; Radice, M.A.; Castañeda-Méndez, P.; Jaime-Villalón, D.A.; Gales, A.C.; Munita, J.M.; Villegas, M.V. In Vitro Susceptibility to Ceftazidime/Avibactam and Comparators in Clinical Isolates of Enterobacterales from Five Latin American Countries. *Antibiotics (Basel)* **2020**, *9*, 62, doi:10.3390/antibiotics9020062.
16. Zarakolu, P.; Eser, Ö.K.; Otlu, B.; Gürpınar, Ö.; Özakın, C.; Akalın, H.; Köksal, İ.; Ünal, S. In-Vitro Activity of Fosfomycin against Escherichia Coli and Klebsiella Pneumoniae Bloodstream Isolates and Frequency of OXA-48, NDM, KPC, VIM, IMP Types of Carbapenemases in the Carbapenem-Resistant Groups. *J Chemother* **2022**, *34*, 235–240, doi:10.1080/1120009X.2021.1963618.
17. Galani, I.; Adamou, P.; Karaiskos, I.; Giamarellou, H.; Souli, M. Evaluation of ComASP™ Colistin (Formerly SensiTest™ Colistin), a Commercial Broth Microdilution-Based Method to Evaluate the Colistin Minimum Inhibitory Concentration for Carbapenem-Resistant Klebsiella Pneumoniae Isolates. *J Glob Antimicrob Resist* **2018**, *15*, 123–126, doi:10.1016/j.jgar.2018.07.006.
18. Pruss, A.; Kwiatkowski, P.; Masiuk, H.; Jursa-Kulesza, J.; Bilska, I.; Lubecka, A.; Cettler, M.; Roszkowska, P.; Dołęgowska, B. Analysis of the Prevalence of Colistin Resistance among Clinical Strains of Klebsiella Pneumoniae. *Ann Agric Environ Med.* **2022**, *29*, 518–522, doi:10.26444/aaem/155253.
19. Kazmierczak, K.M.; de Jonge, B.L.M.; Stone, G.G.; Sahm, D.F. In Vitro Activity of Ceftazidime/Avibactam against Isolates of Enterobacteriaceae Collected in European Countries: INFORM Global Surveillance 2012–15. *Journal of Antimicrobial Chemotherapy* **2018**, *73*, 2782–2788, doi:10.1093/jac/dky266.
20. Pena, I.; Picazo, J.J.; Rodríguez-Avial, C.; Rodríguez-Avial, I. Carbapenemase-Producing Enterobacteriaceae in a Tertiary Hospital in Madrid, Spain: High Percentage of Colistin Resistance among VIM-1-Producing Klebsiella Pneumoniae ST11 Isolates. *Int J Antimicrob Agents* **2014**, *43*, 460–464, doi:10.1016/j.ijantimicag.2014.01.021.

21. Lu, Y.; Feng, Y.; McNally, A.; Zong, Z. Occurrence of Colistin-Resistant Hypervirulent Klebsiella Variicola. *J Antimicrob Chemother* **2018**, *73*, 3001–3004, doi:10.1093/jac/dky301.
22. Kim, S.J.; Jo, J.; Ko, K.S. Lipid A Modification-Induced Colistin-Resistant Klebsiella Variicola from Healthy Adults. *J Med Microbiol* **2023**, *72*, doi:10.1099/jmm.0.001680.
23. Sękowska, A. Wrażliwość wielolekoopornych szczepów Klebsiella pneumoniae na połączenie ceftazydymu z awibaktamem. *Forum Zakażeń* **2019**, *10*, 269–273, doi:10.15374/FZ2019055.
24. Wilson, W.R.; Kline, E.G.; Jones, C.E.; Morder, K.T.; Mettus, R.T.; Doi, Y.; Nguyen, M.H.; Clancy, C.J.; Shields, R.K. Effects of KPC Variant and Porin Genotype on the In Vitro Activity of Meropenem-Vaborbactam against Carbapenem-Resistant Enterobacteriaceae. *Antimicrob Agents Chemother* **2019**, *63*, e02048-18, doi:10.1128/AAC.02048-18.
25. Bianco, G.; Boattini, M.; Comini, S.; Casale, R.; Iannaccone, M.; Cavallo, R.; Costa, C. Occurrence of Multi-Carbapenemases Producers among Carbapenemase-Producing Enterobacterales and in Vitro Activity of Combinations Including Cefiderocol, Ceftazidime-Avibactam, Meropenem-Vaborbactam, and Aztreonam in the COVID-19 Era. *Eur J Clin Microbiol Infect Dis* **2022**, *41*, 573–580, doi:10.1007/s10096-022-04408-5.
26. Sader, H.S.; Mendes, R.E.; Duncan, L.; Kimbrough, J.H.; Carvalhaes, C.G.; Castanheira, M. Ceftazidime-Avibactam, Meropenem-Vaborbactam, and Imipenem-Relebactam Activities against Multidrug-Resistant Enterobacterales from United States Medical Centers (2018-2022). *Diagn Microbiol Infect Dis* **2023**, *106*, 115945, doi:10.1016/j.diagmicrobio.2023.115945.
27. Gaibani, P.; Lombardo, D.; Bussini, L.; Bovo, F.; Munari, B.; Giannella, M.; Bartoletti, M.; Viale, P.; Lazzarotto, T.; Ambretti, S. Epidemiology of Meropenem/Vaborbactam Resistance in KPC-Producing Klebsiella Pneumoniae Causing Bloodstream Infections in Northern Italy, 2018. *Antibiotics (Basel)* **2021**, *10*, 536, doi:10.3390/antibiotics10050536.
28. Camargo, C.H.; Yamada, A.Y.; de Souza, A.R.; Reis, A.D.; Santos, M.B.N.; de Assis, D.B.; de Carvalho, E.; Takagi, E.H.; Cunha, M.P.V.; Tiba-Casas, M.R. Genomic Diversity of NDM-Producing Klebsiella Species from Brazil, 2013–2022. *Antibiotics (Basel)* **2022**, *11*, 1395, doi:10.3390/antibiotics11101395.
29. Ge, H.; Qiao, J.; Xu, H.; Liu, R.; Zhao, J.; Chen, R.; Li, C.; Chen, M.; Guo, X. Emergence of OXA-484-Producing Klebsiella Variicola</Em> in China. *IDR* **2023**, *16*, 1767–1775, doi:10.2147/IDR.S404551.
30. Huang, L.; Fu, L.; Hu, X.; Liang, X.; Gong, G.; Xie, C.; Zhang, F.; Wang, Y.; Zhou, Y. Co-Occurrence of Klebsiella Variicola and Klebsiella Pneumoniae Both Carrying Bla KPC from a Respiratory Intensive Care Unit Patient. *Infect Drug Resist* **2021**, *14*, 4503–4510, doi:10.2147/IDR.S330977.
31. Zurfluh, K.; Poirel, L.; Nordmann, P.; Klumpp, J.; Stephan, R. First Detection of Klebsiella Variicola Producing OXA-181 Carbapenemase in Fresh Vegetable Imported from Asia to Switzerland. *Antimicrob Resist Infect Control* **2015**, *4*, 38, doi:10.1186/s13756-015-0080-5.