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ORIGINAL ARTICLE

Analysis of the prevalence of colistin resistance among clinical strains of *Klebsiella pneumoniae*

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Abstract

Introduction and Objective. *Klebsiella pneumoniae* is an essential component of the human gut microflora. However, it can pose a threat by causing opportunistic infections, especially in hospitalised or immunocompromised patients. It is a serious problem for health medicine, primarily because of increasing resistance to previously used antibiotics. Infections with multidrug-resistant strains are difficult to treat, creating a challenge for clinicians. Also of growing concern is the increasing resistance to the drug of last resort – colistin (CL). The aim of the study is to determine the prevalence of resistance to CL among clinical *K. pneumoniae* strains.

Materials Method. The study was conducted on 200 clinical strains of *K. pneumoniae*. Drug susceptibility, production of resistance mechanisms, and determination of the minimum inhibitory concentration of CL were evaluated.

Results. Of all isolates, 73.0% produced carbapenemases, while the remainder produced an extended substrate spectrum – β-lactamases (ESBLs). All strains showed a diverse antibiotic resistance profile. Resistance to CL was noted among 14.5% of carbapenemase-producing strains, particularly MBL and OXA-48. ESBL-positive strains showed full susceptibility to CL.

Conclusions. Although a low rate of CL resistance was observed, this was true for strains simultaneously producing carbapenemases. Such strains should be under special epidemiological surveillance due to their potential to cause epidemic outbreaks. Monitoring the prevalence of clinical CL-resistant strains would allow for more effective counteraction against pathogens in various fields, including medicine, agriculture, veterinary medicine and industry.

Key words

Klebsiella pneumoniae, colistin resistance, clinical

INTRODUCTION

Klebsiella pneumoniae is common in the environment, inhabiting bodies of water, soil, sewage, as well as plants. In humans, it is part of the intestinal physiological flora and colonises the nasopharyngeal cavity and mucous membranes [1]. *K. pneumoniae* can cause numerous infections, both nosocomial and non-hospital. Due to the ease of spread of *K. pneumoniae* strains, infections associated with prolonged hospitalisation are a particular problem. These microorganisms can cause pneumonia, urinary tract infections (UTIs), liver abscesses, meningitis, and wound infections [2]. Bacteraemia can be a severe consequence of such infections [3].

Bacterial resistance to antibiotics has been observed almost since the beginning of the antibiotic era. Factors favouring the development of this phenomenon are the exchange of genes encoding resistance mechanisms between bacterial populations, thanks to their location on mobile genetic elements. Also unfavourable is the tendency to use antibiotics excessively and often unjustifiably [4].

K. pneumoniae has multiple resistance mechanisms, the most common of which is the production of extended substrate spectrum β-lactamases (ESBLs), and the most dangerous is the production of carbapenemases. *K. pneumoniae* is a producer of enzymes such as KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Delhi metallo-β-lactamase) and OXA-48 [5]. It poses a particular therapeutic challenge, especially in severe systemic infections.

The increasing drug resistance of bacteria has become a global problem in modern medicine. Infections with such pathogens are difficult to treat and are also fraught with high mortality rates [6]. Previously effectively used antibiotics are no longer effective which has resulted in the use of older antibiotics being resumed. One such antibiotic is colistin (CL), which was discontinued after 1970 due to its toxic properties. It began to be used again in the 1990s as a drug

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of last resort, mainly for infections with *Enterobacteriaceae*, which show resistance to carbapenems [7].

CL belongs to the group of polymyxins, otherwise known as polymyxin E. Its antimicrobial activity and toxicity are closely related to the concentration it reaches in the patient's serum. CL's spectrum of action primarily includes Gram-negative bacteria of the *Enterobacteriaceae* family, particularly *K. pneumoniae* or *Escherichia coli*, and non-fermenting bacilli such as *Acinetobacter* and *Pseudomonas* [8]. The mechanism of action of CL is based on the electrostatic interaction between the cationic residues of the drug's diaminobutyric acid, and the anionic phosphate groups of lipid A found in bacterial lipopolysaccharide (LPS). It destabilises the LPS, resulting in increased cell membrane permeability and bacterial cell death [9]. The absorbability of CL from the gastrointestinal tract is poor. Two primary administration forms of this drug are known, acting orally and topically as CL sulphate and the more commonly used parenteral form, CL methanesulfonate. CL sulphate is widely used in veterinary medicine and agriculture as a feed additive for pigs and farmed fish diets [10]. The mechanisms of resistance to CL vary depending on the bacterial species. Most commonly, they are associated with changes in LPS through modification of lipid A. The attachment of phosphoethanolamine and 4-amino-4-deoxy-L-arabinose reduces the negative charge of the outer membrane and limits the binding of CL to the bacterial LPS. In addition, the mechanisms are linked to the over-expression of the outer membrane protein OprH or the action of efflux pumps [11].

Plasmid-encoded CL resistance is associated with the presence of the *mcr-1* gene which encodes a phosphoethanolamine transferase, which in turn catalyses the attachment of phosphoethanolamine to lipid A. In 2015, *mcr-1* was first described in an animal isolate of *E. coli* in China [12]. The systematic identification of the animal-derived *mcr-1* gene and the relatively much lower percentage of such isolates from human clinical specimens suggests that the resistance most likely occurred in animals and then spread among humans. It is conjectured that this may be related to the widespread use of CL in agriculture and veterinary medicine as a therapeutic agent and animal feed additive. The *mcr-1* gene has also been confirmed in *Shigella* spp., *Salmonella* spp. and *Enterobacter* spp. [13]. Of great concern is the discovery of *K. pneumoniae* strains with a concomitant CL resistance mechanism and the production of carbapenemases [14].

Inappropriate use and overuse of antibiotics have resulted in the development of multidrug resistance in bacteria, which has limited therapeutic options. Of most concern is the increasingly reported lack of sensitivity of multidrug-resistant strains (MDR) to the drug of last resort – CL. Thus, the presented study aimed to determine the prevalence of clinical *K. pneumoniae* resistant to CL.

MATERIALS AND METHOD

Bacterial strains and growth conditions. In the current study, 200 strains of *K. pneumoniae* collected from 2019 – 2021, isolated from patients hospitalised in various wards of three major hospitals in Szczecin (*Independent* Public Clinical *Hospital no. 1, Independent* Public Clinical *Hospital no. 2, and Independent Provincial Public* Integrated *Hospital* "*Zdunowo*") and one in Zielona Góra (*University Hospital*), Poland, were used. *K. pneumoniae* strains were isolated from the following sources: urine, cerebrospinal fluid (CSF), blood, pus, central cannula, sputum, surgical material, bronchial tree aspirate, rectal swab, wound swab, nose swab, endotracheal tube, bile, and drain.

All strains were cultivated for 18 h at 37 °C in an aerobic atmosphere on Columbia agar with 5% sheep blood and MacConkey agar (bioMérieux, Poland).

Microbiological diagnostic. *K. pneumoniae* strains were identified using VITEK 2 Compact (bioMerieux, France). Antimicrobial susceptibility testing (AST) for amoxicillin/clavulanic acid (AMC, 20/10 μg), piperacillin/tazobactam (TZP, 100/10 μg), meropeneme (MEM, 10 μg), imipeneme (IMP, 10 μg), cefotaxime (CTX, 10 μg), ceftazidime (CAZ, 10 μg), cefepime (FEP, 30 μg), gentamicin (GE, 10 μg), amikacin (AN, 30 μg), ciprofloxacin (CIP, 5 μg), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg) (Argenta, Poland) was performed using the Kirby-Bauer agar disc diffusion method according to EUCAST guidelines [15]. The presence of ESBL was confirmed with the double disc method. The production of carbapenemases was confirmed by the NG Test Carba 5 test (NG Biotech, France).

Determination of minimal inhibitory concentration (MIC) of colistin (CL). The MIC of CL was determined by the microdilution method in broth using the MIC COL kit (Diagnostics, Slovakia) according to the procedure guideline. Briefly, a single test consisted of 1 strip containing 7 wells with different concentrations of CL and 1 control well. The MIC value was taken as the first well in purple, starting the reading from the lowest concentrations. The obtained MIC for CL was interpreted according to the criteria for determining drug susceptibility according to EUCAST recommendations [15]. Strains with MIC ≤ 2 mg/L and MIC > 2 mg/L were classified as CL-susceptible and CL-resistant, respectively. All tests were performed in triplicate.

Statistical analysis. The description of calculations includes the number of cases (*n*) and the percentage (%).

RESULTS

It was observed that most of *K. pneumoniae* strains were isolated from the following sources: rectal swabs (*n*=96, 48.0%), blood (*n* =33, 16.5%), urine (*n*=30, 15.0%), bronchial tree aspirate (*n*=20, 10.0%), and wound swabs (*n*=12, 6.0%). Single strains were cultured from other clinical materials (Tab. 1).

Based on the results, it was found that 146 (73.0%) strains were *K. pneumoniae*-produced carbapenemases (CP-KP). Of these, the highest percentage were isolates producing NDM-type enzymes (*n*=130, 89.0%), followed by KPC (*n*=8, 5.5%), and OXA-48 (*n*=8, 5.5%). The remaining strains (*n*=54, 27.0%) produced only an ESBL-type resistance mechanism. ESBL-positive *K. pneumoniae* (ESBL-KP) strains showed high resistance to penicillins, cephalosporins, fluoroquinolones, and SXT. However, most strains were susceptible to IMP, MEM, and AN. CP-KP strains exhibited a full or high degree of resistance to most labelled antibiotics (Tab. 2).

All ESBL-KP strains were susceptible to CL (MIC ≤ 2 mg/l). The most frequently observed MIC value was 1 mg/l

Table 2. The antimicrobial susceptibility of extended substrate spectrum β-lactamase-positive K. pneumoniae (ESBL-KP) and carbapenemase-producing K. pneumoniae (CP-KP) strains

Antibiotic	ESBL-KP n=54		CP-KP n=146	
	Susceptibility n (%)	Resistance n (%)	Susceptibility n (%)	Resistance n (%)
AMC	0 (0.0)	54 (100.0)	0 (0.0)	146 (100.0)
TZP	7 (13.0)	47 (87.0)	0 (0.0)	146 (100.0)
CTX	0 (0.0)	54 (100.0)	0 (0.0)	146 (100.0)
CAZ	0 (0.0)	54 (100.0)	0 (0.0)	146 (100.0)
FEP	0 (0.0)	54 (100.0)	0 (0.0)	146 (100.0)
IMP	52 (96.3)	2 (3.7)	16 (11.0)	130 (89.0)
MEM	51 (94.4)	3 (5.6)	4 (2.7)	142 (97.3)
GE	26 (48.1)	28 (51.9)	53 (36.0)	93 (64.0)
AN	41 (75.9)	13 (24.1)	64 (43.9)	82 (56.1)
CIP	13 (24.1)	41 (75.9)	4 (2.7)	142 (97.3)
SXT	8 (14.8)	46 (85.2)	12 (8.0)	134 (92.0)

AMC – amoxicillin/clavulanic acid; TZP – piperacillin/tazobactam; CTX – cefotaxime; CAZ – ceftazidime; FEP – cefepime; IMP – imipeneme; MEM – meropeneme; GE – gentamicin; AN – amikacin; CIP – ciprofloxacin; SXT – trimethoprim/sulfamethoxazole.

Table 1. Origin of K. pneumoniae strains used in this study

Origin	Hospital 1 (n=70)	Hospital 2 (n=37)	Hospital 3 (n=51)	Hospital 4 (n=42)	Total (n=200)
	n (%)				
Rectal swab	33 (47.1)	14 (38.0)	18 (35.3)	31 (73.8)	96 (48.0)
Wound swab	3 (4.3)	6 (16.2)	1 (2.0)	2 (4.8)	12 (6.0)
Blood	21 (30.0)	9 (24.3)	1 (2.0)	2 (4.8)	33 (16.5)
Urine	9 (12.3)	5 (13.6)	11 (21.6)	5 (12.0)	30 (15.0)
Bronchial tree aspirate	1 (1.5)	1 (2.7)	18 (35.3)	0 (0.0)	20 (10.0)
Cerebrospinal fluid	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Pus	0 (0.0)	1 (2.7)	0 (0.0)	0 (0.0)	1 (0.5)
Central cannula	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)	1 (0.5)
Sputum	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	1 (0.5)
Surgical material	0 (0.0)	1 (2.7)	0 (0.0)	0 (0.0)	1 (0.5)
Endotracheal tube	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)	1 (0.5)
Bile	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Drain	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Nose swab	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	1 (0.5)

Hospital 1 – Independent Public Clinical *Hospital No. 1 in Szczecin; Hospital 2 – Independent* Public Clinical *Hospital No. 2 in Szczecin; Hospital 3 – Independent Provincial Public* Integrated *Hospital 'Zdunowo'* in Szczecin; Hospital 4 – *University Hospital* in Zielona Góra

(*n*=43, 79.6%); also, the majority of CP-KP strains showed susceptibility to CL. The most common recurrent MIC was 0.5 mg/l (*n*=63, 43.2%), followed by 1.0 mg/l (*n*=56, 38.4%). CL-resistant CP-KP isolates (MIC of CL > 2 mg/l) accounted for 21 (14.4%) strains (Fig. 1).

All *K. pneumoniae* KPC-positive strains were susceptible to CL. In most (*n*=6, 75.0%), the MIC value was 0.5 mg/l. 4 (50.0%) *K. pneumoniae* OXA-48-positive strains exhibited MIC of CL ≥ 16 mg/l, whereas the remainder of isolates showed susceptibility to CL. Of the NDM-positive strains, 107 (82.3%) strains showed susceptibility to CL. CL-resistant strains accounted for 17 (13.1%) isolates, with MICs most often ≥ 16 mg/l (Fig. 2).

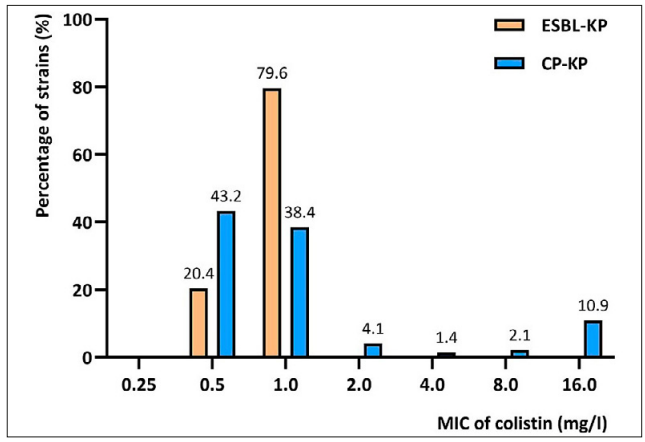

Figure 1. Colistin susceptibility among extended substrate spectrum β-lactamase-positive K. pneumoniae (ESBL-KP) and carbapenemase-producing K. pneumoniae (CP-KP) strains.
MIC – minimum inhibitory concentration

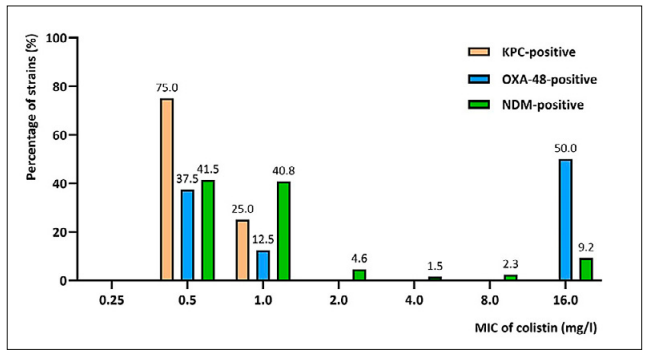

Figure 2. Colistin susceptibility among Klebsiella pneumoniae carbapenemase-(KPC), New Delhi metallo-β-lactamase- (NDM), and OXA-48-positive K. pneumoniae strains

DISCUSSION

Multidrug resistance is one of the biggest problems in medicine worldwide. Microorganisms produce resistance mechanisms that evolve very rapidly and spread between species. Of particular concern is the increasing frequency of resistance to drugs of last resort, such as CL.

K. pneumoniae causes various infections, the most common being UTI, pneumonia or bacteremia [16]. It explains the high number of strains isolated from materials such as urine, bronchial tree aspirate or blood. At the same time, they can reside in the gastrointestinal tract without causing infections, which is the reason for epidemic outbreaks in various hospital wards. The carriage of these strains is confirmed by screening. A high percentage of strains from rectal swabs (40.4%) was obtained by Fils et al., followed by those from urine (37%) and blood (6.2%) [17]. In contrast, a study by Saadatian et al. showed that most isolates came from urine (60.5%) and blood (19.7%) [18]. In the current study, almost half of the collected strains (48%) of *K. pneumoniae* were isolated from rectal swabs. These data confirm the advisability of screening in intensive care units.

K. pneumoniae are characterised by varying resistance profiles to different antibiotic groups and effectiveness in causing infections. Currently, among the etiologic agents of nosocomial infections, strains of *Enterobacteriaceae* producing ESBL-type resistance predominate [19]. It is

believed that microorganisms with this phenotype show the highest sensitivity to antibiotics of the carbapenem group, which has been confirmed by Fils et al. in which sensitivity to IMP among ESBL-positive strains was 99.3%. However, a high resistance rate to CTX and FEP was also observed, which was 98% and 90.4%, respectively [17]. The current study confirmed the complete resistance of ESBL-positive strains to cephalosporins. At the same time, high sensitivity to carbapenems was observed. The strains were primarily sensitive to aminoglycosides but resistant to fluoroquinolones and SXT. A cohort study led by Xercavins et al. on ESBL-KP showed a resistance rate of 91.6% to CIP and 88.3% to SXT [20]. However, a lower percentage of resistance to CIP (37.5%) was obtained by Eftekhar et al. [21].

Carbapenemase-positive *K. pneumoniae,* due to their extensive hydrolytic properties, are resistant to many antimicrobial agents. Unlu et al. showed that CP-KP were fully resistant to cephalosporins and carbapenems [22]. Similar results were obtained in the current study which showed complete resistance to cephalosporins, and a high percentage of resistance to carbapenems. The strains studied also exhibited high resistance levels to other groups of antibiotic aminoglycosides, fluoroquinolones and SXT. Similar resistance profiles were observed by other researchers [19, 23].

The problem of increasing resistance to CL has been reported worldwide [8]. Due to the prominent role of CL in treating infections caused by MDR strains, close monitoring of the growing resistance to this antibiotic is recommended. A study in Italy by Calia et al. among hospitalised patients from whom CP-KP were isolated, showed CL resistance of 17.3% [24]. A retrospective analysis of European studies revealed the growing concern of CL resistance prevalence over the past decade. Since 2013 in Europe, resistance in up to one-third of tested isolates has been observed [25], a situation particularly applicable to countries like as Spain and Italy. A study by Pena et al. confirmed the high rate of CL resistance (31.7%) in *K. pneumoniae* showing simultaneous resistance to other antibiotic groups [26]. Capone et al. conducted a cross-sectional study isolating CP-KP from patients in 9 hospitals in Italy where a CL resistance rate of 36.1% was reported. In addition, infection with a CL-resistant CP-KP was found to be a factor in increased mortality risk among patients [27]. A similar conclusion was also made by Rojas et al. in the United States, who estimated a CL resistance rate of 13% [28]. The high mortality rate (61%) among patients infected with CL-resistant *K pneumoniae* strains was confirmed by a cohort study conducted in 2015–2016 in Turkey [29]. Unfortunately, epidemic outbreaks caused by CP-KP, which additionally exhibit CL resistance, are increasingly being reported [11, 30]. Over the past few years, this phenomenon has been documented in a 2019 study by Jafari et al. in the Middle East. They showed that 50% of *K. pneumoniae* isolates resistant to carbapenems also developed resistance to CL [31].

It is noteworthy that among the KPC-positive *K. pneumoniae* strains isolated in Poland, the results of CL resistance have changed in different years. This was noted by the studies of Baraniak et al. and Sękowska et al. who observed the absence of resistance to CL in 2008 – 2009, and an increase in resistance to this antibiotic in 2020, respectively [32, 33]. In the current study, complete sensitivity to CL was reported among ESBL-positive strains, but at the same time, CL resistance was reported at 14% among carbapenemase-producing strains. It is certain that the increasing resistance to CL is a direct result of the use of this drug in therapy. On the other hand, however, in the situation of isolation of a strain resistant to almost all available antibiotics, CL becomes the only available option.

Disturbing reports worldwide present the overall epidemiological situation in pessimistic terms. Bacterial strains additionally resistant to the drug of last resort leave no therapeutic hope; therefore, the search for appropriate countermeasures and the search for effective treatment therapies should be a priority. To achieve improvements, it is also necessary to raise awareness in the medical, agricultural, veterinary and industrial communities, and promote knowledge about antibiotic therapy.

CONCLUSIONS

The highest percentage of *K. pneumoniae* strains came from rectal, blood and urine swabs. The strains most often produced carbapenemases, followed by an ESBL-type resistance mechanism. All ESBL-positive strains presented sensitivity to CL. Resistance to this antibiotic was observed among 14.5% of carbapenemase-producing isolates. Among the tested *K. pneumoniae* strains isolated from patients of various hospitals and hospital wards, sensitivity to the 'drug of last resort' – CL, remains high.

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