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Emergence of colistin-resistant *Klebsiella pneumoniae* in Poland

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ORIGINAL ARTICLE



ABSTRACT

In recent years, colistin has been the drug of choice for treatment of nosocomial infections, especially in bloodstream infections, lower respiratory tract infections, or urinary tract infections. In this study, 65 multidrug-resistant *Klebsiella pneumoniae* isolated from different clinical samples were included. Minimum inhibitory concentration (MIC) of colistin was detected by broth microdilution method in three different ways. For selected *K. pneumoniae* strains, eazyplex SuperBug mcr-1 test was performed. This test detects *mcr-1* gene, which encodes a colistin-resistance determinant. Most of the analyzed *K. pneumoniae* strains were resistant to colistin in all applied methods. The exception was two strains, where MIC of colistin was 2 mg/L in SensiTest Colistin and MIC-Strip Colistin tests. In MIC COL test, MIC for these strains was 4 mg/L. All analyzed strains produced extended-spectrum beta-lactamases and 11 (16.9%) metallo-beta-lactamases. Eleven (16.9%) *K. pneumoniae* strains were resistant to all antibiotics, whereas 17 (26.1%) were susceptible to only one drug. Colistin MIC values varied from 2 to >64 mg/L in MIC-Strip Colistin test; from 2 to >16 mg/L in SensiTest Colistin and from 4 to >16 mg/L in MIC COL test. None of the analyzed *K. pneumoniae* strains carried *mcr-1* gene. Data of this work suggest that resistance to colistin emerged among multidrug-resistant *K. pneumoniae* strains. The tests allowed for reliable estimation of susceptibility to colistin and could be used in microbiological diagnostics.

KEYWORDS

susceptibility, colistin, detection of resistance, *Klebsiella pneumoniae*, *mcr-1* gene

INTRODUCTION

Klebsiella spp. are among frequent bacteria causing hospital infections. These bacteria easily acquire resistance to antibiotics, and one strain can produce several mechanisms of resistance; therefore, colistin often remains as the only therapeutic option. The most common mechanism of antibiotic resistance in *Klebsiella pneumoniae* is the production of the extended-spectrum beta-lactamases (ESBLs). The first ESBL-positive strain was detected more than 30 years ago, but these enzymes and their constant evolution remain a significant therapeutic problem. In Poland, ESBLs range from 40% to 70% depending on the regional location, type of ward/hospital, and species of bacteria [Żabicka, unpublished data]. In our hospital, above 60% of the *K. pneumoniae* strains produced ESBLs. In the past years, strains producing carbapenemases were more often isolated. In Poland, the predominant carbapenemases are NDM and VIM. According to the data of the European Antimicrobial Resistance Surveillance System [1] in Poland, the percentage of *K. pneumoniae* strains resistant to carbapenems increased from 1%–5% in 2015 to 5%–<10% in 2017. In our hospital, 2%–3% of the *K. pneumoniae* strains produced carbapenemases. Strains producing carbapenemases are often susceptible only to colistin. At present, in Poland, *Enterobacteriaceae* strains resistant to colistin are rarely 1%–2% (Żabicka, unpublished data).

Colistin was discovered in 1940s. This drug is a polycationic antibiotic peptide and is active only against Gram-negative rods. Because of neurotoxicity and nephrotoxicity, it was not used

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in treatment of infections, but irrational use of common antibiotics and more frequent emergence of carbapenem-resistant strains have made it the most frequent use of drug as last choice. Colistin resistance may result from chromosomal mutations that cause change in the external lipopolysaccharide (modification of lipid A). This modification decreases the negative charge of the outer membrane, reducing its interaction with colistin [2]. In *K. pneumoniae* strains, resistant to colistin chromosomal mutations occurs in the *mcrB* (deactivation of lipid A), *pmrE* *pbpP*, *pmrC*, *pagP* (neutralization of lipid A), *pmrB* (overexpression of lipid A), *lpxM* (maturation of lipid A), *phoP/phoQ*, *pmrA*, *pmrC*, and *crrABC* genes [3–5]. The most common chromosomal gene mutations occur in *mcrB* [6]. Colistin resistance could be associated with horizontal transfer of the *mcr* gene. The *mcr* gene was discovered in China in *E. coli* and *K. pneumoniae* strains isolated from human and animals [7]. The resistance associated with the *mcr* gene is mainly plasmid coded. At present, it is estimated that the *mcr* gene is present in eight variants from *mcr-1* to *mcr-8*, but the *mcr-1* is the most predominant gene [6, 8–11]. The *mcr-1* gene encodes a phosphoethanolamine transferase enzyme family and modifies lipid A [5]. Other strategies for colistin resistance include the utilization of an efflux pump AcrAB and capsule synthesis [4, 12]. Capsule polysaccharides may mask charged molecules on the outer membrane and an increased expression of efflux pumps AcrAB-TolC and KpnEF confers colistin resistance [5]. In turn, Helander et al. [13] observed a greater degree of acylation in *K. pneumoniae* strains resistant to colistin.

Thus, the aim of this study was to evaluate and compare selected methods of resistance to colistin of *K. pneumoniae* strains.

MATERIALS AND METHODS

Strain collection

Strains were collected within 4 months in 2017 from patients in Dr. A. Jurasz University Hospital in Bydgoszcz (Poland). During this period, 321 *K. pneumoniae* strains were isolated, of which 65 (20.2%) were resistant to colistin. The study included 65 non-replicate *K. pneumoniae* strains isolated from clinical samples. The analyzed strains were isolated from different specimens: bronchoalveolar lavage (21), blood (16), wound swabs (8), urine (8), and biomaterials (2). Ten (15.4%) strains were isolated from colonization: rectal swabs, throat swabs, and stool. The multidrug resistance strains were selected for this study. Eighteen (27.7%) patients from whom *K. pneumoniae* strains had been isolated were treated with colistin. Fifteen (23.0%) patients were not treated with antibiotics. The strains were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker, Billerica, USA). The susceptibility to antibiotics was tested by automated method in Phoenix system using NMIC-402 cart (Becton-Dickinson, New Jersey, USA).

Phenotypic detection of beta-lactam resistance

A double disk synergy test was performed for *K. pneumoniae* strains to detect ESBLs [14]. The ability to produce metallo-beta-lactamases (MBLs) was determined by Carba NP test [15] and double disk synergy test with ethylenediaminetetraacetic acid. Both tests were performed in accordance with the recommendations of National Centre for Susceptibility Testing [16].

Phenotypic detection of colistin resistance

Minimum inhibitory concentration (MIC) of colistin was determined by microdilution method applying three different tests: SensiTest Colistin (Liofilchem, Via Scozia, Italy) [17–19], MIC-Strip (Merlin, Berlin, Germany) [18, 19], and MIC COL (Diagnostics, Brno, Czech Republic) [19, 20]. Colistin MICs ranged from 0.25 to 16 mg/L in SensiTest Colistin and MIC COL tests and from 0.0625 to 64 mg/L in MIC-Strip test.

Molecular detection of colistin resistance

For selected strains, eazyplex® SuperBug CRE test (Amplex Diagnostics, Gars AM Inn, Germany) was carried out. This test is based on the loop mediated isothermal amplification method (LAMP), and can detect gene encoding resistance to colistin associated with *mcr-1* gene [18].

Escherichia coli NCTC 13440 (colistin-resistant, *mcr-1* positive) was used as positive control strain.

RESULTS

All analyzed *K. pneumoniae* strains produced ESBL. Eleven (16.9%) *K. pneumoniae* strains produced MBLs. These strains were resistant to all antibiotics. Seventeen (26.1%) strains were susceptible only to one drug, tigecycline, imipenem, or gentamicin. Twenty-two (33.9%) strains were susceptible to carbapenems (imipenem and meropenem). The results of susceptibility to selected antibiotics are presented in Table I. Other *K. pneumoniae* strains were susceptible to imipenem, meropenem, and tigecycline. Among analyzed *K. pneumoniae* strains MIC₅₀ was 16 mg/L, and MIC₉₀ was 16 mg/L in SensiTest Colistin and MIC COL tests, respectively. In MIC-Strip test, value of MIC₅₀ was 16 mg/L and MIC₉₀ was 32 mg/L. MICs for colistin in SensiTest Colistin ranged from 2 to >16 mg/L; in MIC-Strip test from 1 to >64 mg/L, and in MIC COL from 4 to >16 mg/L. The results of colistin MICs are presented in Table II. The MIC values for colistin were highest for strains isolated from patients treated with this drug.

DISCUSSION

In recent years, strains resistant to all available drugs have been increasingly isolated. This is due to the excessive and



Table I. Susceptibility to selected antimicrobials of *K. pneumoniae* strains ($n = 65$)

Imipenem	Meropenem	Gentamicin	Tigecycline	No. (%) of strains
Susceptible	Susceptible	Resistant	Resistant	22 (33.9)
Susceptible	Susceptible	Resistant	Susceptible	15 (23.1)
Resistant	Resistant	Resistant	Resistant	11 (16.9)
Susceptible	Resistant	Resistant	Resistant	10 (15.4)
Resistant	Resistant	Resistant	Susceptible	4 (6.1)
Resistant	Resistant	Susceptible	Resistant	3 (4.6)

Table II. Colistin MIC values of *K. pneumoniae* strains ($n = 65$)

Colistin MIC (mg/L)	No. of <i>K. pneumoniae</i> strains		
	SensiTest Colistin (Liofilchem)	MIC-Strip (Merlin)	MIC COL (Diagnostics)
>64	–	13	–
64	–	8	–
32	–	14	–
>16	43	–	27
16	5	16	22
8	10	7	10
4	5	3	6
2	2	4	–

Note: MIC: minimum inhibitory concentration.

uncontrolled use of antibiotics, but also the high epidemic potential of multidrug-resistant strains. Since the discovery of the first *K. pneumoniae* ESBL-positive strain in 1983, resistance to subsequent antibiotics has progressed quickly. In particular, the emergence of carbapenem-resistant strains made this situation even worrisome; therefore, therapy options returned to “old-fashion” drugs.

Colistin was isolated from *Paenibacillus polymyxa* subsp. *colistinus* in 1947 [21]. For many years, it was discontinued in clinical practice due to its high toxicity. In the era of multidrug resistance, however, a return to its use is observed. Therefore, the appearance of colistin-resistant strains is disturbing, because there are no effective antibacterial therapies available.

The incidence of acquired colistin-resistant strains among *Enterobacteriaceae* varies widely and ranges from 1% [22] to 15.9% [20]. Qamar et al. [20], when assessing the MIC of colistin against strains of the *Enterobacteriaceae*

family, obtained MIC₅₀ – 0.5 mg/L and MIC₉₀ – 16 mg/L. *K. pneumoniae* strains were accounting for over 50% of the tested strains. All analyzed strains produced carbapenemases. In turn, Galani et al. [23] reported MIC₅₀ – 1 mg/L and MIC₉₀ > 16 mg/L. They used SensiTest Colistin (Liofilchem). Obtained results in this test were higher for MIC₅₀ – 16 mg/L. Bosacka et al. [19] reported categorical agreements for 96.1% of analyzed Gram-negative strains in SensiTest colistin (Liofilchem), 94.1% in MIC-Strip (Merlin), and 93.1% – MIC COL (Diagnostics). In turn, Sekyere [18] obtained categorical agreements for 89.0%–98.9% of analyzed *Enterobacteriaceae* and non-fermenter rods in SensiTest colistin (Liofilchem) and 91.0% in MIC-Strip (Merlin). In the presented work, three different tests were applied using the broth microdilution method for determining the sensitivity to colistin. At present, this method is recommended by EUCAST. In all tests, the results were similar. Differences were observed in two strains, where the MIC values were different, which affected the interpretation. Sensitive result was obtained in SensiTest Colistin and MIC-Strip tests (MIC was 2 mg/L) and resistant MIC COL test (MIC 4 mg/L). The broth microdilution tests are simple and relatively cheap [18]. At work, almost 28% of patients were previously treated with colistin, so colistin therapy is an important risk factor for acquiring resistance to this drug.

None of the tested strains carried the *mcr-1* gene; therefore, resistance to colistin was probably related to chromosomal mutations. Casselli et al. [24], when estimating the presence of *mcr-1* gene, obtained 8.3% of *Enterobacteriaceae* strains. In turn, Sekyere [18] reported 100% sensitivity and 100% specificity using eazyplex® SuperBug CRE test. The author examined 104 strains of *Enterobacteriaceae* family. Eazyplex® SuperBug CRE test is simple to perform and short, but expensive.

The emergence of multidrug-resistant strains often leads to difficult choices regarding antibiotic therapy. Limited or no therapeutic options force the use of drugs with reduced susceptibility, non-standard combination therapies, or increasing the dose of the drug. Therefore, it is necessary to monitor the emergence of resistant strains and rapid detection of resistance to colistin.

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Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. EARSS: West Nile virus infection. Available at <http://ecdc.europa.eu>
2. Velkov, T., Deris, Z. Z., Huang, J. X., Azad, M. A. K., Butler, M., Sivanesan, S., Kaminskas, L. M., Dong, Y. D., Boyd, B., Baker, M. A., Cooper, M. A., Nation, R. L., Li, J.: Surface changes and polymyxin interactions with a resistant strain of *Klebsiella pneumoniae*. *Innate Immunol* **20**, 350–363 (2014).
3. Cannatelli, A., Giani, T., Antonelli, A., Principe, L., Luzzaro, F., Rossolini, G. M.: First detection of the *mcr-1* colistin resistance gene in *Escherichia coli* in Italy. *Antimicrob Agents Chemother* **60**, 3257–3258 (2016).
4. Olaitan, A. O., Diene, S. M., Kempf, M., Berrazeg, M., Bakour, S., Gupta, S. K., Thongmalayvong, B., Akkhavong, K., Somphavong, S., Paboriboune, P., Chaisiri, K., Komalamisra, C., Adelowo, O. O., Fagade, O. E., Banjo, O. A., Oke, A. J., Adler, A., Assous, M. V., Morand, S., Raoult, D., Rolain, J. M.: Worldwide emergence of colistin resistance in *Klebsiella pneumoniae* from health humans and patients in Lao PDR, Thailand, Israel, Nigeria and France owing to inactivation of the PhoP/PhoQ regulator mgrB: An epidemiological and molecular study. *Int J Antimicrob Agents* **44**, 500–507 (2014).
5. Navon-Venezia, S., Kondratyeva, K., Carattoli, A.: *Klebsiella pneumoniae*: A major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev* **41**, 252–275 (2017).
6. Pragasam, A. K., Shankar, C., Veerarghavan, B., Biswas, I., Nabarro, L. E. B., Inbanathan, F. Y., George, B., Vergese, S.: Molecular mechanism of colistin resistance in *Klebsiella pneumoniae* causing bacteremia from India – A first report. *Front Microbiol* **7**, 2135 (2017).
7. Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X.: Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *Lancet Infect Dis* **16**, 161–168 (2016).
8. Carattoli, A., Villa, L., Feudi, C., Curcio, L., Orsini, S., Luppi, A.: Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. *Euro Surveill* **22**, 30589 (2017).
9. Xavier, B. B., Lammens, C., Ruhel, R., Kumar-Singh, S., Butaye, P., Goossens, H.: Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill* **21**, 30280 (2016).
10. Roer, L., Hansen, F., Stegger, M., Sönksen, U. W., Hasman, H., Hammerum, A. M.: Novel *mcr-3* variant, encoding mobile colistin resistance, in an ST131 *Escherichia coli* isolate from bloodstream infection, Denmark, 2014. *Euro Surveill* **22**, 30584 (2017).
11. Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S., Zhang, S., Shen, J., Shen, Z., Wang, Y.: Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. *Emerg Microbes Infect* **7**, 122 (2018).
12. Padilla, E., Llobet, E., Domenech-Sanchez, A., Martinez-Martinez, L., Bengoechea, J. A., Alberti, S.: *Klebsiella pneumoniae* AcrB efflux pump contributes to antimicrobial resistance and virulence. *Antimicrob Agents Chemother* **54**, 177–183 (2010).
13. Helander, I. M., Kato, Y., Kilpelainen, I., Kostainen, R., Lindner, B., Nummila, K.: Characterization of lipopolysaccharides of polymyxin-resistant and polymyxin-sensitive *Klebsiella pneumoniae* O3. *Eur J Biochem* **237**, 272–278 (1996).
14. Jarlier, V., Nicolas, M. H., Fournier, G., Philippon, A.: Extended broad-spectrum β -lactamases conferring resistance to newer β -lactam agents in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. *Rev Infect Dis* **10**, 867–878 (1998)
15. Nordmann, P., Poirel, L., Dortet, L.: Rapid detection of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* **18**, 1503–1507 (2012)
16. Narodowy Program Ochrony Antybiotyków: Zalecenia dotyczące postępowania w przypadku zachorowań sporadycznych i ognisk epidemicznych wywołanych przez Gram-ujemne pałeczki z rodziny Enterobacteriaceae. Zalecenia Ministra Zdrowia dotyczące postępowania w przypadku identyfikacji szczepów bakteryjnych Enterobacteriaceae wytwarzających karbapenemazy typu KPC, MBL lub OXA-48 [National Antibiotic Protection Program: Recommendations for sporadic cases and epidemic outbreaks cause by Enterobacteriaceae rods. Recommendations of the Minister of Health. *Enterobacteriaceae* producing carbapenemases: KPC, MBL or OXA-48]. Available at <http://www.antybiotyki.edu.pl>
17. Izdebski, R., Baraniak, A., Bojarska, K., Urbanowicz, P., Fiett, J., Pomorska-Wesołowska, M., Hryniewicz, W., Gniadkowski, M., Żabicka, D.: Mobile *mcr-1*-associated resistance to colistin in Poland. *J Antimicrob Chemother* **71**, 2331–2333 (2016).
18. Sekyere, J. O.: Mcr colistin resistance gene: A systematic review of current diagnostics and detection methods. *Microbiologyopen* **8**, e00682 (2019).
19. Bosacka, K., Kozińska, A., Stefaniuk, E., Rybicka, J., Mikołajczyk, A., Młodzińska, E., Hryniewicz, W.: Colistin antimicrobial susceptibility testing of Gram-negative bacteria – Evaluation of test available in Poland. In 28th ECCMID, Madrid Spain, E0116, 2018.
20. Qamar, S., Shaheen, N., Shakoor, S., Farooqi, J., Jabeen, K., Hasan, R.: Frequency of colistin and fosfomycin resistance in carbapenem-resistant *Enterobacteriaceae* from a tertiary care hospital in Karachi. *Infect Drug Resist* **10**, 231–236 (2017).
21. Poirel, L., Jayol, A., Nordmann, P.: Polymyxins: Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microb Rev* **30**, 557–596 (2017).
22. Saavedra, S. Y., Diaz, L., Wiesner, M., Correa, A., Arevalo, S. A., Reves, J., Hidalgo, A. M., de la Cadena, E., Perenguez, M., Montano, L. A.: Genomic and molecular characterization of



- clinical isolates of *Enterobacteriaceae* harboring *mcr-1* in Colombia, 2002 to 2016. *Antimicrob Agents Chemother* **61**, pii: e00841-17 (2017).
23. Galani, I., Adamou, P., Karaikos, 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* **15**, 123–126 (2018).
24. Casselli, E., D'Accolti, M., Soffritti, I., Piffanelli, M., Mazzacane, S.: Spread of *mcr-1*-driven colistin resistance on hospital surfaces, Italy. *Emerg Infect Dis* **24**, 1752–1753 (2018).