Polish Journal of Microbiology
2019, Vol. 68, No 4, 417–427
https://doi.org/10.33073/pjm-2019-055

MINIREVIEW

Colistin Resistance in Enterobacterales Strains – A Current View

ELŻBIETA M. STEFANIUK[1]* and STEFAN TYSKI[1, 2]

[1] Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland
[2] Department of Pharmaceutical and Microbiology, Medical University of Warsaw, Warsaw, Poland

Submitted 18 June 2019, revised 5 November 2019, accepted 5 November 2019

Abstract

Colistin is a member of cationic polypeptide antibiotics known as polymyxins. It is widely used in animal husbandry, plant cultivation, animal and human medicine and is increasingly used as one of the last available treatment options for patients with severe infections with carbapenem-resistant Gram-negative bacilli. Due to the increased use of colistin in treating infections caused by multidrug-resistant (MDR) bacteria, the resistance to this antibiotic ought to be monitored. Bacterial resistance to colistin may be encoded on transposable genetic elements (e.g. plasmids with the *mcr* genes). Thus far, nine variants of the *mcr* gene, *mcr-1 – mcr-9*, have been identified. Chromosomal resistance to colistin is associated with the modification of lipopolysaccharide (LPS). Various methods, from classical microbiology to molecular biology methods, are used to detect the colistin-resistant bacterial strains and to identify resistance mechanisms. The broth dilution method is recommended for susceptibility testing of bacteria to colistin.

Key words: Enterobacterales, polymyxins and their use, colistin-resistance and detection methods, treatment options

Pharmacology and application

Colistin is a cationic polypeptide antibiotic, a member of the polymyxin family of molecules. It was isolated for the first time in 1949 as a product of *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*), which is an industrially significant facultative anaerobic, non-pathogenic, and endospore-forming bacillus. The polymyxin molecule consists of a peptide and a fatty acid residue. Based on the amino acid sequence of the peptide, five polymyxin (A – E) variants can be distinguished but only two variants are used in medicine, B and E (colistin). The antibacterial effect of colistin is concentration-dependent (Li 2005; Das et al. 2017). Colistin is only active against Gram-negative bacteria (GNB), such as the aerobic Enterobacterales ord. nov. (except *Proteus* spp., *Providencia* spp., *Serratia* spp., *Edwardsiella* spp., *Morganella* spp., and *Hafnia* spp.), non-fermenting rods of *Pseudomonas*, *Acinetobacter* and *Burkholderia*, and anaerobic bacteria, e.g. *Fusobacterium* and *Bacteroides* (except *Bacteroides fragilis*) (Li et al. 2005). Its antibacterial mechanism is based on the electrostatic interaction between colistin amino groups and lipid A subunits of lipopolysaccharide (LPS). Colistin displaces Mg^{2+} and Ca^{2+} ions from LPS, leading to disturbances in the outer membrane structure of the cell. This leads to increased permeability of the cell membrane and, consequently, to cell death (Schindler and Osborn 1979).

As polymyxins are poorly absorbed from the digestive tract, orally administered polymyxins are only active on bacteria in the gastrointestinal system. Polymyxins do not diffuse well into tissues and do not penetrate the cerebrospinal fluid or the pleural and peritoneal cavity. Colistin has numerous side effects, including nephrotoxicity and neurotoxicity; therefore, it cannot be used in patients with renal failure (Kostowski and Herman 2010). The levels of nephrotoxicity and neurotoxicity were the reason for its discontinued use in human medicine after 1970 (Tullu and Dhariwal 2013).

In medicine, two physical forms of colistin are available, colistin sulphate (CS) for oral and topical use, and colistin methanesulphonate (CMS) for parenteral use (Kwa et al. 2005; Li et al. 2005). Nephrotoxicity and neurotoxicity are dose-dependent (Ordooei Javan et al. 2015). Risk factors for nephrotoxicity include colistin plasma levels > 2.5–3 g/l, concomitant administration of other nephrotoxic drugs (such as anti-inflammatory

* Corresponding author: Elżbieta M. Stefaniuk, Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland; **e-mail: e.stefaniuk@nil.gov.pl**

drugs, vancomycin and aminoglycosides), the advanced age of patient, and severity of the disease (rates of nephrotoxicity 14–53%) (Kwon et al. 2010; Pogue et al. 2011). Neurotoxicity is reversible and manifests itself, among others, in the form of peripheral and facial paresthesia, dizziness/vertigo, weakness, visual disturbances, and ataxia (4–6% of patients) (Koch-Weser et al. 1970; Spapen et al. 2011). Colistin methanesulphonate has no antimicrobial activity and acts as a colistin pro-drug that does not bind to plasma proteins. After parenteral administration, approximately 20% of CMS is hydrolyzed to colistin. This is an important feature in reducing toxicity, especially nephrotoxicity (Falagas et al. 2005). When given intravenously, a large portion of CMS is eliminated mainly through the kidneys by glomerular filtration and tubular secretion, which allows the use of CMS in urinary tract infections (Kostowski and Herman 2010). The intravenous (IV) form of the drug may also be administered by inhalation (Li et al. 2005). Inhaled colistin is used for treating pneumonia and ventilator-associated pneumonia (VAP) caused by multidrug-resistant (MDR) Gram-negative microorganisms, while it is also used prophylactically in patients with cystic fibrosis. Colistin also causes the release of histamine and serotonin by monocytes, which can lead to acute respiratory failure; therefore, care is needed when administering this drug in the form of an aerosol (Dzierżanowska 2018).

In the last 20 years, the emergence of MDR Gram-negative bacilli has led to polymyxins B and E being used once again, as a "salvage" therapy in the patients with CRE (carbapenems-resistant *Enterobacteriaceae*) infections for which we do not have the better treatment options (Li et al. 2006; Nation and Li 2009; Lim et al. 2010). Orally and topically administered colistin sulphate and parenteral colistin methanesulphonate sodium are designed for the treatment of life-threatening human infections caused by Gram-negative rods. Colistin has been approved by the American Thoracic Society and Infectious Diseases Society of America, who have provided guidelines for the treatment of VAP caused by MDR Gram-negative rods (American Thoracic Society and Infectious Diseases Society of America 2005). The parenteral form of colistin has also been evaluated for the treatment of other serious infections caused by MDR *P. aeruginosa*, *A. baumannii*, and *Enterobacteriaceae*, such as sepsis, abdominal infections, bone and joint infections, urinary tract infections, and meningitis (Falagas et al. 2005; Walkty et al. 2009; Batirel et al. 2014). Recent studies have demonstrated acceptable effectiveness and considerably less toxicity than had been reported on polymyxins in older studies (Ordooei Javan et al. 2015). However, randomized controlled trials are urgently needed to further clarify the issues surrounding the efficacy and safety of polymyxins.

Colistin resistance mechanisms

Bacteria acquire resistance to colistin as a result of mutations and adaptation mechanisms. Different molecular mechanisms are associated with colistin resistance in Gram-negative bacteria; there are, among others, changes in the two-component systems: *pmrA*/*pmrB (Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Acinetobacter baumannii,* and *Pseudomonas aeruginosa)*, *phoP*/*phoQ (K. pneumoniae, Salmonella* spp.), *parR*/*parS* (*P. aeruginosa*), *colR*/*colS* (*P. aeruginosa*), and *cprR*/*cprS* (*Campylobacter jejuni*) (Olaitan et al. 2014b). Mechanisms of resistance differ among Gram-negative bacterial species. The most important chromosomal mechanism of colistin resistance in *K. pneumoniae* is an alteration of the *mgrB* gene, which encodes a negative regulator of *phoP*/*phoQ* system (Jayol et al. 2015). Colistin resistance is mainly achieved by modification of LPS, which is the main target of colistin in the bacterial cell. Mutations that lead to the addition of cationic groups to lipid A weaken the binding of polymyxins (Olaitan et al. 2014b; Baron et al. 2016). In the case of *A. baumannii*, similar changes in the *lpxA*, *lpxC* and *lpxD* genes as described above, cause inhibition of lipid A biosynthesis and thus loss of the polymyxin target in the bacteria (Moffatt et al. 2010). There is the hypothesis that colistin resistance of clinical isolates results from a combination of porin mutations and overexpression of efflux pump systems (Olaitan et al. 2014b).

Bacterial colistin resistance may be coded on transposable genetic elements (mostly plasmids with the *mcr* genes). Thus far, nine variants of the *mcr* genes, *mcr-1* – *mcr-9*, have been identified in various Enterobacterales and *Moraxella* species. The first plasmid-mediated colistin resistance was detected in an *E. coli* strain collected from food animals in China in 2015 (Liu et al. 2016). Since then, the plasmid-mediated colistin resistance in Enterobacterales has been reported worldwide, including human infections, also from Poland (Izdebski et al. 2016). The *mcr-1* gene modifies LPS by encoding phosphoethanolamine transferase (pEtN transferase), which mediates the addition of pEtN to lipid A (Baron et al. 2016). Generally, *E. coli* strains with the *mcr-1* gene are characterized by the low-level colistin resistance with a minimum inhibitory concentration (MIC) in the range of 2–8 mg/l. Zhang et al. (2019) have shown that the expression of the *mcr-1* gene in *E. coli* led to a higher mutation rate in the chromosomal polymyxin resistance cascade genes and produced higher MIC values (≥ 64 mg/l).

The *mcr-2* gene was first identified by Xavier and colleagues in *E. coli* strains isolated from calves and pigs in Belgium; MCR-1 and MCR-2 proteins showed 80.65% identity (Xavier et al. 2016). In 2017, a third mobile

colistin resistance gene, *mcr-3,* was described in *E. coli* by Yin et al. (2017). The amino acid sequence of the *mcr-3* gene product, MCR-3, showed 32.5 and 31.7% amino acid identity to MCR-1 and MCR-2, respectively (Yin et al. 2017). Also, Carrattoli et al. (2017) in 2017 detected a new plasmid-mediated colistin gene, *mcr-4*, in *Salmonella* on a small, not self-conjugative plasmid. For the first time, Borowiak et al. (2017) described a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, which conferred colistin resistance in d-tartrate-fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. In 2018, further variants, the *mcr-6 – mcr-8* genes, were described (AbuOun et al. 2017; 2018; Wang et al. 2018; Yang et al. 2018).

Recently, Carroll et al. (2019) have described the *mcr-9* gene, a novel *mcr* homologue detected in MDR colistin-susceptible *Salmonella enterica* serovar Typhimurium strain isolated from a patient in the Washington State in 2010. This strain was phenotypically sensitive to colistin with a MIC value of 2 mg/l, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. The *mcr-9* gene was cloned into colistin-sensitive *E. coli* and the expression-conferred *E. coli* NEB5α strain with resistance to 1, 2 and 2.5 mg/l colistin. Pairwise comparison of the predicted protein structures of all nine *mcr* homologues (*mcr-1* to *mcr-9*) revealed that the *mcr-3*, *mcr-4*, *mcr-7*, and *mcr-9* genes share a high degree of similarity at the structural level (Carroll et al. 2019).

Colistin uses in human medicine

Colistin is used for treating infections with carbapenem-resistant *Enterobacteriaceae* (CRE) that belong to multi-resistant isolates and have already been reported worldwide (Grundmann et al. 2010). The seriousness of the problem is underlined by high (> 30%) mortality of hospitalized patients infected with carbapenem-resistant strains (Capone et al. 2013; Ghafur et al. 2014; Guducuoglu et al. 2018; Zhang et al. 2018). Such infections are difficult to treat and with limited therapeutic options (Parisi et al. 2015; Baraniak et al. 2016). Tumbarello et al. (2012) analyzed the course of *K. pneumoniae* KPC-positive infection in the patients; combination therapy with tigecycline, colistin, and meropenem was associated with a lower risk of mortality (12.5%). The authors also indicate that incorrect empirical therapy is a significant factor in the mortality rate of the patients infected with carbapenem-resistant *K. pneumoniae* (Tumbarello et al. 2012; Tumbarello et al. 2015).

Moreover, the development of outbreaks by colistin-resistant Gram-negative bacilli producing carbapenemases is a great problem (Antoniadou et al. 2007; Marchaim et al. 2011; Monaco et al. 2014; Olaitan et al. 2014a; Parisi et al. 2015; Jayol et al. 2016; Gundogdu et al. 2018). In 2013, the colistin resistance rate has risen to an average of over 30% of CRE isolates, including Italy, Spain, and Greece, and constituted accordingly 43, 31, and 20.8%, respectively (ECDC 2014; Monaco et al. 2014; Pena et al. 2014; Meletis et al. 2015). The increased mortality is also related to infections with colistin-resistant strains (Capone et al. 2013). Colistin resistance makes the choice of antimicrobial agents difficult, and the use of therapeutic options for colistin-resistant MDR isolates depends on the sensitivity phenotype of the isolates, the infection type and site, antimicrobial PK/PD properties, and potential side effects (Petrosillo et al. 2019).

Due to the increasing role of colistin in the treatment of human infections with MDR bacteria, the resistance to this antibiotic should be carefully monitored. The use of colistin in human medicine is assumed to be a cause for the occurrence of colistin resistance in Enterobacterales, particularly in *K. pneumoniae* (Sandri et al. 2013; Vicari et al. 2013).

Ceftazidime/avibactam, as a combination of β-lactam and β-lactamases inhibitor, plays an important role in the treatment of MDR *K. pneumoniae* infections, including colistin-resistant isolates producing KPC (Jayol et al. 2018a). It is registered for the treatment of abdominal infections, urinary tract infections and nosocomial pneumonia (Zhanel et al. 2013). Avibactam inhibits class A, C, and D β-lactamases, including KPC and OXA-48 carbapenemases (Shields et al. 2015; Pogue et al. 2019), but does not inhibit metallo-β-lactamases (Ambler class B) due to the absence of the active-site serine residue in these enzymes (Davido et al. 2017). The combination of ceftazidime/avibactam with aztreonam showed activity against *K. pneumoniae* strains, regardless of the type of carbapenemase produced (Davido et al. 2017; Jayol et al. 2018a). Ceftazidime/avibactam therapy is less nephrotoxic compared to aminoglycosides or colistin (Zhanel et al. 2013). However, it has been reported that *K. pneumoniae* acquired resistance to ceftazidime with avibactam during treatment (Shields et al. 2017; Gaibani et al. 2018).

The combination of meropenem with vaborbactam is the new antimicrobial agent active against KPC-positive *K. pneumoniae* (Pfaller et al. 2018; Pogue et al. 2019), and it is registered for the treatment of respiratory pneumonia and bacteraemia (U.S. National Library from Medicine 2019). Vaborbactam does not inhibit class D or class B carbapenemases and due to the risk of developing resistance, meropenem/vaborbactam should be reserved for the treatment of infections caused by MDR strains, including colistin-resistant *K. pneumoniae* KPC-positive (Lomovskaya et al. 2017).

Methods for susceptibility testing

It is highly important to develop phenotypic tests capable of detecting the colistin resistance in Gram-negative rods. Until recently, there was no consensus as to the methodology for colistin susceptibility testing. The disc diffusion method and gradient tests proved to be unreliable due to the poor diffusion of colistin in agar (Galani et al. 2008; Behera et al. 2010; Dafopoupolu et al. 2015; Chew et al. 2017; Vasoo 2017; Giske and Kahlameter 2018). Therefore, disk diffusion and gradient diffusion are not valid techniques for the determination of susceptibility to polymyxins.

In 2016, both EUCAST and the Clinical and Laboratory Standards Institute (CLSI) recommended the International Standard Organization (ISO) 20776 standard broth dilution method for testing of the MIC values of colistin (CLSI 2016; EUCAST 2016). However, the reference broth microdilution method is difficult to apply in routine microbiological diagnostics. The EUCAST does not recommend the use of automated systems to determine the phenotype of bacterial sensitivity such as Vitek 2, (bioMerieux, France), BD Phoenix (Becton Dickinson, USA), as well as WalkAway (Beckman Coulter, USA) for the analysis of the sensitivity of Gram-negative bacilli to colistin. This is because these systems have fairly limited accuracy in determining colistin MIC, particularly in the range of 2–4 mg/l when compared to the reference method (Nordmann et al. 2016b; Bosacka et al. 2018; Matuschek et al. 2018b; Lellouche et al. 2019).

The literature data indicate the usefulness of several commercially available systems that are based on the broth microdilution method, such as the MIC-Strip Colistin (Merlin,Germany), Microlatest MIC Colistin (Erba Lachema, Czech Republic), Sensitest Colistin (Liofilchem, Italy), and MIC COL (Diagnostics, Slovakia) for the evaluation of the sensitivity of Enterobacterales and non-fermenting rods to colistin (Nordmann et al. 2016b; Matuschek et al. 2018a; Bosacka et al. 2018; Lellouche et al. 2019). Members of colistin-resistant bacilli are usually correctly categorized as resistant using the above-mentioned methods (Chew et al. 2017; Poirel et al. 2017). An increasing number of recent reports point to the heterogeneity of strains detected *via* microdilution in broth (Chew et al. 2017).

Methods for the detection of colistin resistance

The innovatory technique for the identification of colistin resistance is the Rapid Polymyxin NP (Nordmann/Poirel) test. It was developed by the Nordmann's group for the colistin susceptibility testing in Enterobacterales (Nordmann et al. 2016b). Currently, the researchers are underway to use this test for the detection of colistin resistance in non-fermenting bacilli. The Rapid Polymyxin NP test detects fermentation of glucose associated with bacterial growth in the presence of a defined concentration of polymyxin E or B; the presence of acid metabolites is evidenced by the change in the pH and the indicator (red phenol) color from orange to yellow. The sensitivity and specificity of the test are highly comparable to the reference broth microdilution method (99.3 and 95.4%, respectively). This test is easy to perform and provides a result in less than 2 hours (Nordmann et al. 2016b).

Chromogenic media are commonly used for screening; they allow the growth of sought bacteria as properly colored colonies. The first agar medium for detecting colistin-resistant Gram-negative rods from bacterial cultures and rectal swab samples was the SuperPolymyxin screening medium (Nordmann et al. 2016a); the commercial version of this medium is SuperPolymyxin medium (ELITechGroup, Puteaux, France) for detecting colistin-resistant Enterobacterales strains, including these with the low MIC values (mg/l) that harbor the *mcr-1* gene (Jayol et al. 2018). It is composed of eosin methylene blue (EMB) agar and includes colistin, daptomycin, and amphotericin B (3.5, 10, and 5 µg/ml, respectively). The other medium, CHROMagar COLAPSE medium for the detection of colistin-resistant strains was compared to the SuperPolymyxin medium (Abdul Momin et al. 2017); this medium differentiates colistin-resistant Enterobacterales strains from non-fermenting rods. Bardet et al. (2017) described the LBJMR medium, a new polyvalent culture medium for the isolation and selection of colistin-resistant bacteria and vancomycin-resistant bacteria. This medium was developed by the addition of colistin sulphate salt (4 µg/ml), vancomycin (50 µg/ml), and a substrate for fermentation (7.5 g/l of glucose) to a Purple Agar Base (31 g/l). In early 2018, a new chromogenic medium, CHROMID Colistin R agar (COLR; bioMérieux, France) came into the market and allowed the screening of colistin-resistant *Enterobacteriaceae* in clinical samples, such as stools and rectal swabs. The COLR is a manual qualitative diagnostic test, which allows colistin-resistant isolates to be distinguished from those that are susceptible. Colistin-resistant strains forming colored colonies on chromogenic media and their color depends on the species. By contrast, colistin-susceptible isolates do not grow on the COLR plate (García-Fernández et al. 2019).

The chromogenic method is based on the dilution in agar, although EUCAST does not recommend this procedure for the determination of bacterial susceptibility to colistin, as the threshold of the detectability increases with the growth of the bacterial inoculum (Matuschek et al. 2018b). However, Turlej-Rogacka et al. (2018) reported that when compared to broth

dilution methods, the method of the dilution in agar yields more accurate results in the evaluation of the colistin MIC values (Turlej-Rogacka et al. 2018). Behera and colleagues (2010) confirmed the high correlation of results between the reference method and the agar dilution method (Behera et al. 2010; Dafopouolu et al. 2015). The greatest challenge in colistin handling is its binding to plastic (Humphries 2015; Matuschek et al. 2018b). According to the above-mentioned authors, the agar dilution method significantly reduces the phenomenon of colistin-plastic binding, and the MICs results obtained with this method are characterized by a high accuracy (Behera et al. 2010; Humphries 2015; Matuschek et al. 2018).

The COLR medium uses the borderline colistin concentrations that allow qualification of the strains studied as susceptible or resistant. This chromogenic medium is a qualitative method of Enterobacterales detection and does not allow the determination of the colistin MIC values against the bacterial strains analyzed. As such, it should only be regarded as a screening test. On the other hand, in treating the infections caused by colistin-resistant bacteria, the clinical interpretation is significant. This entails the categorization of colistin resistance rather than the determination of the specific MIC value since maximum dosages of the medication are prescribed independently of precise susceptibility levels. However, the MIC values are important in monitoring the increase in the resistance to colistin observed in the intestinal bacteria.

Other new-generation methods have been developed recently to detect colistin-resistant strains: the loop-mediated isothermal amplification (LAMP) for nucleic acid detection (Zou et al. 2017), and a microarray CT103XL (Bernasconi et al. 2017). Zou et al. (2017) showed that the LAMP test is ten times more sensitive than the conventional PCR and confirmed its usefulness for the detection of the *mcr-1* gene in Enterobacterales strains from stool samples. Similarly, Bernascini et al. (2017) demonstrated the usefulness of the new CT103XL microarray for the rapid characterization of multidrug-resistant Gram-negative bacteria through simultaneously identifying the *mcr-1*, *mcr-2*, and clinically important *bla* genes.

Colistin in veterinary medicine and agriculture

Colistin sulphate has also been widely and heavily used for decades in veterinary medicine for the treatment of intestinal infections in pigs, poultry, and cattle, which were caused by Enterobacterales strains, mainly *E. coli* and *Salmonella* spp. (Liu et al. 2016). In these situations, colistin is chiefly used in an oral form, and its usage varies widely among different countries. In Spain, it is used during gestation and lactation, the post-weaning period, and for metaphylactic intestinal disease control (Casal et al. 2007). During the post-weaning period, it is used in 50% and 35% of pig farms in France and Austria, respectively (Kempf et al. 2013; Trauffler et al. 2014). In Sweden, colistin was the most frequently used antibiotic in 18% of weaned piglet herds (Sjölund et al. 2015). A German study on antimicrobial use in pigs has revealed that polypeptides accounted for 4.2% of the total use per kg but regarding treatment units, they were among the three most frequently used antimicrobial classes (van Rennings et al. 2015). A Netherland study has shown that colistin, as one of the most used antimicrobials next to tetracyclines, trimethoprim/sulfonamides, macrolides, and lincosamides was available on the prescription and deliveries for pigs, veal calves, and broilers in the country (Bos et al. 2013).

In Asian countries, the use of antibiotics, particularly colistin, in animal husbandry also takes place on a large scale. China is one of the world's largest users of colistin in agriculture; over 11 thousand tons of colistin is being used (QYResearch Medical Research Centre 2015). Considering this upward trend, the consumption of colistin in Chinese agriculture is estimated to reach more than 16 thousand tons in 2021 (QYResearch Medical Research Centre 2015). China remains the largest user of colistin in agriculture worldwide. In the Red River Delta region of Vietnam, colistin was also used as a feed additive for growth promotion in pig production (Kim et al. 2013). This was a cause of concern because colistin is an unapproved antibiotic for growth promotion in Vietnam (MARD 2006; 2009). These facts illustrate the sheer scale of antibiotic consumption in animal and poultry husbandry.

Alarming data on the use of antibiotics in veterinary medicine, in particular colistin, has led to efforts to limit their use. The different monitoring systems for the use of antibiotics in animals and the surveillance of resistance to antibiotics were established in European countries (BTK 2015; Borck Høg et al. 2017; SDa Autoriteit Diergenesmiddelen 2018; SWEDRES/SVARM 2018; MARAN 2019). In 2015, Nunan and Young (2015) reported that antibiotics, particularly colistin, should not be routinely used as prophylactics in animal farms in the United Kingdom (UK). The authors added that colistin accounted for only 0.2% of all antibiotics that were used in breeding in the UK and was only used by veterinarians to treat sick animals (EMA/CVMP 2010; Nunan and Young 2015; Catry et al. 2015).

In a national report on antibiotics consumption in the Australian pig industry, Jordan et al. (2009) found that colistin was not used during the study period in the production of pigs.

Until recently, there were no recommendations on the need of conducting the screening tests to find

the carriage of colistin-resistant bacteria, but under a 'One Health' perspective, it is necessary to monitor the colistin resistance among Gram-negative bacteria in veterinary and human medicine. Currently, at least in the veterinarian sector in Germany, screening for colistin resistance is recommended and carried out routinely, and efforts are being made to implement colistin screening also for human isolates. It, therefore, seems justified to develop a chromogenic agar medium for detecting colistin-resistant rods directly from clinical material other than stools and rectal swabs, e.g. samples from the lower respiratory tract or urine samples.

Colistin-resistant strains in plant food and the environment

There are progressively more and more reports on the culture of the colistin-resistant Enterobacterales strains from vegetables and fruits samples (Liu et al. 2014; Jones-Dias et al. 2016; Luo et al. 2017). A study by Zhon et al. (2017) showed that water, where live bacteria may have come from the excrements, can be the source of plant contamination with Gram-negative bacilli (Zhon et al. 2017). Jung et al. (2014) analyzed the relationship between the plant food production chain and the incidence of foodborne disease outbreaks, and the consumption of contaminated raw vegetables has been linked with these outbreaks (Jung et al. 2014). Liu et al. (2014) studied the samples of vegetables (carrots, pak choi, green peppers, and leaf lettuce) from supermarkets or farmers' markets in nine provinces of China; about 4% of the vegetable samples (3.6%) carried one or more the *mcr*-positive isolates (*E. coli* and *Enterobacter cloacae*); the dissemination of the *mcr-1* gene was mediated by plasmids. All isolates were MDR; however, they were susceptible to meropenem and tigecycline (Liu et al. 2014). Jones-Dias et al. (2016) showed the presence of the *mcr-1* gene in lettuce samples in Portugal (Jones-Dias et al. 2016).

Zurfuh et al. (2016) reported the presence of the plasmid-borne *mcr-1* colistin resistance gene in the extended-spectrum β-lactamase (ESBL) producing *E. coli* strains from rivers and lakes in Switzerland, and the ready-to-eat imported vegetables from Asian countries (Zurfuh et al. 2016). The *E. coli* strains with the *mcr-1* genes belonged to different multilocus sequence types (MLSTs), which harbored different the bla_{ESBL} genes. This suggests that the *mcr-1* gene can be spread on different plasmids. Luo et al. (2017) described the identification of the *mcr-1* gene in *E. coli* and *Raoultella ornithinolytica* ESBL-producing isolates collected from fresh vegetable samples in Guangzhou, China. *Raoultella ornithinolytica* belongs to a genus closely related to *Klebsiella* and is an environmental microorganism associated with community-acquired infections, but the number of *R. ornithinolytica* infections might have been underestimated due to its misidentification as a *Klebsiella* spp. (Luo et al. 2017). Li et al. (2017) showed that the *mcr-1* gene in isolates from Guangzhou was located on IncHI2/ST3, IncI2, and IncX4 plasmids in both isolates from animals and humans. The studies cited here differ in the number and variety of the vegetables examined; however, as vegetables are often consumed raw, the presence of bacteria carrying the *mcr-1* gene may pose a threat to public health.

Summary

The resistance of Gram-negative rods to colistin, including Enterobacterales, is a serious public health problem. The colistin use in animal husbandry and agriculture has an impact on the spread of colistin resistance (Catry et al. 2015). The *mcr* genes were found in bacteria isolated from various food sources as animal meat and vegetables as well as the environment (including rivers and lakes water), infected patients, and asymptomatic human carriers. The detection of the antimicrobial resistance genes is critical for the prevention of the spread of bacterial resistance. There are several phenotypic and genotypic methods to detect colistin-resistant strains, determine the colistin MIC values and identify colistin resistance mechanisms. The easy transmission of resistance genes among microorganisms poses a challenge to the therapy of MDR bacterial infections, especially caused by carbapenem-resistant Enterobacterales. Therefore, resistance to colistin in the members of the Enterobacterales should be perceived as an important global health problem, requiring multisectoral, further research as well as a proper monitoring and surveillance systems.

ORCID
Elżbieta M. Stefaniuk 0000-0002-8169-6964
Stefan Tyski 0000-0003-3352-038X

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Abdul Momin MHF, Bean DC, Hendriksen RS, Haenni M, Phee LM, Wareham DW. CHROMagar COL-APSE: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogens. J Med Microbiol. 2017 Nov 01;66(11):1554–1561. https://doi.org/10.1099/jmm.0.000602

AbuOun M, Stubberfield EJ, Duggett NA, Kirchner M, Dormer L, Nunez-Garcia J, Randall LP, Lemma F, Crook DW, Teale C, et al. *mcr-1* and *mcr-2* (*mcr-6.1*) variant genes identified in *Moraxella* species isolated from pigs in Great Britain from 2014 to 2015. J Antimicrob Chemother. 2017 Oct 01;72(10):2745–2749. https://doi.org/10.1093/jac/dkx286

AbuOun M, Stubberfield EJ, Duggett NA, Kirchner M, Dormer L, Nunez-Garcia J, Randall LP, Lemma F, Crook DW, Teale C, et al. *mcr-1* and *mcr-2* (*mcr-6.1*) variant genes identified in *Moraxella* species isolated from pigs in Great Britain from 2014 to 2015. J Antimicrob Chemother. 2018 Oct 01;73(10):2904. https://doi.org/10.1093/jac/dky272

American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med. 2005 Feb 15;171(4):388–416. https://doi.org/10.1164/rccm.200405-644ST

Antoniadou A, Kontopidou F, Poulakou G, Koratzanis E, Galani I, Papadomichelakis E, Kopterides P, Souli M, Armaganidis A, Giamarellou H. Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster. J Antimicrob Chemother. 2007 Apr 01;59(4):786–790. https://doi.org/10.1093/jac/dkl562

Baraniak A, Izdebski R, Fiett J, Gawryszewska I, Bojarska K, Herda M, Literacka E, Żabicka D, Tomczak H, Pewińska N, et al. NDM-producing Enterobacteriaceae in Poland, 2012–14: inter-regional outbreak of *Klebsiella pneumoniae* ST11 and sporadic cases. J Antimicrob Chemother. 2016 Jan;71(1):85–91. https://doi.org/10.1093/jac/dkv282

Bardet L, Le Page S, Leangapichart T, Rolain JM. LBJMR medium: a new polyvalent culture medium for isolating and selecting vancomycin and colistin-resistant bacteria. BMC Microbiol. 2017 Dec; 17(1):220. https://doi.org/10.1186/s12866-017-1128-x

Baron S, Hadjadj L, Rolain JM, Olaitan AO. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int J Antimicrob Agents. 2016 Dec;48(6):583–591. https://doi.org/10.1016/j.ijantimicag.2016.06.023

Batirel A, Balkan II, Karabay O, Agalar C, Akalin S, Alici O, Alp E, Altay FA, Altin N, Arslan F, et al. Comparison of colistin-carbapenem, colistin-sulbactam, and colistin plus other antibacterial agents for the treatment of extremely drug-resistant *Acinetobacter baumannii* bloodstream infections. Eur J Clin Microbiol Infect Dis. 2014 Aug;33(8):1311–1322. https://doi.org/10.1007/s10096-014-2070-6

Behera B, Mathur P, Das A, Kapil A, Gupta B, Bhoi S, Farooque K, Sharma V, Misra MC. Evaluation of susceptibility testing methods for polymyxin. Int J Infect Dis. 2010 Jul;14(7):e596–e601. https://doi.org/10.1016/j.ijid.2009.09.001

Bernasconi OJ, Principe L, Tinguely R, Karczmarek A, Perreten V, Luzzaro F, Endimiani A. Evaluation of a new commercial microarray platform for the simultaneous detection of β-lactamase and *mcr-1* and *mcr-2* genes in *Enterobacteriaceae.* J Clin Microbiol. 2017 Oct;55(10):3138–3141. https://doi.org/10.1128/JCM.01056-17

Borck Høg B, Korsgaard HB, Wolff Sönksen U, Bager H, Boratolaia V, Ellis-Iversen J, Hendriksen RS, Jensen LB, Korsgaard HB, Vorobieva V. DANMAP 2016 – Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Statens Serum Institut, National Veterinary Institute, Technical University of Denmark National Food Institute, Technical University of Denmark. 2017. https://orbit.dtu.dk/ws/files/161713656/Rapport_DANMAP_2017.pdf

Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. J Antimicrob Chemother. 2017 Dec 01;72(12): 3317–3324. https://doi.org/10.1093/jac/dkx327

Bos ME, Taverne FJ, van Geijlswijk IM, Mouton JW, Mevius DJ, Heederik DJ; Netherlands Veterinary Medicines Authority SDa. Consumption of antimicrobials in pigs, veal calves, and broilers in the Netherlands: quantitative results of nationwide collection of data in 2011. PLoS One. 2013 Oct 21;21;8(10):e77525. https://doi.org/10.1371/journal.pone.0077525

Bosacka K, Kozińska A, Stefaniuk E, Rybicka J, Mikołajczyk A, Młodzińska E, Hryniewicz W. Colistin antimicrobial susceptibility testing of Gram-negative bacteria – evaluation of tests available in Poland. ECCMID 2018 Abstract publication E0116, 21–24 April 2018; Available from https://www.escmid.org/escmid_publications/escmid_elibrary/?q=Bosacka+K&id=2173&L=0&x=28&y=20

BTK. Guidelines for the prudent use of veterinary antimicrobial drugs – with notes for guidance. Federal Veterinary Surgeons' Association (BTK = Bundestierärztekammer). Addendum to the German Veterinary Gazette 2015;(3/2015). Available from https://www.bundestieraerztekammer.de/tieraerzte/leitlinien/

Capone A, Giannella M, Fortini D, Giordano A, Meledandri M, Ballardini M, Venditti M, Bordi E, Capozzi D, Balice MP, et al. High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. Clin Microbiol Infect. 2013 Jan;19(1):E23–E30. , https://doi.org/10.1111/1469-0691.12070

Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, Pezzotti G, Magistrali CF. Novel plasmid-mediated colistin resistance gene *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill. 2017 Aug 3;22(31). https://doi.org/10.2807/1560-7917.ES.2017.22.31.30589

Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. Identification of novel mobilized colistin resistance gene *mcr-9* in a multidrug-resistant, colistin-susceptible *Salmonella enterica* serotype Typhimurium isolate. MBio. 2019;10(3):e00853-19. https://doi.org/10.1128/mBio.00853-19

Casal J, Mateu E, Mejía W, Martín M. Factors associated with routine mass antimicrobial usage in fattening pig units in a high pig-density area. Vet Res. 2007 May;38(3):481–492. https://doi.org/10.1051/vetres:2007010

Catry B, Cavaleri M, Baptiste K, Grave K, Grein K, Holm A, Jukes H, Liebana E, Navas AL, Mackay D, et al. Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. Int J Antimicrob Agents. 2015 Sep;46(3):297–306. https://doi.org/10.1016/j.ijantimicag.2015.06.005

Chew KL, La MV, Lin RTP, Teo JWP. Colistin and polymyxin B susceptibility testing for carbapenem-resistant and *mcr* -positive Enterobacteriaceae: comparison of sensititre, MicroScan, Vitek 2, and Etest with broth microdilution. J Clin Microbiol. 2017 Sep; 55(9):2609–2616. https://doi.org/10.1128/JCM.00268-17

CLSI. Performance standards for antimicrobial susceptibility testing. 26th ed. CLSI supplement M100S. Wayane (PA): Clinical and Laboratory Standards Institute; 2016.

Dafopoulou K, Zarkotou O, Dimitroulia E, Hadjichristodoulou C, Gennimata V, Pournaras S, Tsakris A. Comparative evaluation of colistin susceptibility testing methods among carbapenem-non-susceptible *Klebsiella pneumoniae* and *Acinetobacter baumannii* clinical isolates. Antimicrob Agents Chemother. 2015 Aug;59(8): 4625–4630. https://doi.org/10.1128/AAC.00868-15

Das P, Sengupta K, Goel G, Bhattacharya S. Colistin: Pharmacology, drug resistance and clinical applications. J Acad Clin Microbiol. 2017;19(2):77–85. https://doi.org/10.4103/jacm.jacm_31_17

Davido B, Fellous L, Lawrence C, Maxime V, Rottman M, Dinh A. Ceftazidime-avibactam and aztreonam, an interesting

strategy to overcome b-lactam resistance conferred by metallo-b-lactamases in *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2017 Sep;61(9):e01008-17. https://doi.org/10.1128/AAC.01008-17

Dzierżanowska D. Antybiotykoterapia praktyczna (in Polish). Bielsko-Biała (Poland): α-medica Press; 2018. p. 206–209.

ECDC. Antimicrobial Resistance Surveillance in Europe, 2013; Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm (Sweden): European Centre for Disease Prevention and Control; 2014 [cited 2019 Jun 11]. Available from https://ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2013

EMA/CVMP. Opinion following an Article 35 referral for veterinary medicinal formulations containing colistin at 2 000 000 IU per ml and intended for administration in drinking water to food producing species. Amsterdam (The Netherlands): European Medicines Agency, Committee for Medical Products for Veterinary Use; 2010 [cited 2019 Jun 11]. Available from http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Colistin_25/WC500093733.pdf.

EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016.; Basel (Switzerland): The European Committee on Antimicrobial Susceptibility Testing; 2016 [cited 2019 Jun 11]. Available from htpp://www.eucast.org

Falagas ME, Kasiakou SK, Saravolatz LD. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis. 2005 May 01;40(9):1333–1341. https://doi.org/10.1086/429323

Gaibani P, Campoli C, Lewis RE, Volpe SL, Scaltriti E, Giannella M, Pongolini S, Berlingeri A, Cristini F, Bartoletti M, et al. *In vivo* evolution of resistant subpopulations of KPC-producing *Klebsiella pneumoniae* during ceftazidime/avibactam treatment. J Antimicrob Chemother. 2018 Jun 01;73(6):1525–1529. https://doi.org/10.1093/jac/dky082

Galani I, Kontopidou F, Souli M, Rekatsina PD, Koratzanis E, Deliolanis J, Giamarellou H. Colistin susceptibility testing by Etest and disk diffusion methods. Int J Antimicrob Agents. 2008 May;31(5):434–439. https://doi.org/10.1016/j.ijantimicag.2008.01.011

Gao B, Li X, Yang F, Chen W, Zhao Y, Bai G, Zhang Z. Molecular epidemiology and riskfactors of ventilator-associated pneumonia infection caused by carbapenem-resistant *Enterobacteriaceae*. Front Pharmacol. 2019 Mar 22;10:262. https://doi.org/10.3389/fphar.2019.00262

García-Fernández S, García-Castillo M, Ruiz-Garbajosa P, Morosini MI, Bala Y, Zambardi G, Cantón R. Performance of CHROMID® Colistin R agar, a new chromogenic medium for screening of colistin-resistant Enterobacterales. Diagn Microbiol Infect Dis. 2019 Jan;93(1):1–4. https://doi.org/10.1016/j.diagmicrobio.2018.07.008

Ghafur A, Vidyalakshimi PR, Murali A, Priyadarshini K, Thirunarayan MA. Emergence of Pan-drug resistance amongst gram negative bacteria! The First case series from India. J Microbiol Infect Dis. 2014 Sep 01;4(3):86–91. https://doi.org/10.5799/ahinjs.02.2014.03.0145

Giske CG, Kahlmeter G. Colistin antimicrobial susceptibility testing – can the slow and challenging be replaced by the rapid and convenient? Clin Microbiol Infect. 2018 Feb;24(2):93–94. https://doi.org/10.1016/j.cmi.2017.10.007

Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos J, Vatopoulos A, Gniadkowski M, Toth A, Pfeifer Y, Jarlier V, Carmeli Y; CNSE Working Group. Carbapenem-non-susceptible *Enterobacteriaceae* in Europe: conclusions from a meeting of national experts. Euro Surveill. 2010 Nov 18;15(46):19711.

Guducuoglu H, Gursoy NC, Yakupogullari Y, Parlak M, Karasin G, Sunnetcioglu M, Otlu B. Hospital outbreak of a colistin-resistant, NDM-1- and OXA-48-producing *Klebsiella pneumoniae*: high mortality from pandrug resistance. Microb Drug Resist. 2018 Sep;24(7):966–972. https://doi.org/10.1089/mdr.2017.0173

Gundogdu A, Ulu-Kilic A, Kilic H, Ozhan E, Altun D, Cakir O, Alp E. Could frequent carbapenem use be a risk factor for colistin resistance? Microb Drug Resist. 2018 Jul;24(6):774–781. https://doi.org/10.1089/mdr.2016.0321

Humphries RM. Susceptibility testing of the polymyxins: where are we now? Pharmacotherapy. 2015 Jan;35(1):22–27. https://doi.org/10.1002/phar.1505

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: Table 1. J Antimicrob Chemother. 2016 Aug;71(8):2331–2333. https://doi.org/10.1093/jac/dkw261

Jayol A, Nordmann P, Brink A, Poirel L. Heteroresistance to colistin in *Klebsiella pneumoniae* associated with alterations in the PhoPQ regulatory system. Antimicrob Agents Chemother. 2015 May;59(5):2780–2784. https://doi.org/10.1128/AAC.05055-14

Jayol A, Nordmann P, Poirel L, Dubois V. Ceftazidime/avibactam alone or in combination with aztreonam against colistin-resistant and carbapenemase-producing *Klebsiella pneumoniae*. J Antimicrob Chemother. 2018 Feb 01;73(2):542–544. https://doi.org/10.1093/jac/dkx393

Jayol A, Poirel L, André C, Dubois V, Nordmann P. Detection of colistin-resistant Gram-negative rods by using the SuperPolymyxin medium. Diagn Microbiol Infect Dis. 2018 Oct;92(2):95–101. https://doi.org/10.1016/j.diagmicrobio.2018.05.008

Jayol A, Poirel L, Dortet L, Nordmann P. National survey of colistin resistance among carbapenemase-producing *Enterobacteriaceae* and outbreak caused by colistin-resistant OXA-48-producing *Klebsiella pneumoniae*, France, 2014. Euro Surveill. 2016;21(37):30339. https://doi.org/10.2807/1560-7917.ES.2016.21.37.30339

Jones-Dias D, Manageiro V, Ferreira E, Barreiro P, Vieira L, Moura IB, Caniça M. Architecture of class 1, 2, and 3 integrons from Gram negative bacteria recovered among fruits and vegetables. Front Microbiol. 2016;7:1400. https://doi.org/10.3389/fmicb.2016.01400

Jordan D, Chin JJ-C, Fahy VA, Barton MD, Smith MG, Trott DJ. Antimicrobial use in the Australian pig industry: results of a national survey. Aust Vet J. 2009 Jun;87(6):222–229. https://doi.org/10.1111/j.1751-0813.2009.00430.x

Jung Y, Jang H, Matthews KR. Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microb Biotechnol. 2014 Nov;7(6):517–527. https://doi.org/10.1111/1751-7915.12178

Kempf I, Fleury MA, Drider D, Bruneau M, Sanders P, Chauvin C, Madec JY, Jouy E. What do we know about resistance to colistin in *Enterobacteriaceae* in avian and pig production in Europe? Int J Antimicrob Agents. 2013 Nov;42(5):379–383. https://doi.org/10.1016/j.ijantimicag.2013.06.012

Kim DP, Saegerman C, Douny C, Dinh TV, Xuan BH, Vu BD, Hong NP, Scippo M-L. First survey on the use of antibiotics in pig and poultry production in the Red River Delta region of Vietnam. Food Public Health. 2013;3(5):247–256. https://doi.org/10.5923/j.fph.20130305.03

Koch-Weser J, Sidel VW, Federman EB, Kanarek P, Finer DC, Eaton AE. Adverse effects of sodium colistimethate. Manifestations and specific reaction rates during 317 courses of therapy. Ann Intern Med. 1970 Jun 01;72(6):857–868. https://doi.org/10.7326/0003-4819-72-6-857

Kostowski W, Herman ZS. Farmakologia (in Polish). Warsaw (Poland): PZWL Press; 2010. p. 341–342.

Kwa ALH, Loh C, Low JGH, Kurup A, Tam VH. Nebulized colistin in the treatment of pneumonia due to multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa.* Clin Infect Dis. 2005 Sep 01;41(5):754–757. https://doi.org/10.1086/432583

Kwon JA, Lee JE, Huh W, Peck KR, Kim YG, Kim DJ, Oh HY. Predictors of acute kidney injury associated with intravenous colistin treatment. Int J Antimicrob Agents. 2010 May;35(5):473–477. https://doi.org/10.1016/j.ijantimicag.2009.12.002

Lellouche J, Schwartz D, Elmalech N, Ben Dalak MA, Temkin E, Paul M, Geffen Y, Yahav D, Eliakim-Raz N, Durante-Mangoni E, et al.; AIDA study group. Combining VITEK® 2 with colistin agar dilution screening assist timely reporting of colistin susceptibility. Clin Microbiol Infect. 2019 Jun;25(6):711–716. https://doi.org/10.1016/j.cmi.2018.09.014

Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K. Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. Int J Antimicrob Agents. 2005 Jan;25(1):11–25. https://doi.org/10.1016/j.ijantimicag.2004.10.001

Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, Paterson DL. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis. 2006 Sep;6(9):589–601. https://doi.org/10.1016/S1473-3099(06)70580-1

Li J. Difficulty in assaying colistin methanesulphonate. Clin Microbiol Infect. 2005 Sep;11(9):773–774. https://doi.org/10.1111/j.1469-0691.2005.01218.x

Li R, Xie M, Zhang J, Yang Z, Liu L, Liu X, Zheng Z, Chan EWC, Chen S. Genetic characterization of *mcr-1* -bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance determinant. J Antimicrob Chemother. 2017 Feb;72(2):393–401. https://doi.org/10.1093/jac/dkw411

Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski A 3rd, Forrest A, Bulitta JB, Tsuji BT. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. Pharmacotherapy. 2010 Dec;30(12):1279–1291. https://doi.org/10.1592/phco.30.12.1279

Liu B-T, Li X, Zhang Q, Shan H, Zou M, Song F-J. Colistin-resistant *mcr*-positive *Enterobacteriaceae* in fresh vegetables, an increasing infectious threat in China. Int J Antimicrob Agents. 2019;54(1): 89–94. https://doi.org/10.1016/j.ijantimicag.2019.04.013

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, et al. 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. 2016 Feb;16(2):161–168. https://doi.org/10.1016/S1473-3099(15)00424-7

Lomovskaya O, Sun D, Rubio-Aparicio D, Nelson K, Tsivkovski R, Griffith DC, Dudley MN. Vaborbactam: spectrum of beta-lactamase inhibition and impact of resistance mechanisms on activity in *Enterobacteriaceae.* Antimicrob Agents Chemother. 2017 Nov;61(11):e01443-17. https://doi.org/10.1128/AAC.01443-17

Luo J, Yao X, Lv L, Doi Y, Huang X, Huang S, Liu JH. Emergence of *mcr-1* in *Raoultella ornithinolytica* and *Escherichia coli* isolates from retail vegetables in China. Antimicrob Agents Chemother. 2017 Oct;61(10):e01139-17. https://doi.org/10.1128/AAC.01139-17

MARAN. NethMap 2019: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands/MARAN 2019: Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2018. Wageningen (The Netherlands): Wageningen University & Research; 2019 [cited 2019 Jun 11]. Available from https://www.wur.nl/upload_mm/a/7/9/89640bbc-53a2-40f0-ba4a-a9a34a7bf416_Nethmap%20Maran%202019.pdf

Marchaim D, Chopra T, Pogue JM, Perez F, Hujer AM, Rudin S, Endimiani A, Navon-Venezia S, Hothi J, Slim J, et al. Outbreak of colistin-resistant, carbapenem-resistant *Klebsiella pneumoniae* in metropolitan Detroit, Michigan. Antimicrob Agents Chemother. 2011 Feb;55(2):593–599. https://doi.org/10.1128/AAC.01020-10

MARD (Ministry of Agriculture and Rural Development). 2009. Livestock development strategy to 2020, Amendedand Reprinted in the first time. Publishing House for Scienceand Technology.

MARD. (Ministry of Agriculture and Rural Development). Standard TCN. 2006;861:2006. [Animal feeding stuffs – Maximum levels of antibiotics and drugs in complete feed.].

Matuschek E, Åhman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin – evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Acinetobacter* spp. Clin Microbiol Infect. 2018a Aug;24(8):865–870. https://doi.org/10.1016/j.cmi.2017.11.020

Matuschek E, Davies L, Ahman J, Kahlmeter G, Wootton M. Can agar dilution be used for colistin MIC determination? Session: Colistin antimicrobial susceptibility testing – which methods are available? ECCMID 2018, 24 April 2018, Abstract publication O0954. 2018b [cited 2019 Jun 11]. Available from https://www.escmid.org/escmid_publications/escmid_elibrary/?q=Matuschek+E&id=2173&L=0&x=21&y=24

Meletis G, Oustas E, Botziori C, Kakasi E, Koteli A. Containment of carbapenem resistance rates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* in a Greek hospital with a concomitant increase in colistin, gentamicin and tigecycline resistance. New Microbiol. 2015 Jul;38(3):417–421.

Moffatt JH, Harper M, Harrison P, Hale JD, Vinogradov E, Seemann T, Henry R, Crane B, St Michael F, Cox AD, et al. Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. Antimicrob Agents Chemother. 2010;54(12):4971–4977. https://doi.org/10.1128/AAC.00834-10

Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S; Network EuSCAPE-Italy, Grundmann H, Pantosti A, Rossolini GM. Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. Euro Surveill. 2014;19(42): 20939. https://doi.org/10.2807/1560-7917.es2014.19.42.20939

Nation RL, Li J. Colistin in the 21st century. Curr Opin Infect Dis. 2009 Dec;22(6):535–543. https://doi.org/10.1097/QCO.0b013e328332e672

Nordmann P, Jayol A, Poirel L. A universal culture medium for screening polymyxin-resistant Gram-negative isolates. J Clin Microbiol. 2016 May;54(5):1395–1399. https://doi.org/10.1128/JCM.00446-16

Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in *Enterobacteriaceae.* Emerg Infect Dis. 2016 Jun;22(6): 1038–1043. https://doi.org/10.3201/eid2206.151840

Nunan C, Young R. Use of antibiotics in animals and people. Vet Rec. 2015 Nov 07;177(18):468.2–470. https://doi.org/10.1136/vr.h5934

Olaitan AO, Diene SM, Kempf M, Berrazeg M, Bakour S, Gupta SK, Thongmalayvong B, Akkhavong K, Somphavong S, Paboriboune P, et al. Worldwide emergence of colistin resistance in *Klebsiella pneumoniae* from healthy 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. 2014 Dec;44(6):500–507. https://doi.org/10.1016/j.ijantimicag.2014.07.020

Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014 Nov 26;5:643. https://doi.org/10.3389/fmicb.2014.00643

Ordooei Javan A, Shokouhi S, Sahraei Z. A review on colistin nephrotoxicity. Eur J Clin Pharmacol. 2015 Jul;71(7):801–810. https://doi.org/10.1007/s00228-015-1865-4

Parisi SG, Bartolini A, Santacatterina E, Castellani E, Ghirardo R, Berto A, Franchin E, Menegotto N, De Canale E, Tommasini T, et al. Prevalence of *Klebsiella pneumoniae* strains producing carbapenemases and increase of resistance to colistin in an Italian teaching hospital from January 2012 To December 2014. BMC Infect Dis. 2015 Dec;15(1):244. https://doi.org/10.1186/s12879-015-0996-7

Pena I, Picazo JJ, 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 May;43(5):460–464. https://doi.org/10.1016/j.ijantimicag.2014.01.021

Petrosillo N, Taglietti F, Granata G. Treatment Options for Colistin Resistant *Klebsiella pneumoniae*: Present and Future. J Clin Med. 2019;28;8(7):E934. https://doi.org/10.3390/jcm8070934

Pfaller MA, Huband MD, Mendes RE, Flamm RK, Castanheira M. *In vitro* activity of meropenem/vaborbactam and characterisation of carbapenem resistance mechanisms among carbapenem-resistant Enterobacteriaceae from the 2015 meropenem/vaborbactam surveillance programme. Int J Antimicrob Agents. 2018 Aug;52(2): 144–150. https://doi.org/10.1016/j.ijantimicag.2018.02.021

Pogue JM, Bonomo RA, Kaye KS. Ceftazidime/avibactam, meropenem/vaborbactam, or both? Clinical and formulary considerations. Clin Infect Dis. 2019 Jan 18;68(3):519–524. https://doi.org/10.1093/cid/ciy576

Pogue JM, Lee J, Marchaim D, Yee V, Zhao JJ, Chopra T, Lephart P, Kaye KS. Incidence of and risk factors for colistin-associated nephrotoxicity in a large academic health system. Clin Infect Dis. 2011 Nov 01;53(9):879–884. https://doi.org/10.1093/cid/cir611

Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev. 2017 Apr;30(2): 557–596. https://doi.org/10.1128/CMR.00064-16

QYResearch Medical Research Centre. The global polymyxin industry report 2015. Ottawa (Canada): QYResearch Medical Research Centre; 2015 [cited 2019 Jun 11]. Available from http://www.qyresearch.com

Sandri AM, Landersdorfer CB, Jacob J, Boniatti MM, Dalarosa MG, Falci DR, Behle TF, Bordinhão RC, Wang J, Forrest A, et al. Population pharmacokinetics of intravenous polymyxin B in critically ill patients: implications for selection of dosage regimens. Clin Infect Dis. 2013 Aug 15;57(4):524–531. https://doi.org/10.1093/cid/cit334

Schindler M, Osborn MJ. Interaction of divalent cations and polymyxin B with lipopolysaccharide. Biochemistry. 1979 Oct 02;18(20):4425–4430. https://doi.org/10.1021/bi00587a024

SDa Autoriteit Diergeneesmiddelen. Usage of antibiotics in agricultural livestock in the Netherlands in 2017. Trends and benchmarking of livestock farms and veterinarians. Utreht (The Netherlands): SDa Autoriteit Diergeneesmiddelen; 2018 [cited 2019 Jun 11]. Available from https://www.autoriteitdiergeneesmiddelen.nl/en/publications

Shields RK, Chen L, Cheng S, Chavda KD, Press EG, Snyder A, Pandey R, Doi Y, Kreiswirth BN, Nguyen MH, et al. Emergence of ceftazidime-avibactam resistance due to plasmid-borne bla_{KPC-3} mutations during treatment of carbapenem-resistant *Klebsiella pneumoniae* infections. Antimicrob Agents Chemother. 2017 Mar; 61(3):e02097-16. https://doi.org/10.1128/AAC.02097-16

Shields RK, Clancy CJ, Hao B, Chen L, Press EG, Iovine NM, Kreiswirth BN, Nguyen MH. Effects of *Klebsiella pneumoniae* carbapenemase subtypes, extended-spectrum β-lactamases, and porin mutations on the in vitro activity of ceftazidime-avibactam against carbapenem-resistant *K. pneumoniae.* Antimicrob Agents Chemother. 2015 Sep;59(9):5793–5797. https://doi.org/10.1128/AAC.00548-15

Sjölund M, Backhans A, Greko C, Emanuelson U, Lindberg A. Antimicrobial usage in 60 Swedish farrow-to-finish pig herds. Prev Vet Med. 2015 Oct;121(3-4):257–264. https://doi.org/10.1016/j.prevetmed.2015.07.005

Spapen H, Jacobs R, Van Gorp V, Troubleyn J, Honoré PM. Renal and neurological side effects of colistin in critically ill patients. Ann Intensive Care. 2011 Dec;1(1):14. https://doi.org/10.1186/2110-5820-1-14

SWEDRES/SVARM. Consumption of antibiotics and occurrence of antibiotic resistance in Sweden. Solna/Uppsala (Sweden): Public Health Agency of Sweden and National Veterinary Institute; 2018 [cited 2019 Jun 11]. Available from https://www.folkhalsomyndigheten.se/contentassets/d8f6b3d187a94682a1d50a48f0a4fb3d/swedres-svarm-2018.pdf

Trauffler M, Griesbacher A, Fuchs K, Köfer J. Antimicrobial drug use in Austrian pig farms: plausibility check of electronic on-farm records and estimation of consumption. Vet Rec. 2014 Oct 25;175(16):402. https://doi.org/10.1136/vr.102520

Tullu MS, Dhariwal AK. Colistin: re-emergence of the 'forgotten' antimicrobial agent. J Postgrad Med. 2013;59(3):208–215. https://doi.org/10.4103/0022-3859.118040

Tumbarello M, Trecarichi EM, De Rosa FG, Giannella M, Giacobbe DR, Bassetti M, Losito AR, Bartoletti M, Del Bono V, Corcione S, et al.; ISGRI-SITA (Italian Study Group on Resistant Infections of the Società Italiana Terapia Antinfettiva). Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentre study. J Antimicrob Chemother. 2015 Jul 01;70(7):2133–2143. https://doi.org/10.1093/jac/dkv086

Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, Spanu T, Ambretti S, Ginocchio F, Cristini F, et al. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. Clin Infect Dis. 2012 Oct 01;55(7):943–950. https://doi.org/10.1093/cid/cis588

Turlej-Rogacka A, Xavier BB, Janssens L, Lammens C, Zarkotou O, Pournaras S, Goossens H, Malhotra-Kumar S. Evaluation of colistin stability in agar and comparison of four methods for MIC testing of colistin. Eur J Clin Microbiol Infect Dis. 2018 Feb;37(2):345–353. https://doi.org/10.1007/s10096-017-3140-3

U.S. National Library of Medicine. A study of meropenem-vaborbactam versus piperacillin/tazobactam in participants with hospital-acquired and ventilator-associated bacterial pneumonia (TANGOIII). https://clinicaltrials.gov/ct2/show/NCT03006679

van Rennings L, von Münchhausen C, Ottilie H, Hartmann M, Merle R, Honscha W, Käsbohrer A, Kreienbrock L. Cross-sectional study on antibiotic usage in pigs in Germany. PLoS ONE. 2015; 0(3): e0119114. https://doi.org/10.1371/journal.pone.0119114

Vasoo S. Susceptibility testing for the polymyxins: two steps back, three steps forward? J Clin Microbiol. 2017;55(9):2573–2582. https://doi.org/10.1128/JCM.00888-17

Vicari G, Bauer SR, Neuner EA, Lam SW. Association between colistin dose and microbiologic outcomes in patients with multidrugresistant Gram-negative bacteremia. Clin Infect Dis. 2013;56: 398–404. https://doi.org/10.1093/cid/cis909

Walkty A, DeCorby M, Nichol K, Karlowsky JA, Hoban DJ, Zhanel GG. *In vitro* activity of colistin (polymyxin E) against 3,480 isolates of gram-negative bacilli obtained from patients in Canadian

hospitals in the CANWARD study, 2007–2008. Antimicrob Agents Chemother. 2009 Nov 01;53(11):4924–4926.
https://doi.org/10.1128/AAC.00786-09

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. 2018 Dec;7(1):1–9.
https://doi.org/10.1038/s41426-018-0124-z

Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. Identification of a novel plasmid-madiated colistin-resistance gene, *mcr-2*, in *Escherichia coli*. Euro Surveill. 2016;21(27):30280.
https://doi.org/10.2807/1560-7917.ES.2016.21.27.30280

Yang Y-Q, Li Y-X, Lei C-W, Zhang A-Y, Wang H-N. 2018. Novel plasmid mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. J Antimocrob Chemother. 73(7):1791–1795.
https://doi.org/10.1093/jac/dky111

Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh RT, Shen J, Wang Y. 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. MBio. 8:e0054317.
https://doi.org/10.1128/mBio.00543-17

Zhanel GG, Lawson CD, Adam H, Schweizer F, Zelenitsky S, Lagacé-Wiens PR, Denisuik A, Rubinstein E, Gin AS, Hoban DJ, et al. Ceftazidime-avibactam: a novel cephalosporin/β-lactamase inhibitor combination. Drugs. 2013;73(2):159–77.
https://doi.org/10.1007/s40265-013-0013-7

Zhang H, Zhao D, Quan J, Hua X, Yu Y. *mcr-1* facilitated selection of high-level colistin-resistant mutants in *Escherichia coli*. Clin Microbiol Infect. 2019;25(4):517.e1–517.e4.
https://doi.org/10.1016/j.cmi.2018.12.014

Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, Xie L, Yang C, Ma X, Li H, et al. Epidemiology of carbapenem-resistant *Enterobacteriaceae* infections: report from the China CRE Network. Antimicrob Agents Chemother. 2018;62(2): e01882-17.
https://doi.org/10.1128/AAC.01882-17

Zhon HW, Zhang T, Ma JH, Fang Y, Wang HY, Huang ZX, Wang Y, Wu C, Chen GX. Occurrence of plasmid- and chromosome-carried *mcr-1* in water-borne *Enterobacteriaceae* in China. Antimicrob Agents Chemother. 2017;61(8):e00017-17.
https://doi.org/10.1128/AAC.00017-17

Zou D, Huang S, Lei H, Yang Z, Su Y, He X, Zhao Q, Wang Y, Liu W, Huang L. Sensitive and rapid detection of the plasmid-encoded colistin-resistance gene *mcr-1* in *Enterobacteriaceae* isolates by loop-mediated isothermal amplification. Front Microbiol. 2017;8:2356. https://doi.org/10.3389/fmicb.2017.02356

Zurfuh K, Poirel L, Nordmann P, Nuesch-Inderbinen M, Hachler H, Stefan R. Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* in river water and imported vegetables was identified in Switzerland. Antimicrob Agents Chemother. 2016;60(4):2594–2595.
https://doi.org/10.1128/AAC.00066-16