

Colistin Resistance in Enterobacterales Strains – A Current View

ELŻBIETA M. STEFANIUK^{1*} and STEFAN TYSKI^{1,2}¹Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland²Department 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). Colis-

tin displaces Mg²⁺ and Ca²⁺ 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 (Ordoeji 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

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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 (Ordoeji 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 Enterobacteriales 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 Enterobacteriales 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* NEB5 α 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; Dafopoulou 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, (bioMérieux, France), BD Phoenix (Becton Dickinson, USA), as well as Walk-Away (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, Bernasconi 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 Høeg 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 multi-sectoral, further research as well as a proper monitoring and surveillance systems.

ORCID

Elżbieta M. Stefaniuk [0000-0002-8169-6964](https://orcid.org/0000-0002-8169-6964)

Stefan Tyski [0000-0003-3352-038X](https://orcid.org/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.

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