Open Forum Infectious Diseases

REVIEW ARTICLE (INVITED)

Emergent Polymyxin Resistance: End of an Era?

Zekun Li,[1,2,3] Yuping Cao,[4] Lingxian Yi,[4] Jian-Hua Liu,[4] and Qiwen Yang[1,2]

[1]Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing; [2]Beijing Key Laboratory for Mechanisms Research and Precision Diagnosis of Invasive Fungal Diseases, China; [3]Department of Laboratory Medicine, Xiangya School of Medicine, Central South University, Changsha, China; [4]College of Veterinary Medicine, South China Agricultural University, Guangzhou

Until recently, the polymyxin antibiotics were used sparingly due to dose limiting toxicities. However, the lack of therapeutic alternatives for infections caused by highly resistant Gram-negative bacteria has led to the increased use of the polymyxins. Unfortunately, the world has witnessed increased rates of polymyxin resistance in the last decade, which is likely in part due to its irrational use in human and veterinary medicine. The spread of polymyxin resistance has been aided by the dissemination of the transferable polymyxin-resistance gene, *mcr,* in humans and the environment. The mortality of colistin-resistant bacteria (CoRB) infections varies in different reports. However, poor clinical outcome was associated with prior colistin treatment, illness severity, complications, and multidrug resistance. Detection of polymyxin resistance in the clinic is possible through multiple robust and practical tests, including broth microdilution susceptibility testing, chromogenic agar testing, and molecular biology assays. There are multiple risk factors that increase a person's risk for infection with a polymyxin-resistant bacteria, including age, prior colistin treatment, hospitalization, and ventilator support. For patients that are determined to be infected by polymyxin-resistant bacteria, various antibiotic treatment options currently exist. The rising trend of polymyxin resistance threatens patient care and warrants effective control.

Key words. colistin; *mcr-1*; polymyxin; resistance.

INTRODUCTION

Polymyxins have been used for more than 50 years in both human and veterinary medicine. There exist 5 kinds of polymyxins, from A to E, and to date only polymyxin B and E are used in clinical treatment of Gram-negative bacteria infections. Colistin, also known as polymyxin E, is a cationic circular peptide that interacts with the negatively charged phosphate group of lipid A and consequently destructs the outer membrane of bacteria. Previously, the use of polymyxins in human medicine was predominantly restricted to topical administrations due to its systemic toxicity. However, systemic polymyxins recently have reignited significant interest due to the increasing incidence of infections caused by multidrug-resistant (MDR) Gram-negative bacteria. In clinical medicine, polymyxins, together with carbapenems, now serve as last-resort drugs for infections caused by MDR Gram-negative bacteria, such as *Enterobacteriaceae* (*Escherichia coli* and *Klebsiella pneumoniae*), *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* [1–3]. At the same time, colistin has been utilized extensively for bacterial infection control and growth promotion in veterinary medicine [4]. To date, research groups have made great efforts to elucidate the colistin resistance mechanism. However, incomplete comprehension still hinders solving the question of extremely resistant infections and, thus, requires further investigation. Several chromosomal mutations have been related to colistin resistance due to their modifications on essential outer membrane constituents.Liu et al described for the first time a plasmid-mediated colistin resistance mechanism (*mcr-1*) in November 2015, and, subsequently, a mass of studies confirmed *mcr-1* and its variants in a growing number of countries [5]. Thus, we provide an overview of polymyxin resistance in bacteria, rapid detection techniques, and clinical treatment regimens for infections due to polymyxin-resistant bacteria.

COLISTIN RESISTANCE MECHANISM

Historically, resistance to polymyxins has been attributed to chromosomal mutations. Such resistance arises due to lipopolysaccharide (LPS) modification mediated by operon *pmrCAB*, the 2 components system *phoPQ* and its regulator *mgrB*, operon *pmrHFIJKLM* and *pmrE* gene, and the *crrAB* operon [6–9]. Of note, the inactivation of *mgrB* by insertion sequences or mutations is responsible mostly for polymyxin resistance in clinical *K. pneumoniae* isolates [10]. Susceptibility to colistin is based

Received 21 May 2019; editorial decision 8 August 2019; accepted 10 August 2019.

Correspondence: Q. Yang, Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China (yangqiwen81@vip.163.com).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofz368

on inactivation of operon *arnBCADTEF* and *pmrCAB*, which is normally maintained by *phoPQ* and *pmrAB*. When stimuli like high Fe^{3+} arise, cascade activation of *pmrB*, *pmrA*, and *arnBCADTEF* will enhance expression of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine (PEtN) and modify lipid A of the bacterial outer membrane [11]. Decline of anionic charge results in less electrostatic binding of colistin to bacterial outer membranes. A significant increase of *arnBCADTEF* expression was noticed in polymyxin-resistant bacteria in a recent report [12]. Participation of *mgrB* in the feedback control of *phoPQ*means mutations in the *mgrB* sequence also contribute to polymyxin resistance. Mutations in *pmrAB* lead to polymyxin resistance in a similar way. Chlorhexidine exposure recently was found to increase the colistin resistance rate of *K. pneumoniae*, which might result from a point mutation in *pmrB* [13]. Another report discovered that high expression of *pmrC* occurred after a point mutation in the *pmrB* gene in *K. pneumoniae*, which subsequently resulted in PEtN modification of lipid A [14]. Modification of LPS is the major resistance mechanism caused by chromosomal mutations in *Salmonella* and *E. coli* as well[15]. Mutations of *CrrB* induce *crrC* expression and further affect the *pmrAB* system. Finally, resistance was formed by *pmrC* hyper-expression [16]. On the other hand, although LPS, a predominant component of the outer membrane, is essential for some bacteria, loss of LPS in *A. baumannii* would bring on resistance instead of death. Boll et al reported multidrug resistance phenotypes appeared after colistin screening in *A. baumannii* without LPS, which might be due to an extremely high-level expression of transporters and efflux pumps as rescue mechanisms. Furthermore, knockdown of PBP1A hampers colistin resistance of *A. baumannii* with deficiency of lipid synthesis. It is noteworthy that *A. baumannii* lacking lipid A were more susceptible to tobramycin, suggesting the potential clinical utility of tobramycin for these bacteria [17]. Additional culprits in the development of colistin resistance include loss of OmpW, expression of EptA, and production of *dedA* [18]. At the same time, there are more regulatory systems involved with colistin resistance, like *vprAB* in *Vibrio cholerae*, *cprRS* in *P. aeruginosa,* and *parRS*, which impacts cation peptides in outer membrane [19, 20]. Some chromosomal mechanisms mediating colistin resistance were illustrated in Figure 1.

The gene *mcr-1* encodes MCR-1, a PEtN transferase that modifies lipid A, altering its electric charge, causing colistin resistance. Liu et al also reported that plasmids containing the *mcr-1* gene persisted stably and can confer polymyxin resistance to *K. pneumoniae* after transformation [5]. A retrospective study characterized the early existence of *mcr-1* in isolates of chicken origin from 1980s, when colistin was first introduced into China [21]. After the first report of *mcr-1* in IncI2 plasmid, a series of plasmids were reported to be carriers of *mcr-1*, including IncX4, IncHI2, IncP, IncFII, F18:A-:B-, phage P7-like plasmids, and so on [22–24]. Subsequently, a series of *mcr* genes were identified, including *mcr-1.2* to *mcr-1.18*, *mcr-2* to *mcr-2.9*, *mcr-3* to *mcr-3.18*, *mcr-4 to mcr-4.5*, *mcr-5* to *mcr-5.3*, *mcr-6*, *mcr-7* and *mcr-8*. Identification of *mcr* genes provides new insight into horizontal and cross-dissemination of colistin resistance. Significant progress has been made to better understand polymyxin-resistance mechanisms, but our comprehension is insufficient still and this area needs further research.

EPIDEMIOLOGY OF COLISTIN RESISTANCE

Colistin is an effective antibiotic against Gram-negative rods and has been used both in the clinic and as a growth promoter for veterinary purposes. However, colistin resistance has been found in *Enterobacteriaceae*, *P. aeruginosa*, *A. baumannii*, *Campylobacter* spp., and in some other patient- or environment-originated strains [4, 25]. Colistin resistance caused by the plasmid-borne *mcr-1* gene has been detected in natural waters, soil, manure, and city drainage [26–28]. Accumulating evidence demonstrates that there is an increasing trend of colistin resistance in both humans and the surrounding environment.

In clinical Gram-negative isolates, the rate of colistin resistance differs in strains, with *K. pneumoniae* and *A. baumannii* showing high resistance rates [29, 30]. The first reported colistin-resistant (CoR) *K. pneumoniae* was isolated in Athens in 2004 and CoR *Enterobacteriaceae* has spread all over the world since then [31]. A Tunisian epidemiological study analyzed CoR *K. pneumoniae* data in a national medical facility from 2002–2013 and found that the rate of colistin resistance in *K. pneumoniae* increased from 3.57% in 2002 to 9.68% in 2013 [32]. In Europe, the rate increased from 1.1% in 2003 to 2.2% in 2009. A high colistin resistance rate also was found in Romania (25.8%) [33]. In China, Lu et al investigated 112 clinical isolates from bloodstream infection patients and reported 5 (0.4%, 5 of112) colistin-resistant isolates, which indicated that the prevalence of colistin resistance in hypervirulent *K. pneumoniae* in China is relatively high [34]. Recent evidence indicates that carbapenem-resistant *K. pneumoniae* has a high colistin resistance rate, which is unsettling and restricts treatment options. The European Antimicrobial Resistance Surveillance Network (EARS-Net) reported that 29% of carbapenem-resistant *K. pneumoniae* were CoR, yet only 3% of carbapenem-susceptible *K. pneumoniae* was CoR [35]. A retrospective study in Dubai found 27% of carbapenem-resistant *K. pneumoniae* was resistant to colistin in 5 major hospitals. A higher resistance rate was discovered only in Italy (43%) [36]. An epidemiological survey on carbapenem-resistant *Enterobacteriaceae* isolates from 25 provinces confirmed that 1.4% of carbapenem-resistant *Klebsiella pneumoniae* were colistin-resistant, whereas the colistin resistance rate in *E. coli*, *Enterobacter cloacae*, and *Citrobacter freundii* was 4%, 2.9%, and 2.4%, respectively [37]. Wang et al investigated farms in 4 provinces and found out colistin susceptibility of *K. pneumoniae* in healthy animals and

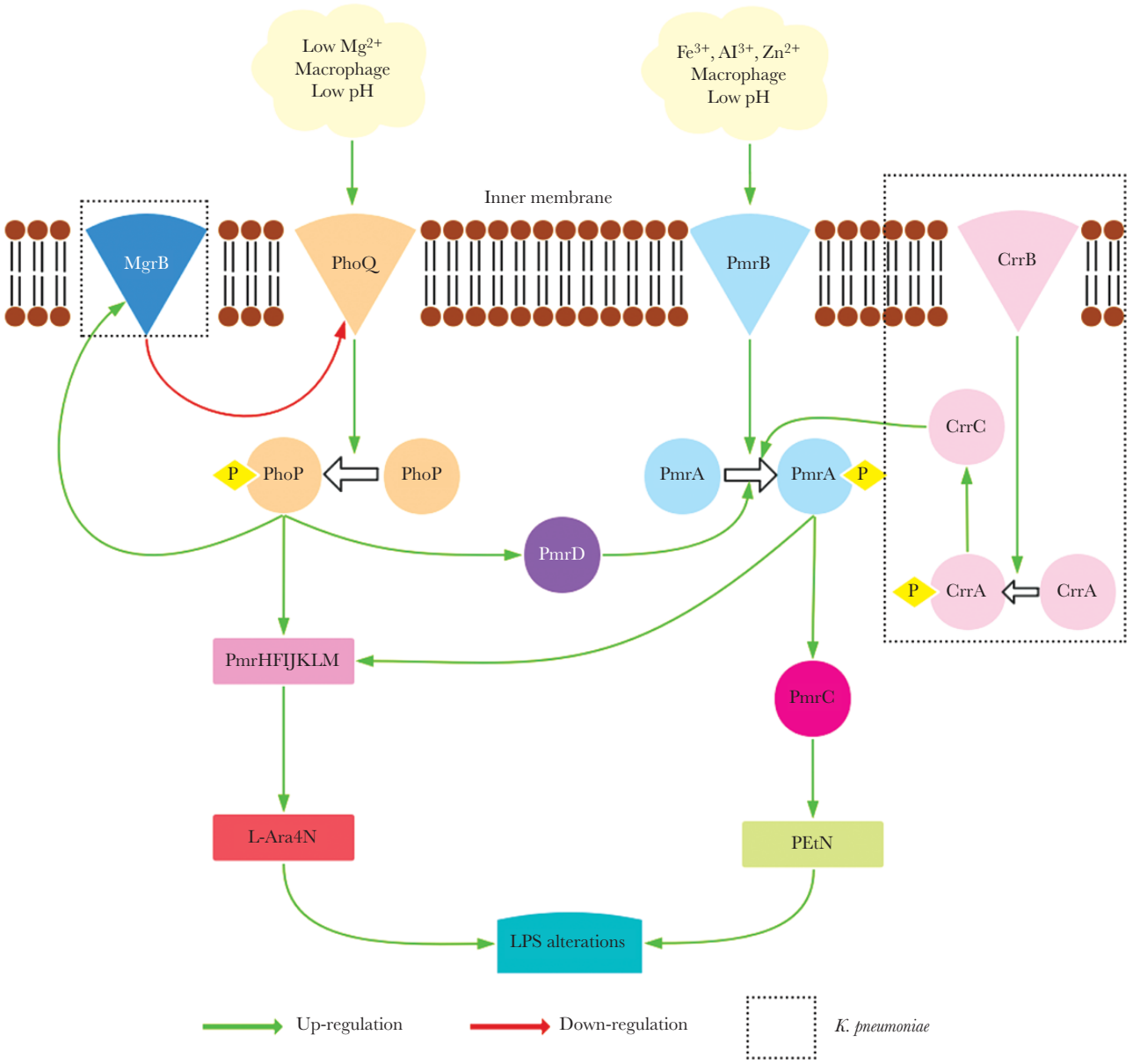

Figure 1. Illustration of Chromosomal Colistin-resistance Mechanisms Multiple mutations contribute to the development of colistin resistance based on subsequent lipopolysaccharide modifications.

organs of sick animals was 74.2% and 69.2%, respectively. Also, 9.3% of *K. pneumoniae* was found to be resistant to carbapenem [38]. Overall, polymyxin-resistance rates in *K. pneumoniae* vary among different regions, but it seems likely that the prevalence is increasing worldwide.

Similarly, for *A. baumannii,* since the first reported clinical CoR isolate in the Czech Republic in 1999, CoR rates have increased over the last few decades [39]. A retrospective study in French Guiana, including 441 intensive care unit (ICU)-associated outbreaks of carbapenem-resistant *A. baumannii* reported a 4.4% CoR rate [40]. According to a multicenter study, MARATHON (multicenter epidemiological surveillance study of the antibiotic resistance of nosocomial pathogens), the CoR rate in Russian hospital-acquired *A. baumannii* was 1.9% (10 of 527) [41]. Collected EARS-Net 2013 annual data from 17 countries and showed a resistance rate of 5% on average among the countries, with high levels of CoR strains (>80%) collected from Greece and Italy [42]. In comparison, the highest CoR rate according to SENTRY data from 2006–2009 was 30.6% in Korea [29]. Studies in Iran, Bulgaria, and French Guiana reported a CoR rate of 0 [40, 43, 44]. Polymyxin-resistance rates are extremely variable for *A. baumannii* isolates in different parts of the world.

In contrast to *K. pneumoniae* and *A. baumannii,* colistin resistance generally is not common in clinical *E. coli* isolates, with a moderate CoR rate of 0.2%–0.6%. However, environmental

studies revealed an alarming prevalence of CoR *E. coli* in food-producing animals and from the surrounding environment. Wasyl et al investigated fecal samples from wild deer in Poland in 2012 to 2014 and found 0.3% (2 of 542) antibiotic-resistant isolates exhibited a CoR phenotype [45]. A Swiss study assessed CoR rate of *E. coli* in a local slaughterhouse and found 4% (13 of 325) of *E. coli* in pig feces and 3.3% (8 of 241) of *E. coli* in bovine feces were CoR [46]. In Taiwan, Kuo et al studied the rates of *mcr-1* positive isolates from clinical and commercial meat samples. Their data showed an increasing trend of *mcr-1*-positive *E. coli* isolates from 2010 (0.2%) to 2014 (0.9%). Market meat samples had a more marked increase from 2012 (1.1%) to 2015 (8.7%) [47]. Another study in China collected *E. coli* from patients with diarrhea and analyzed polymyxin B susceptibility of these isolates. The study found that 7.3% (9 of 123) of *E. coli* isolates were polymyxin resistant, and further polymerase chain reaction (PCR) tests determined 5.7% (7 of 123) were *mcr-1* positive [48]. Another recent study by Yang et al revealed that *E. coli* encoding *mcr-1* can be disseminated through whole-market chicken product lines [49]. This mechanism might contribute to contamination of human food and human infection. Colistin resistance in clinical practice in China is still uncommon but alarming when compared to the known reported CoR rate around the world. Political prioritization and enforced management are needed urgently to prevent further spread of CoR strains.

Relatively few studies have investigated the prevalence of polymyxin resistance in *P. aeruginosa*. According to EARS-Net surveillance data, colistin resistance for *P. aeruginosa* in Europe rose from 1% in 2013 to 4% in 2016 [50]. Most CoR isolates in 2016 were from Greece and Italy. In China, colistin susceptibility in *P. aeruginosa* was reported to be around 93%–99.6% [51–53]. Both methodological differences and a relatively minor proportion of colistin susceptibility identified in total *P. aeruginosa* isolates might contribute to regionally high CoR rates.

Besides polymyxin resistance mediated by chromosomal mutations, acquired plasmid-mediated resistance is worrisome because of the potential horizontal spread. Since the first *mcr* gene reported in China, investigations began to realize the importance of *mcr-1* gene. The positive rate of *mcr-1* in *E. coli* isolated from slaughterhouse swine and market raw meat samples was 15% (78 of 523) and 21% (166 of 804), respectively [5]. This rate is slightly higher than that of Japan. The latter study investigated pathogenic *E. coli* in swine from Japan and found that 13% (90 of 684) harbored the *mcr-1* gene. The rate of *E. coli* carrying *mcr-1* was 0.02% in healthy swine in Japan while it was 30% in diseased pigs [51]. In an investigation conducted in Portugal, Kieffer et al isolated 108 colistin-resistant strains from 100 rectal swabs of swine and observed a high prevalence (98 of 100) of plasmid-induced CoR phenotypes [54]. In Tunisia, the highest *mcr-1* prevalence of chicken (83%) was reported in a farm [55]. The *mcr-1* gene has been detected in sullage, boot swabs, dog feces, soil, and manure [26, 56]. The wide distribution of the *mcr-1* gene in environmental samples increases the risk that pathogenic bacteria acquire colistin resistance. The polymyxin-resistant bacteria then may colonize or infect humans via the food chain or through fecal-oral transmission. The *mcr* gene also has been detected in humans. A concerning study in China showed a high prevalence of the *mcr-1* gene in healthy children without history of colistin treatment [57]. Some large-scale investigations determined approximately 0%–1% genotypic CoR rates in *Enterobacteriaceae* from human [58, 59]. After the discovery of *mcr-1* in China, subsequent studies found a series of *mcr*-like genes, such as *mcr-1.2* to *mcr-1.13*, *mcr-2* to *mcr-2.9*, and *mcr-3* to *mcr-8*. Among these *mcr* genes, *mcr-1* has the highest prevalence in both human and in the environment. In addition, *mcr* genes may have variable genetic backgrounds, meaning they might be found in different plasmids. Due to the diversity of *mcr* genes, a detection method that is quick, affordable, convenient, and compatible with multiple targets is needed urgently. Colistin resistance rate in common microbes isolated from human and food-producing animals was listed in Table 1.

RISK FACTORS AND PROGNOSIS FOR CoRB INFECTION

Previous studies deciphered several factors associated with CoRB infections and poor treatment outcome. Recently, it has been shown that colistin resistance may arise due to subtherapeutic polymyxin treatment [25]. Except innately CoR species like *Hafnia alvei* and *Hafnia paralvei,* colistin resistance also was associated with the history of colistin. A multicenter study collected colistin-resistant Klebsiella pneumoniae carbapenemase (KPC)-producing *K. pneumoniae* from the bloodstreams of patients and found that previous treatment with colistin, a preceding colonization of resistant *K. pneumoniae*, and a Charlson score of ≥3 were correlated with CoRB infection [60, 61]. Previous treatment with colistin also has been associated with CoR *Enterobacteriaceae* infection in a matched case control study by Drozdinsky et al [62]. A possible relationship exists between neurological disorders and CoR *K. pneumoniae* infections [63], though this phenomenon was described only in few researches and needs further verification. Intensive care unit treatment and ST101 *K. pneumoniae* were considered independent risk factors of infection with polymyxin-resistant bacteria [64–66]. Colistin-resistant *A. baumannii* (CoR-AB) infection or colonization was associated with age and treatment history of carbapenem or colistin [67]. Ventilator support also was determined to be a risk factor of Gram-negative CoR microorganism infection [63]. In summary, patients with previous polymyxin exposure as well as those that are critically ill may be at increased risk for colonization or infection with polymyxin-resistant bacteria.

Prognosis for patients with infections caused by polymyxin-resistant bacteria also needs to be considered. CoRB infections

in critically-ill patients (including pneumonia, urine tract infection, bacteremia, infection with underlying diseases as transplantation, stroke, chronic obstructive pulmonary disease [COPD], and so on) are associated with high mortality rates (30%–37%) [68–70]. Patients infected with CoRB but without previous exposure to colistin would have a significantly better outcome [71]. Also, high mortality rates with CoRB infection was associated with dialysis (63.6%, 7 of 11), septic shock (37.5%–60%, 9 of 24–9 of15), and intraabdominal infection (83.3%, 10 of 12) [68, 72]. For patients with respiratory system infection, COPD and lower respiratory tract infection contributed to a nearly 3-fold higher mortality rate than the control group, which could be explained by several increased factors, such as medical ventilation, damage of pulmonary function, treatment with corticosteroids, and so on [73]. Infection caused by carbapenem- and colistin-resistant *K. pneumoniae* exhibited remarkably higher mortality rates and was confirmed to be an independent risk factor bound to poor outcome [74]. In nosocomial infection, mortality rates increased significantly to 100% (6 of 6) for patients in anesthesia and reanimation ICU, who were-infected with pan-drug resistant (including colistin) *K. pneumoniae* [75]. At the same time, control of removable infectious resource and isolates susceptible to aminoglycosides produced favorable treatment outcomes. Partial synergetic effect of colistin and rifampicin was correlated with the markedly higher rate of microbiological response (100% of culture negative conversion) and clinical response (100% of symptom elimination) in CoR-AB–infected pneumonia patients. However, high response rates might need further validation due to the relatively small sample sizes [76, 77].

DETECTION OF POLYMYXIN RESISTANCE

Rapid and accurate detection of CoRB infection in clinical practice is important. With a precise microbiology report, clinicians can adjust treatment regimens and personalize patient therapies. Primary detection methods can be categorized as (1) classical microbiology assays, (2) molecular biology assays, and (3) novel methods. EUCAST (European Committee on Antimicrobial Susceptibility Testing) recommends broth microdilution for antimicrobial susceptibility testing and determined that minimal inhibitory concentration (MIC) value of >2 mg/L as colistin resistance for *Enterobacteriaceae*, *Pseudomonas spp.* and *Acinetobacter spp*. Several commercial broth microdilution-based colistin detection systems have been established. Nevertheless, this predominant assay has some limitations. The Umic (Biocentric, NJ) system is flexible and user-friendly but lacks accuracy when it comes to extremely low or high MICs. Also, it has a high very major error (VME) rate. Sensititre (ThermoFisherDiagnostics, Thermo Fisher, Waltham, MA) has a VME rate of 3%. MicroScan (Beckman Coulter, Brea, CA) has the lowest VME rate among these detection systems, yet a narrow range of MIC and a high VME rate in nonfermenting bacteria limits its application [78]. Despite the time consumption of time-kill assay, broth microdilution, disk diffusion, Gradient diffusion, and other tests, chromogenic medias, such as Superpolymyxin (ELITechGroup, Puteaux, France), COL-APSE (CHROMagar, Paris, France), and ChromID (bioMerieux, NC) are accessible and convenient in differentiation, isolation, and culture of CoRB infection. Superpolymyxin performs well for CoR Gram-negative bacteria and COL-APSE has proved efficient in separating and differentiating *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Enterobacteriaceae*, and even nonfermentive Gram-negative bacteria [79]. Additionally, variant tests based on a chromogenic principle have been established. Resazurin was employed recently as a colorant to test colistin resistance of *A. baumannii* and *P. aeruginosa*. The specificity and sensitivity of this assay were 95% and 100%, respectively, for each bacteria [80]. The newly developed LBJMR medium precisely identified 143 pure culturing strains of clinical isolates and 68 stool samples with 100% specificity and sensitivity in both [81]. Nordmann et al reported a detection system based on acid metabolites produced by Enterobacteriaceae bacteria during growth in colistin-contained culture medium for which the sensitivity and specificity were 99.3% and 95.4%, respectively. Chromogenic culturing medium is practical in the laboratory due to the fact that it works well with different types of clinical samples, especially the ones with complex biological backgrounds (eg, feces), without requiring complicated instruments. Yet, it takes a long time to cultivate the isolates with this method despite its convenience and low cost.

Molecular biological assays on antibiotic resistant phenotype are based generally on known genetic loci. Real-time PCR and multiplex PCR have been used universally in routine clinical work and epidemiology research. The real-time PCR method is rapid and sensitive in resistance determination, and it is simple to operate and read. Nevertheless, due to its methodological limitations, the test results given by PCR may need broth microdilution testing for further result validation when the result is unreliable or a clinical physician requests a repeat test. Although plasmid-mediated resistance is blamed mainly on the *mcr* gene, there might be unknown variants contributing to the polymyxin-resistance phenotype, which would not be detected by PCR methods [5]. Also, molecular tests have no consideration for the penetrance of antibiotic genes, which could affect false negative or positive rates as PCR assays are based on genotype instead of phenotype. Next-generation sequencing provides full-scale genetic data of well-prepared samples. A recent study used a whole-genome sequencing method to detect plasmid-induced resistance, such as *mcr* genes, and intrinsic hereditable chromosome mutations related with polymyxin resistance, such as *mgrB* and *pmrB* [82]. Whole-genome sequencing is more accurate and can cover all

known variants, which makes it an optimal tool in research. However, antibiotic gene penetrance, high cost, and intricate instruments limit its routine use in hospital. There is increasing evidence that modifications to the outer membrane contribute to colistin resistance. In *A. baumannii*, lipid A enzymatically modifies L-Ara4N, PEtN, or galactosamine and will drastically increase polymyxin MICs. More specifically, polymyxin resistance is induced by *pmrA* and *pmrB* binary system when Fe^{3+}, Al^{3+}, or low pH value is present [83]. Similar modifications can be initiated by *pmrA/B*, *phoP/Q*, or *parR/S* systems in *P. aeruginosa*. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be employed for detection of lipid A modifications that confer colistin resistance. Park et al recommended to use methods that can decipher phenotypical changes, such as MALDI-TOF MS or lipid A construction, because of the mask effect of population heterogeneity [84]. Lipid A structure analysis is quick and accurate in predicting resistance phenotypes caused by lipid A modifications, regardless of strains or mechanisms. For researchers, pulsed field gel electrophoresis provides an efficient tool in homology analysis, though it was not suitable for clinical detection [4].

Novel detection methods are based mainly on newly discovered characteristics of CoR-related genes or resistant isolates. Structural studies of *mcr-1* phosphoethanolamine transferase domain emphasized that zinc cation plays an important role in the resistant phenotype, which enables the zinc-constraint system to be utilized in detection. Fernanda et al evaluated 4 different assays based on ethylenediaminetetraacetic acid (EDTA)-induced zinc-deprivation systems and indicated that the modified rapid polymyxin Nordmann-Poirel test has the highest sensitivity and specificity in phenotypic detection of colistin resistance among *Enterobacteriaceae*. The study also indicated that an accurate and inexpensive method can be developed based on zeta potential alteration caused by lipid A modification [85]. In addition, He et al developed new antibodies against MCR-1, which can be used in phenotypical detection of *mcr-1* and *mcr-2* in some species to originate resistance [86]. However, this method has been used only in meat samples and still needs further evaluation in human sample applications. Methods for colistin resistance detection are listed in Table 2.

TREATMENT FOR CoRB INFECTIONS

With increasing resistance to the polymyxin antibiotics, clinicians need to define effective therapies for CoRB infections. An accumulating body of evidence indicates that 3 groups of treatment can be effective in CoRB infections: (1) monotherapy, (2) combination chemotherapy (including combinations with or without colistin), and (3) new therapeutic methods. Despite resistance to colistin, CoRB infections typically remain susceptible to some other antibiotics. In a multicenter study, Quan et al found that 21 of 26 CoRB strains were susceptible to several antibiotics, including amikacin, piperacillin-tazobactam, cefoperazone-sulbactam, meropenem, imipenem, and tigecycline. Notably, the patients in this study had not had colistin treatment, and there was a 0% mortality rate [71]. Plazomicin was reported previously to be bactericidal in CoR *Enterobacteriaceae* in vitro [90]. Lertsrisatit et al has shown that colistin monotherapy is not optimal for treatment of CoRB. In this paper, all (17 of 17) CoR-AB isolates were susceptible to tigecycline and were partially susceptible to sulbactam, imipenem, meropenem, and cotrimoxazole in vitro [91]. A retrospective cohort study revealed gentamicin markedly reduced mortality caused by CoR-AB triggered sepsis, especially in urinary tract infections [63]. Patients in the study received empirical antibiotics first and then targeted antimicrobial therapy within 5 days. A retrospective study including 19 newborns infected with *K. pneumoniae* who were resistant to colistin and carbapenems discovered that all isolates were susceptible to tigecycline and chloramphenicol [32]. An epidemiological report in China, which revealed a high prevalence of *mcr-1*-positive strains in the stool samples of children, appealed a more rational prescription of colistin in gut infection in children [57]. Alternative antibiotic regimens have been shown to perform better than colistin for treatment of infections of carbapenem-resistant Gram-negative rods, which could help reduce colistin usage. Ceftazidime-avibactam showed lower renal injury rates and all-cause 30 days mortality rates compared to colistin-contained regimen (18% versus 57% and 9% versus 32%, respectively) [92, 93]. Plazomicin also was determined as a new effective therapeutic prescription with less severe side effects (50%, 9 of 18) compared to colistin (81%, 17 of 21) [94]. Nontraditional chemotherapeutic agents, such as zidovudine, also have been considered for polymyxin-resistant bacteria. Zidovudine has been used in antiretroviral treatment in patients with HIV. It also was effective in the elimination of CoR *E. coli* and *K. pneumoniae* clinical isolates in vitro. Because of the potential harm to hemopoiesis during long-term administration, Zidovudine might only be considered as an alternative short-term salvage chemotherapy in CoRB infection patients [95].

Combination therapy utilizing synergetic effects of multi-antibiotics also may be a practical treatment option for polymyxin-resistant bacteria. Colistin in combination with the following antibiotics have been reported to be synergetic with in vivo or in vitro trials against CoRB infection: chloramphenicol, netropsin, meropenem, rifampicin, gentamicin, and resveratrol [32, 71, 84, 96, 97]. In vitro synergy of triple-drug combinations of polymyxin B, aztreonam, and amikacin also was confirmed by Bulman et al [98]. Research by Chung et al established an alternative therapy in which netropsin combined with colistin worked effectively against CoR-AB, *E. coli*, *Shigella flexneri*,

Table 1. Colistin Resistance Rate in Common Microbes Isolated From Human and Food-Producing Animals [5, 26, 36, 42, 47, 50]

	K. pneumoniae	*A. baumannii*	*E. coli*	*Salmonella.spp*
Food-producing animal	0%–74.2%	Insufficient data	0.2%–21%	1.4% (overall), 0%–18.2%
Human	1.5%–6.8%, 13%–31.4% (Carbapenem R)	0%–6.45% , 4.4%(Carbapenem R)	0.5%–1.1%	1.5%

Salmonella typhi, and *P. aeruginosa* in the *Galleria mellonella* model [96]. In CoR-AB infected patients, Qureshi et al showed that treatment with colistimethate sodium, a carbapenem and ampicillin-sulbactam produced lower mortality (0%) compared with other regimens (60%) [69]. novel antimicrobials derived from colistin currently are being developed. Polymyxin B nonapeptide, a derivative of colistin, can potentiate rifampicin in treatment of Gram-negative bacterial infections because of its interaction with lipid A [99]. However, insufficient clinical data limits its application and more research is needed. New

Table 2. Detection Methods for Colistin Resistance in Clinical Practice and Scientific Research

		Name	Advance	Limitations	Ref.
Microbiological methods	Broth microdilution assay	UMIC (Biocentric, NJ)	• Manipulable • User-friendly	• Lack of accuracy in bacteria with extreme MICs • High VME rate	[78]
		Sensititre (ThermoFisher Diagnostics, Waltham, MA,)	• Low/moderate VMR rate	• Fixed testing samples per test	
		MicroScan (Beckman Coulter, Brea, CA)	• Low VMR rate	• Small range of MICs • Relatively high VME rate in some nonfermenting species	
	Chromogenic media	Superpolymyxin (ELITechGroup, Puteaux, France)	• Good identification of Gram-negative bacteria • Less sample pretreatment	• Unable to identify species directly	[79, 81, 87]
		COL-APSE (CHROMagar, Paris, France)	• Efficient in some species and nonfermenting Gram-negative bacteria	• Low ability to detect for plasmid-induced resistance	
		ChromID (bioMerieux, NC)	• Rapid identification of colony	• Poor performance in *Enterobacteriaceae*	
		rapid polymyxin NP test	• Easy to perform • Time-saving • Sensitive and specific	• Lack of confirmation in heteroresistant isolates with low MICs • Interference in detections of organism with low-level resistance • Limited target microbe (Enterobacteriaceae)	[101]
	Others	Disk diffusion, gradient diffusion		• Lack of accuracy, NOT RECOMMENDED	[32, 68]
Molecular biological methods	PCR	Real-time PCR	• Time-saving • Simple to operate and read • Sensitive	• Need broth microdilution test to validate • Specific-gene based	[88, 89]
		Multiplex PCR			
	Next generation sequence		• Large-scale genetic data • Accurate • Full coverage of known-resistance-related sequence	• Difficult to operate and read • High cost • Affected by gene penetration	[82, 90]
	Lipid A construction detection		• Phenotype-based	• Unable to detect other mechanism-induced resistance • Mask effect	[84]
Novel methods	Zinc-constraint system		• High sensitivity and specificity for modified Nordmann/Poirel test • Suitable for *Enterobacteriacea*	• Lack of validation	[85]
	MCR-1 antibody		• Phenotypical detection • Compatible for *mcr-1* and *mcr-2*	• Only has been used in meat sample	[86]

Abbreviations: MIC, minimal inhibitory concentration; PCR, polymerase chain reaction; VME, very major error.

therapies have been developed recently and are being tested. Photodynamic therapy is one of the safe neoplasm remedies. Yang et al attempted to add it to a CoRB infection regimen. Ming et al used blue light together with ZnO particles to eliminate CoR *A. baumannii* and *K. pneumoniae*. Thanks to its safety, this system has great potential in clinical regimens [100].

In summary, the polymyxins are a last-resort treatment option for multidrug-resistant Gram-negative bacteria. An increasing number of reports evaluating the mechanism, epidemiology, risk-factors, detection methods, and treatment options for colistin-resistant Gram-negative bacteria have been published. The prevalence of polymyxin resistance is increasing generally worldwide, but it is lower apparently in humans than in animals. However, potential risk of food chain contamination and human-animal cross-infection makes control of polymyxin resistance in the food chain important. Detection of polymyxin resistance is possible through several tests such as rapid NP test [101], but broth microdilution remains the gold standard method for phenotypic detection. It is noticeable that heterogenicity related to methodological diversity may issue in an unreliable result. Monotherapy and combination chemotherapy have been used in the treatment of patients infected with CoR microorganisms. Additional studies are warranted to determine optimal antimicrobial treatment options. Emerging polymyxin resistance raises the risk of treatment failure, especially in the patients with multidrug-resistant Gram-negative bacteria. However, a combination of rapid diagnosis, optimal treatment regimen, and good infection control practice in humans and animals will weaken greatly the threat of this bacteria.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Author contributions. Z. L., Y.C., and L.Y. wrote the manuscript; J.-H.L. and Q.Y. revised it; and all authors commented on the manuscript.

Financial support. This study was supported by the National Key Research and Development Program of China (2018YFC1200100, 2018YFC1200105), the CAMS Initiative for Innovative Medicine (grant no. 2016-I2M-3–014), and the Outstanding Talents Training Funding Project of Dongcheng District, Beijing (2017).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Biswas S, Brunel JM, Dubus JC, Reynaud-Gaubert M, Rolain JM. Colistin: an update on the antibiotic of the 21st century. Expert Rev Anti Infect Ther **2012**; 10:917–34.
2. Li J, Nation RL, Turnidge JD, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis **2006**; 6:589–601.
3. Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev **2017**; 30:557–96.
4. Catry B, Cavaleri M, Baptiste K, 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**; 46:297–306.
5. Liu YY, Wang Y, Walsh TR, 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**; 16:161–8.
6. Gunn JS. The Salmonella PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. Trends Microbiol **2008**; 16:284–90.
7. Cannatelli A, D'Andrea MM, Giani T, et al. In vivo emergence of colistin resistance in *Klebsiella pneumoniae* producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP mgrB regulator. Antimicrob Agents Chemother **2013**; 57:5521–6.
8. Yan A, Guan Z, Raetz CR. An undecaprenyl phosphate-aminoarabinose flippase required for polymyxin resistance in *Escherichia coli*. J Biol Chem **2007**; 282:36077–89.
9. Wright MS, Suzuki Y, Jones MB, et al. Genomic and transcriptomic analyses of colistin-resistant clinical isolates of *Klebsiella pneumoniae* reveal multiple pathways of resistance. Antimicrob Agents Chemother **2015**; 59:536–43.
10. Cannatelli A, Giani T, D'Andrea MM, et al; COLGRIT Study Group. MgrB inactivation is a common mechanism of colistin resistance in KPC-producing *Klebsiella pneumoniae* of clinical origin. Antimicrob Agents Chemother **2014**; 58:5696–703.
11. Baron S, Leulmi Z, Villard C, Olaitan AO, Telke AA, Rolain JM. Inactivation of the *arn* operon and loss of aminoarabinose on lipopolysaccharide as the cause of susceptibility to colistin in an atypical clinical isolate of *proteus vulgaris*. Int J Antimicrob Agents **2018**; 51:450–7.
12. Phan MD, Nhu NTK, Achard MES, et al. Modifications in the pmrB gene are the primary mechanism for the development of chromosomally encoded resistance to polymyxins in uropathogenic *Escherichia coli*. J Antimicrob Chemother **2017**; 72:2729–36.
13. Xu Y, Wei W, Lei S, et al. An evolutionarily conserved mechanism for intrinsic and transferable polymyxin resistance. MBio **2018**; 9.:e02317–17.
14. Pelletier MR, Casella LG, Jones JW, et al. Unique structural modifications are present in the lipopolysaccharide from colistin-resistant strains of *Acinetobacter baumannii*. Antimicrob Agents Chemother **2013**; 57:4831–40.
15. Rhouma M, Beaudry F, Thériault W, Letellier A. Colistin in pig production: chemistry, mechanism of antibacterial action, microbial resistance emergence, and one health perspectives. Front Microbiol **2016**; 7:1789.
16. Cheng YH, Lin TL, Lin YT, Wang JT. A putative RND-type efflux pump, H239_3064, contributes to colistin resistance through CrrB in *Klebsiella pneumoniae*. J Antimicrob Chemother **2018**; 73:1509–16.
17. Boll JM, Crofts AA, Peters K, et al. A penicillin-binding protein inhibits selection of colistin-resistant, lipooligosaccharide-deficient *Acinetobacter baumannii*. Proc Natl Acad Sci U S A **2016**; 113:E6228–37.
18. Lee K, Yong D, Jeong SH, Chong Y. Multidrug-resistant Acinetobacter spp.: increasingly problematic nosocomial pathogens. Yonsei Med J **2011**; 52:879–91.
19. Herrera CM, Crofts AA, Henderson JC, Pingali C, Davies BW, Trent MS. Correction for Herrera et al., The *Vibrio cholerae* VprA-VprB two-component system controls virulence through endotoxin modification. MBio **2015**; 6:e00155-15.
20. Fernández L, Jenssen H, Bains M, Wiegand I, Gooderham WJ, Hancock RE. The two-component system CprRS senses cationic peptides and triggers adaptive resistance in *Pseudomonas aeruginosa* independently of ParRS. Antimicrob Agents Chemother **2012**; 56:6212–22.
21. Shen Z, Wang Y, Shen Y, Shen J, Wu C. Early emergence of *mcr-1* in *Escherichia coli* from food-producing animals. Lancet Infect Dis **2016**; 16:293.
22. Gao R, Hu Y, Li Z, et al. Dissemination and mechanism for the MCR-1 colistin resistance. PLOS Pathog **2016**; 12:e1005957.
23. Zhao F, Feng Y, Lu X, McNally A, Zong Z. IncP plasmid carrying colistin resistance gene *mcr-1* in *Klebsiella pneumoniae* from hospital sewage. Antimicrob Agents Chemother **2017**; 61.:e02229–16.
24. Xavier BB, Lammens C, Butaye P, Goossens H, Malhotra-Kumar S. Complete sequence of an IncFII plasmid harbouring the colistin resistance gene *mcr-1* isolated from Belgian pig farms. J Antimicrob Chemother **2016**; 71:2342–4.
25. Sherry N, Howden B. Emerging Gram negative resistance to last-line antimicrobial agents fosfomycin, colistin and ceftazidime-avibactam - epidemiology, laboratory detection and treatment implications. Expert Rev Anti Infect Ther **2018**; 16:289–306.
26. Lekunberri I, Balcázar JL, Borrego CM. Detection and quantification of the plasmid-mediated *mcr-1* gene conferring colistin resistance in wastewater. Int J Antimicrob Agents **2017**; 50:734–6.

27. Zurfuh K, Poirel L, Nordmann P, Nüesch-Inderbinen M, Hächler H, Stephan R. Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in extended-spectrum-β-lactamase-producing *Enterobacteriaceae* in river water and imported vegetable samples in Switzerland. Antimicrob Agents Chemother **2016**; 60:2594–5.
28. Zheng B, Huang C, Xu H, et al. Occurrence and genomic characterization of ESBL-producing, MCR-1-harboring *Escherichia coli* in farming soil. Front Microbiol **2017**; 8:2510.
29. Gales AC, Jones RN, Sader HS. Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006–09). J Antimicrob Chemother **2011**; 66:2070–4.
30. Kontopidou F, Plachouras D, Papadomichelakis E, et al. Colonization and infection by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients. Clin Microbiol Infect **2011**; 17:E9–E11.
31. Antoniadou A, Kontopidou F, Poulakou G, et al. Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster. J Antimicrob Chemother **2007**; 59:786–90.
32. Battikh H, Harchay C, Dekhili A, et al. Clonal spread of colistin-resistant *Klebsiella pneumoniae* coproducing KPC and VIM carbapenemases in neonates at a Tunisian University Hospital. Microb Drug Resist **2017**; 23:468–72.
33. Maalej SM, Meziou MR, Mahjoubi F, Hammami A. Epidemiological study of *Enterobacteriaceae* resistance to colistin in Sfax (Tunisia). Med Maladies Infect **2012**; 42:256–63.
34. Lu Y, Feng Y, McNally A, Zong Z. The occurence of colistin-resistant hypervirulent *Klebsiella pneumoniae* in China. Front Microbiol **2018**; 9:2568.
35. European Centre for Disease Prevention and Control. Antimicrobial Resistance Surveillance in Europe – Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2014. https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/antimicrobial-resistance-europe-2014.pdf. Published November **2015**. Accessed August 25, 2019.
36. Moubareck CA, Mouftah SF, Pál T, et al. Clonal emergence of *Klebsiella pneumoniae* ST14 co-producing OXA-48-type and NDM carbapenemases with high rate of colistin resistance in Dubai, United Arab Emirates. Int J Antimicrob Agents **2018**; 52:90–5.
37. Wang Q, Wang X, Wang J, et al. Phenotypic and genotypic characterization of carbapenem-resistant *Enterobacteriaceae*: data from a longitudinal large-scale CRE study in China (2012–2016). Clin Infect Dis **2018**; 67(suppl_2):S196–205.
38. Wang R, Liu Y, Zhang Q, et al. The prevalence of colistin resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolated from food animals in China: coexistence of *mcr-1* and blaNDM with low fitness cost. Int J Antimicrob Agents **2018**; 51:739–44.
39. Hejnar P, Kolár M, Hájek V. Characteristics of Acinetobacter strains (phenotype classification, antibiotic susceptibility and production of beta-lactamases) isolated from haemocultures from patients at the Teaching Hospital in Olomouc. Acta Univ Palacki Olomuc Fac Med **1999**; 142:73–7.
40. Mahamat A, Bertrand X, Moreau B, et al. Clinical epidemiology and resistance mechanisms of carbapenem-resistant *Acinetobacter baumannii*, French Guiana, 2008-2014. Int J Antimicrob Agents **2016**; 48:51–5.
41. Sheck EA, Edelstein MV, Sukhorukova MV, et al. Epidemiology and genetic diversity of colistin nonsusceptible nosocomial *Acinetobacter baumannii* strains from Russia for 2013-2014. Can J Infect Dis Med Microbiol **2017**; 2017:1839190.
42. Giamarellou H. Epidemiology of infections caused by polymyxin-resistant pathogens. Int J Antimicrob Agents **2016**; 48:614–21.
43. Mahmoudi S, Mahzari M, Banar M, et al. Antimicrobial resistance patterns of Gram-negative bacteria isolated from bloodstream infections in an Iranian referral paediatric hospital: A 5.5-year study. J Glob Antimicrob Resist **2017**; 11:17–22.
44. Strateva T, Sirakov I, Stoeva T, et al. Carbapenem-resistant *Acinetobacter baumannii*: Current status of the problem in four Bulgarian university hospitals (2014-2016). J Glob Antimicrob Resist **2019**; 16:266–73.
45. Wasyl D, Zając M, Lalak A, et al. Antimicrobial resistance in *Escherichia coli* isolated from wild animals in Poland. Microb Drug Resist **2018**; 24:807–15.
46. Buess S, Nüesch-Inderbinen M, Stephan R, Zurfluh K. Assessment of animals as a reservoir for colistin resistance: No MCR-1/MCR-2-producing *Enterobacteriaceae* detected in Swiss livestock. J Glob Antimicrob Resist **2017**; 8:33–4.
47. Kuo SC, Huang WC, Wang HY, Shiau YR, Cheng MF, Lauderdale TL. Colistin resistance gene *mcr-1* in *Escherichia coli* isolates from humans and retail meats, Taiwan. J Antimicrob Chemother **2016**; 71:2327–9.
48. Li B, Ke B, Zhao X, et al. Antimicrobial resistance profile of *mcr-1* positive clinical isolates of *Escherichia coli* in China from 2013 to 2016. Front Microbiol **2018**; 9:2514.
49. Wang Y, Zhang R, Li J, et al. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nat Microbiol **2017**; 2:16260.
50. European Centre for Disease Prevention and Control. Surveillance of Antimicrobial Resistance in Europe 2017. https://ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2017. Published **November 15, 2018**. Accessed August 25, 2019.
51. Sader HS, Dale GE, Rhomberg PR, Flamm RK. Antimicrobial activity of murepavadin tested against clinical isolates of *Pseudomonas aeruginosa* from the United States, Europe, and China. Antimicrob Agents Chemother **2018**; 62.e00311–18.
52. Chen H, Wang Z, Li H, et al. In vitro analysis of activities of 16 antimicrobial agents against Gram-negative bacteria from six teaching hospitals in China. Jpn J Infect Dis **2015**; 68:263–7.
53. Liu L, Liu B, Li Y, Zhang W. Successful control of resistance in *Pseudomonas aeruginosa* using antibiotic stewardship and infection control programs at a Chinese university hospital: a 6-year prospective study. Infect Drug Resist **2018**; 11:637–46.
54. Kieffer N, Aires-de-Sousa M, Nordmann P, Poirel L. High rate of MCR-1-producing *Escherichia coli* and *Klebsiella pneumoniae* among Pigs, Portugal. Emerg Infect Dis **2017**; 23:2023–9.
55. Grami R, Mansour W, Mehri W, et al. Impact of food animal trade on the spread of *mcr-1*-mediated colistin resistance, Tunisia, July 2015. Euro Surveill **2016**; 21:30144.
56. Guenther S, Falgenhauer L, Semmler T, et al. Environmental emission of multiresistant *Escherichia coli* carrying the colistin resistance gene *mcr-1* from German swine farms. J Antimicrob Chemother **2017**; 72:1289–92.
57. Hu YY, Wang YL, Sun QL, et al. Colistin resistance gene *mcr-1* in gut flora of children. Int J Antimicrob Agents **2017**; 50:593–7.
58. Wang Y, Tian GB, Zhang R, et al. Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive *Enterobacteriaceae* in patients and healthy adults from China: an epidemiological and clinical study. Lancet Infect Dis **2017**; 17:390–9.
59. Zheng B, Xu H, Yu X, et al. Low prevalence of MCR-1-producing *Klebsiella pneumoniae* in bloodstream infections in China. Clin Microbiol Infect **2018**; 24:205–6.
60. Giacobbe DR, Del Bono V, Trecarichi EM, et al; ISGRI-SITA (Italian Study Group on Resistant Infections of the Società Italiana Terapia Antinfettiva). Risk factors for bloodstream infections due to colistin-resistant KPC-producing *Klebsiella pneumoniae*: results from a multicenter case-control-control study. Clin Microbiol Infect **2015**; 21:1106.e1–8.
61. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis **1987**; 40:373–83.
62. Drozdinsky G, Ben-Zvi H, Kushnir S, Leibovici L, Yahav D. Colistin exposure as a risk factor for infections caused by inherently colistin resistant *Enterobacteriaceae*-a case-control study. Clin Microbiol Infect **2018**; 24:896–9.
63. Richter SE, Miller L, Uslan DZ, et al. Risk factors for colistin resistance among Gram-negative rods and *Klebsiella pneumoniae* isolates. J Clin Microbiol **2018**; 56.:e00149–18.
64. Can F, Menekse S, Ispir P, et al. Impact of the ST101 clone on fatality among patients with colistin-resistant *Klebsiella pneumoniae* infection. J Antimicrob Chemother **2018**; 73:1235–41.
65. Novovic K, Trudic A, Brkic S, et al. Molecular epidemiology of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* in Serbia from 2013 to 2016. Antimicrob Agents Chemother **2017**; 61.:e025550–16.
66. Lomonaco S, Crawford MA, Lascols C, et al. Resistome of carbapenem- and colistin-resistant *Klebsiella pneumoniae* clinical isolates. PLOS ONE **2018**; 13:e0198526.
67. Gundogdu A, Ulu-Kilic A, Kilic H, et al. Could frequent carbapenem use be a risk factor for colistin resistance? Microb Drug Resist **2018**; 24:774–81.
68. de Maio Carrillho CM, Gaudereto JJ, Martins RC, et al. Colistin-resistant *Enterobacteriaceae* infections: clinical and molecular characterization and analysis of in vitro synergy. Diagn Microbiol Infect Dis **2017**; 87:253–7.
69. Qureshi ZA, Hittle LE, O'Hara JA, et al. Colistin-resistant *Acinetobacter baumannii*: beyond carbapenem resistance. Clin Infect Dis **2015**; 60:1295–303.
70. Rossi Gonçalves I, Ferreira ML, Araujo BF, et al. Outbreaks of colistin-resistant and colistin-susceptible KPC-producing *Klebsiella pneumoniae* in a Brazilian intensive care unit. J Hosp Infect **2016**; 94:322–9.
71. Quan J, Li X, Chen Y, et al. Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study. Lancet Infect Dis **2017**; 17:400–10.
72. Falcone M, Russo A, Iacovelli A, et al. Predictors of outcome in ICU patients with septic shock caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*. Clin Microbiol Infect **2016**; 22:444–50.
73. Wang YC, Lee YT, Yang YS, et al. Risk factors and outcome for colistin-resistant Acinetobacter nosocomialis bacteraemia in patients without previous colistin exposure. Clin Microbiol Infect **2015**; 21:758–64.

74. Capone A, Giannella M, Fortini D, 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**; 19:E23–30.
75. Guducuoglu H, Gursoy NC, Yakupogullari Y, et al. Hospital outbreak of a colistin-resistant, NDM-1- and OXA-48-producing *Klebsiella pneumoniae*: high mortality from pandrug resistance. Microb Drug Resist **2018**; 24:966–72.
76. Gonzalez-Padilla M, Torre-Cisneros J, Rivera-Espinar F, et al. Gentamicin therapy for sepsis due to carbapenem-resistant and colistin-resistant *Klebsiella pneumoniae*. J Antimicrob Chemother **2015**; 70:905–13.
77. Park HJ, Cho JH, Kim HJ, et al. Colistin monotherapy versus colistin and rifampin combination therapy in pneumonia caused by colistin-resistant *Acinetobacter baumannii*: a randomized controlled trial. J Glob Antimicrob Resist **2018**; 17:66–71.
78. Jayol A, Nordmann P, André C, Poirel L, Dubois V. Evaluation of three broth microdilution systems to determine colistin susceptibility of Gram-negative bacilli. J Antimicrob Chemother **2018**; 73:1272–8.
79. 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**; 66:1554–61.
80. Lescat M, Poirel L, Tinguely C, Nordmann P. A resazurin reduction-based assay for rapid detection of polymyxin resistance in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. J Clin Microbiol **2019**; 57.:e01563–18.
81. 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**; 17:220.
82. Lalaoui R, Bakour S, Livnat K, Assous MV, Diene SM, Rolain JM Spread of carbapenem and colistin-resistant *Klebsiella pneumoniae* ST512 clinical isolates in Israel: a cause for vigilance. Microb Drug Resist **2019**; 25:63–71.
83. Chen HD, Groisman EA. The biology of the PmrA/PmrB two-component system: the major regulator of lipopolysaccharide modifications. Annu Rev Microbiol **2013**; 67:83–112.
84. Park YJ, Hong DJ, Yoon EJ, et al. Differences in colistin-resistant *Acinetobacter baumannii* clinical isolates between patients with and without prior colistin treatment. Ann Lab Med **2018**; 38:545–54.
85. Esposito F, Fernandes MR, Lopes R, et al. Detection of colistin-resistant MCR-1-positive *Escherichia coli* by use of assays based on inhibition by EDTA and zeta potential. J Clin Microbiol **2017**; 55:3454–65.
86. He X, Mavrici D, Patfield S, Rubio FM. Development of novel antibodies for detection of mobile colistin-resistant bacteria contaminated in meats. Sci Rep **2018**; 8:16744.
87. Girlich D, Naas T, Dortet L. Comparison of the superpolymyxin and ChromID colistin R screening media for the detection of colistin-resistant *Enterobacteriaceae* from spiked rectal swabs. Antimicrob Agents Chemother **2019**; 63. doi: 10.1128/AAC.01618-18.
88. Rebelo AR, Bortolaia V, Kjeldgaard JS, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1*, *mcr-2*, *mcr-3, mcr-4* and *mcr-5* for surveillance purposes. Euro Surveill **2018**; 23:29–39.
89. Hatrongjit R, Kerdsin A, Akeda Y, Hamada S. Detection of plasmid-mediated colistin-resistant and carbapenem-resistant genes by multiplex PCR. MethodsX **2018**; 5:532–6.
90. Denervaud-Tendon V, Poirel L, Connolly LE, et al. Plazomicin activity against polymyxin-resistant *Enterobacteriaceae*, including MCR-1-producing isolates. J Antimicrob Chemother **2017**; 72:2787–91.
91. Lertsrisatit Y, Santimaleeworagun W, Thunyaharn S, Traipattanakul J. In vitro activity of colistin mono- and combination therapy against colistin-resistant *Acinetobacter baumannii*, mechanism of resistance, and clinical outcomes of patients infected with colistin-resistant *A. baumannii* at a Thai university hospital. Infect Drug Resist **2017**; 10:437–43.
92. Shields RK, Nguyen MH, Chen L, et al. Ceftazidime-avibactam is superior to other treatment regimens against carbapenem-resistant *Klebsiella pneumoniae* bacteremia. Antimicrob Agents Chemother **2017**; 61:e00883–17.
93. van Duin D, Lok JJ, Earley M, et al; Antibacterial Resistance Leadership Group. Colistin versus ceftazidime-avibactam in the treatment of infections due to carbapenem-resistant *Enterobacteriaceae*. Clin Infect Dis **2018**; 66:163–71.
94. McKinnell JA, Dwyer JP, Talbot GH, et al; CARE Study Group. Plazomicin for infections caused by carbapenem-resistant *Enterobacteriaceae*. N Engl J Med **2019**; 380:791–3.
95. Peyclit L, Baron SA, Yousfi H, Rolain JM. Zidovudine: a salvage therapy for *mcr-1* plasmid-mediated colistin-resistant bacterial infections? Int J Antimicrob Agents **2018**; 52:11–3.
96. Chung JH, Bhat A, Kim CJ, et al. Combination therapy with polymyxin B and netropsin against clinical isolates of multidrug-resistant *Acinetobacter baumannii*. Sci Rep **2016**; 6:28168.
97. Cannatelli A, Principato S, Colavecchio OL, et al. Synergistic activity of colistin in combination with resveratrol against colistin-resistant Gram-negative pathogens. Front Microbiol **2018**; 9:1808.
98. Bulman ZP, Chen L, Walsh TJ, et al. Polymyxin combinations combat *Escherichia coli* harboring *mcr-1* and bla_{NDM-5}: preparation for a postantibiotic era. MBio **2017**; 8:e00540-17.
99. Stokes JM, MacNair CR, Ilyas B, et al. Pentamidine sensitizes Gram-negative pathogens to antibiotics and overcomes acquired colistin resistance. Nat Microbiol **2017**; 2:17028.
100. Yang MY, Chang KC, Chen LY, et al. Blue light irradiation triggers the antimicrobial potential of ZnO nanoparticles on drug-resistant *Acinetobacter baumannii*. J Photochem Photobiol B **2018**; 180:235–42.
101. Nordmann P, Jayol A, Poirel L. Rapid Detection of Polymyxin Resistance in Enterobacteriaceae. Emerg Infect Dis **2016**; 22:1038–43. doi:10.3201/eid2206.151840.