RAPID RISK ASSESSMENT

Plasmid-mediated colistin resistance in Enterobacteriaceae

13 June 2016

Conclusions and options for response

The recently recognised global distribution of a self-transferable plasmid-borne colistin resistance determinant (*mcr-1* gene) poses a substantial public health risk to the EU/EEA. This specific mode of molecular dissemination of drug resistance is an example of a so-called plasmid-mediated gene epidemic.

This plasmid-mediated gene epidemic is of exceptional public health concern because it further limits treatment options in patients with infections caused by multidrug-resistant (MDR) gram-negative bacteria and can spread colistin resistance more easily between bacteria and humans than colistin resistance resulting from chromosomal mutation. MDR gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae strains that acquire the *mcr-1* gene, remain susceptible to only a few antimicrobial agents, which means that infections caused by these strains are very difficult to treat and result in excess mortality. As the limited development of new antimicrobials is unlikely to provide a solution anytime soon, it is crucial to take measures to control the spread of *mcr-1* and thus protect the activity of colistin.

Options for actions to reduce identified risks

Improved laboratory methods for colistin resistance and *mcr-1* detection

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratories Standards Institute (CLSI) recommend MIC determination by microbroth dilution as the reference method for colistin susceptibility testing. For the time being, these organisations do not recommend other methods (e.g. agar dilution, disk diffusion or gradient diffusion) for colistin susceptibility testing until historical data have been reviewed or new study data have been generated. ECDC has added colistin to the priority panel of antimicrobial agents to test for as part of the EU surveillance of antimicrobial-resistant *Salmonella* infections (revised EU surveillance protocol to be published June 2016) and initiated a project with EUCAST to study the gradient strip MIC and disk diffusion methods as an alternative.

PCR for *mcr-1* detection could be conveniently combined with the detection of other resistance gene targets in multiplex PCR-based assays used for the detection of multi-drug resistance determinants of epidemiological and clinical importance in gram-negative bacilli, such as extended-spectrum beta-lactamases and carbapenemases. Whole-genome sequencing (WGS) can be used for the detection or confirmation of the presence of the *mcr-1* gene and offers additional information about the associated plasmid vector, additional resistance genes, and strain type.

Suggested citation: European Centre for Disease Prevention and Control. Plasmid-mediated colistin resistance in Enterobacteriaceae. Stockholm: ECDC; 2016.

Improved surveillance

To gather more information on the extent of the spread and the prevalence of gram-negative bacteria carrying the *mcr-1* gene in the human and animal microbiomes, it is advisable to perform sentinel testing surveys of food, animal, environmental and human isolates to determine the presence of the *mcr-1* gene and characterise the associated mobile genetic vectors. The compulsory monitoring of colistin resistance in zoonotic and commensal bacteria isolated from food-producing animals and their meat is already in place in accordance with EU legislation.

At the local level, healthcare facilities affected by sporadic cases or epidemic outbreaks of carbapenem-resistant and other MDR Enterobacteriaceae should consider prospective, at least sentinel, colistin susceptibility testing followed by PCR detection of the *mcr-1* gene in colistin-resistant isolates to ascertain the introduction of the *mcr-1* gene into their facility and ensure its containment as part of their efforts to prevent and control MDR bacteria. Testing of MDR Enterobacteriaceae isolates from patients with recent contact with healthcare facilities in foreign countries for colistin susceptibility and for presence of the *mcr-1* gene could also be considered, based on available laboratory resources and following current national guidelines.

At the national level, there is a need for performing retrospective and prospective structured molecular testing surveys of the prevalence of the *mcr-1* gene in colistin-resistant isolates of carbapenem-resistant Enterobacteriaceae (CRE) and other MDR Enterobacteriaceae. In the *European Survey on Carbapenemase-Producing Enterobacteriaceae* (EuSCAPE) project, all EU/EEA Member States reported having a national surveillance system for CRE in place supported by national reference identification of carbapenemase genes. Performing additional phenotypic testing for co-resistance to colistin and molecular detection of the *mcr-1* gene on isolates collected within those sentinel, survey-based systems would be therefore valuable and feasible.

ECDC plans to initiate a new EU sentinel WGS-based surveillance module for CRE and extensively drug-resistant Enterobacteriaceae (XDRE) infections, by means of periodically repeating structured, pan-EU surveys following the sampling design of the EuSCAPE project. Analysis of WGS data from collected isolates will determine the genetic complement and predict the phenotype by mobilome/resistome/virulome profiling and the identification of genetic markers of high-risk clones and plasmids. This approach would enable identification of internationally disseminated high-risk colistin-resistant CRE clones that carry the *mcr-1* gene and monitor their geographical distribution across EU/EEA countries.

Clinical management

Timely and reliable laboratory investigation of microbiological samples and timely reporting of the results is essential to avoid a delay in the administration of appropriate antimicrobial treatment, which has been found to be associated with increased morbidity and mortality. Colistin treatment should be accompanied by colistin susceptibility testing. Patients with infections due to colistin-resistant bacteria are likely to benefit from consultations with experts in infectious diseases or clinical microbiology, which ensures the best possible therapeutic regimen considering the limited treatment options.

Actions to prevent transmission in healthcare settings

Infection control precautions

Adherence to standard infection control practices, including hand hygiene, environmental cleaning and adequate reprocessing of medical devices, and adequate capacity of microbiological laboratories are the basis for prevention of transmission of MDR bacteria.

Surveillance and detection of *mcr-1* in healthcare facilities should be conducted as outlined above. Prompt notification of patients who were identified as carriers or infected with colistin-resistant MDR gram-negative bacteria to the clinical team and to the infection prevention and control/hospital hygiene team is essential.

The need for enhanced control measures such as contact isolation precautions, single-room isolation or cohorting, and dedicated nursing for patients who tested positive, should be determined based on the antibiotic resistance pattern of the detected *mcr-1*-carrying Enterobacteriaceae isolates. Such enhanced control measures should be considered in particular for patients colonised or infected with isolates carrying both the *mcr-1* gene and carbapenemase genes. For patients colonised or infected with isolates carrying the *mcr-1* gene in combination with other resistance genes, individual decisions should be taken based on the resistance profile, the likelihood of nosocomial transmission, and previous evidence of association with outbreaks. This decision should take into account evidence on the spread of the strain and on control measures as soon as they become available.

Antimicrobial stewardship

Antimicrobial stewardship refers to coordinated programmes that implement interventions to ensure appropriate antimicrobial prescribing with the aim to improve clinical efficacy of antimicrobial treatment and limit antimicrobial resistance through reducing selective pressure. Although evidence for a specific beneficial effect of antimicrobial stewardship on the emergence and spread of colistin resistance is currently lacking, reduction of the use of broad-spectrum antimicrobials can reduce the emergence of carbapenem-resistant bacteria and therefore obviate the need for colistin treatment. Implementation of comprehensive antimicrobial stewardship programmes is therefore important. Caution should be exercised in using colistin-containing selective digestive tract decontamination as it can promote selection and spread of colistin resistance, especially if plasmid-based.

Nevertheless, targeted and appropriate use of antimicrobial agents is not likely to fully reverse the current resistance trends, and there is an urgent public health need to promote the development of new antibacterial agents active against MDR gram-negative bacteria.

Actions to prevent spread of plasmid-based colistin resistance in the community

One Health approach

It is important to carefully monitor the presence of *mcr-1* and to try to avoid the potential transmission of *mcr-1* via contaminated food. Close cooperation between veterinary medicine and human medicine, combined with regular monitoring in domestically produced and imported foodstuffs, is needed to assess whether consumers may be exposed to *mcr-1*-positive gram-negative bacteria via food.

Reduction of colistin use in animals

Prudent use of antimicrobials, and especially the reduction of colistin use in food-producing animals, would be effective in minimising the further emergence and spread of MDR bacteria, including those carrying *mcr-1*, via the food chain. Detailed guidance on the use of colistin in animals is available in a draft advice from the European Medicines Agency (EMA) dated 26 May 2016. As indicated by EMA, emphasis should also be placed on the improvement of the conditions of animal husbandry (e.g. biosecurity, hygienic conditions) and the implementation of alternative measures that would reduce the need for antimicrobial agents and therefore also reduce the development of antimicrobial-resistant bacteria in food-producing animals.

Source and date of request

ECDC internal decision, 30 May 2016.

Public health issue

The recent discovery of the *mcr-1* gene, encoding the first transferable resistance mechanism to colistin, a last-resort antimicrobial agent for infections by multidrug-resistant (MDR) gram-negative bacteria, raised widespread concern that its dissemination within the human microbiome could lead to nosocomial outbreaks of virtually untreatable infections. Follow-up reports have revealed worldwide spread of the *mcr-1* gene by plasmid transfer to diverse species and strains of Enterobacteriaceae, including MDR strains. In May 2016, the US CDC reported the first human *mcr-1* positive *E. coli* isolate detected in the United States.

This document assesses the risk for the patients and healthcare systems in the EU/EEA due to the global *mcr-1* plasmid-mediated gene epidemic [44,45] and reviews the options for strengthening laboratory-based surveillance and for mitigating further epidemic dissemination of *mcr-1*-positive MDR strains of Enterobacteriaceae in healthcare facilities.

Consulted experts

In alphabetical order: Barbara Albiger, Dragoslav Domanovic, Anke Kohlenberg, Dominique Monnet, Diamantis Plachouras, Marc Struelens

Consulted external experts: Jan Kluytmans (Amphia Hospital Breda), Gian Maria Rossolini (Florence Careggi University Hospital, Florence), Christian Giske (Karolinska University Hospital, Stockholm) and colleagues from the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Commission – Directorate-General for Health and Food Safety (SANTE).

Health issue background information

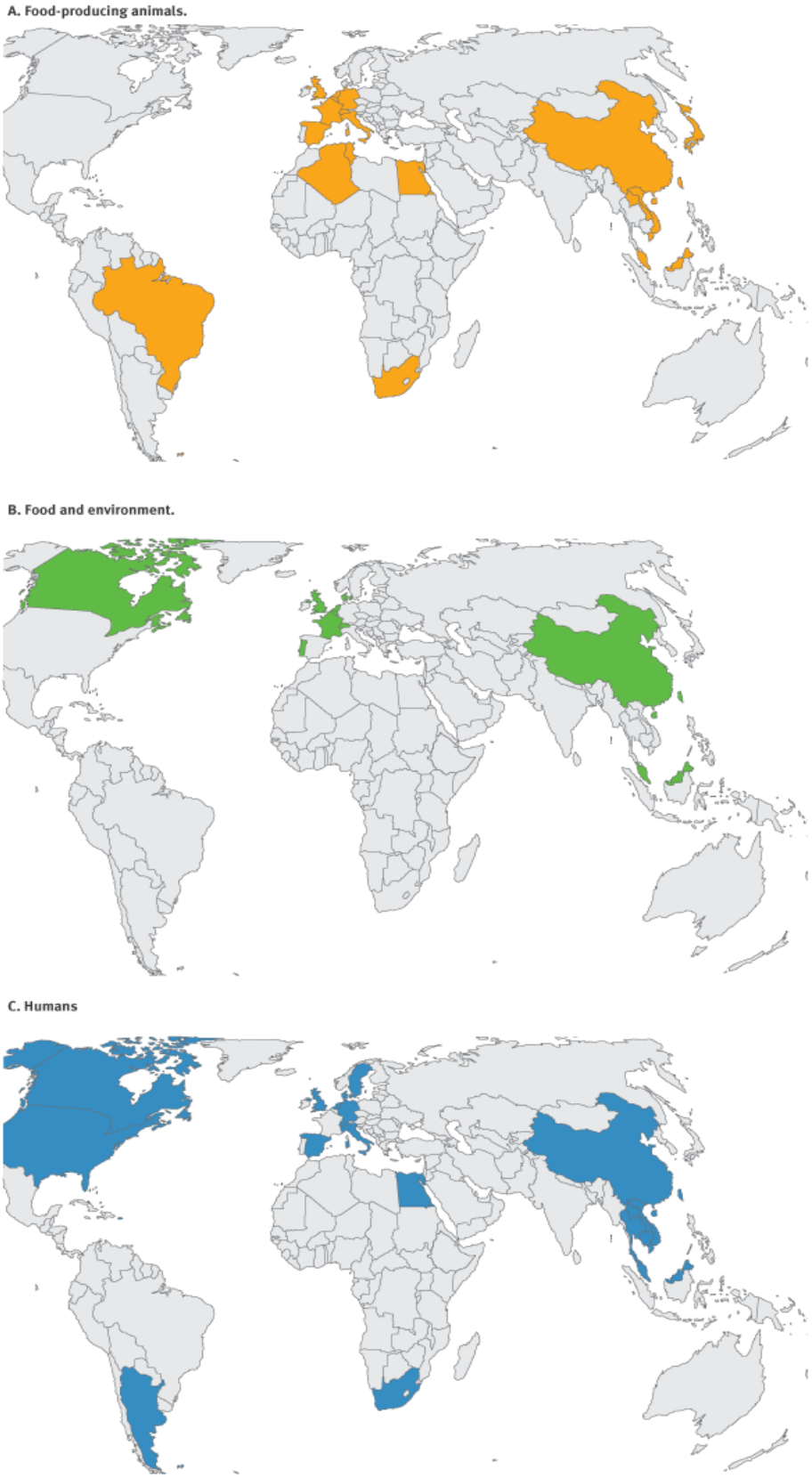
The global rise of MDR gram-negative bacteria is alarming and represents an increasing threat to healthcare delivery and patient safety in Europe and beyond. The past decades have seen a rapid increase of resistance to penicillins, cephalosporins and carbapenems due to the global spread of extended-spectrum beta-lactamases (ESBLs) and carbapenemases in gram-negative bacteria, first in *Klebsiella pneumoniae* and other *Klebsiella* species, then in *Escherichia coli* [1,2]. Treatment alternatives for patients infected with MDR gram-negative bacteria resistant to carbapenems and other key antimicrobial agents are often limited to combination therapy and to older antimicrobial agents such as polymyxins, e.g. colistin.

Colistin (polymixin E) is a cationic, multicomponent lipopeptide antibacterial agent that is effective against most gram-negative bacteria (including *Acinetobacter*, *Pseudomonas, Escherichia* and *Klebsiella* species) by disrupting the bacterial cell membrane. Because of its nephrotoxicity, colistin was restricted to topical use and virtually abandoned for systemic use when less toxic anti-gram-negative agents (e.g. beta-lactams, aminoglycosides) became available. The increasing number of hospital outbreaks with carbapenem-resistant gram-negative bacteria and the lack of new antimicrobial agents active against such MDR bacteria have compelled clinicians to reconsider the value of colistin as a therapeutic option and reintroduce its systemic administration as a last resort treatment for healthcare-associated infections due to carbapenem-resistant MDR gram-negative infections. For this reason, in 2012 WHO reclassified colistin as a critically important antibacterial agent for human medicine. The latest data available from the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) show that consumption of colistin in human health in the EU/EEA almost doubled in Europe between 2010 and 2014 [3]. Colistin is used more frequently for treating infected patients in those EU/EEA countries that have the highest prevalence of carbapenem-resistant Enterobacteriaceae (CRE). In some healthcare settings in these countries, as a result of an increasing number of patients exposed to colistin and varying infection control practices, colistin resistance has rapidly developed in carbapenem-resistant *K. pneumoniae* strains due to chromosomal mutations in the genes involved in lipopolysaccharide biosynthesis.

In November 2015, the discovery of a transferable plasmid-mediated colistin resistance gene, *mcr-1*, in *E. coli* challenged the concept that gram-negative bacteria only develop colistin resistance through chromosomal mutations or adaptive mechanisms (e.g. changes of the bacterial cell outer membrane or presence of an efflux pump). This new determinant of colistin resistance is transferable among bacterial strains and species by horizontal transfer of mobile genetic elements [4]. In vitro self-transfer of the *mcr-1* gene from conjugative plasmids has been shown to occur at high frequency [4].

While the *mcr-1* gene was first detected in Enterobacteriaceae in China, within the six months following its discovery, plasmids carrying the *mcr-1* gene were found in isolates from animals, food, the environment and humans worldwide, including the EU (Figure 1).

Figure 1. Geographic spread of the *mcr-1* gene, updated from [6] **on 2 June 2016**



Note: Maps are based on the latest peer-reviewed publications on the spread of the mcr-1 *gene.*

Event background information

On 18 November 2015, Liu et al. reported the first description of plasmid-mediated colistin resistance (*mcr-1* gene) in food-producing animals, food and humans in China [4]. On 3 March 2016, a literature review published in Eurosurveillance summarised the available information and knowledge on the extent of specimen/infection type, the geographic and longitudinal spread of the *mcr-1* gene, the variety of host species, the associated resistance genes as well as the rate of transferability of the plasmid-mediated *mcr-1* gene and showed that, within three months of its discovery, the *mcr-1* gene had been reported from most continents and found in bacteria from various food animals, from the environment, from various types of meat and vegetables, and from infected patients and asymptomatic human carriers including international travellers [6]. The first report on 26 May 2016 of the *mcr-1* gene in an *E. coli* isolate from a human specimen in the United States further extends the geographic distribution of the gene, which has been reported to date in 27 countries (Figure 1).

A summary of the latest data on detection of the *mcr-1 gene* in animals, food and humans can be found in Table 9 of the updated European Medicine Agency (EMA) draft advice on the use of colistin products in animals within the European Union, dated 26 May 2016 [5].

In the EU/EEA, the *mcr-1* gene was identified in human samples starting from 2011 in Denmark, Germany, Italy, the Netherlands, Spain, Sweden and the United Kingdom; in food-producing animals starting from 2005 in Belgium, France, Germany, Italy, the Netherlands, Spain and the United Kingdom; from food starting from 2009 in Belgium, Denmark, France, Portugal, the Netherlands and the United Kingdom [5,6] (Figure 1). Since the publication of the updated EMA draft advice, there have been several additional reports of detection of the *mcr-1* gene, including reports of detection in isolates from human samples from Malaysia [7], South Africa [8], Egypt [9], the USA [10] and China [11,12] and animal isolates from Brazil [13] and the UK [14].

Following the discovery of this new mechanism of resistance to colistin and given its biological potential for rapid spread, the European Commission requested the Antimicrobial Advice ad hoc Expert Group (AMEG) of EMA to update its 2013 advice on the *Use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health* [15]. This update was endorsed by the Committee for Medicinal Products for Veterinary Use (CVMP) and the Committee for Medicinal Products for Human Use (CHMP) and launched on 26 May 2016 for a public consultation until 26 June 2016 [5]. The update recommends that the sale of colistin for use in animals should be reduced and that its use in animals should be restricted to last-resort treatment, The update also proposes appropriate risk management measures [5].

In line with the EMA update, this rapid risk assessment was initiated to update the information on the dissemination and prevalence of *mcr-1* mediated colistin resistance in humans in the EU/EEA and on new developments in laboratory detection methods. In addition, it reviews the options for strengthening laboratory-based surveillance systems for MDR, surveys the emerging antimicrobial resistant bacterial pathogens in human health in the EU/EEA, and advises on mitigating the risk of further epidemic dissemination of *mcr-1*-positive, MDR strains of Enterobacteriaceae in healthcare facilities.

Colistin resistance mechanisms

Some gram-negative bacteria (e.g. *Morganella*, *Proteus*, *Providencia*, *Serratia* and *Burkholderia* species) are intrinsically resistant to colistin. Colistin resistance can be acquired in other, normally susceptible gram-negative bacteria such as *E. coli* and *K. pneumoniae* by adaptive or mutational mechanisms that alter the bacterial outer membrane, which is the site of action of colistin. Mutations in the genes (e.g. pmrHFIJFKLM operon, lpxACD, pmrAB, phoQ and mgrB) involved in the synthesis of components of the bacterial outer membrane (i.e. lipopolysaccharide [LPS] and its core component lipid A) or in its regulation can change the negative surface charge of the outer membrane such that colistin is no longer able to bind and initiate membrane lysis [16,17]. The recently discovered *mcr-1* gene encodes for a phosphoethanolamine (PEt) transferase that adds PEt to lipid A, which reduces the LPS anionic charge and, subsequently, its affinity to the cationic colistin. While the genes involved in the synthesis of the bacterial outer membrane or in its regulation are usually located on the chromosome, the *mcr-1* gene is carried by mobile genetic elements such as plasmids [4]. Moreover, it was recently found that the insertion of mobile genetic elements, such as insertion sequences (IS) or transposons carrying ESBL/carbapenem-resistance genes into the regulatory gene mgrB, conferred not only resistance to extended-spectrum cephalosporins/carbapenems but concomitantly colistin resistance [18,19]. These findings highlight the critical role of mobile genetic elements carrying resistant genes in amplifying co-resistance and accelerating the emergence of pandrug-resistant (PDR) bacteria.

Laboratory detection of colistin resistance and genetic identification of *mcr-1*

Colistin susceptibility testing is technically challenging due to several methodological issues. Polymyxins bind to a range of plastics and glass, including the most common plastics (polystyrene and polypropylene) used in microdilution trays used in laboratory susceptibility testing, raising reliability and reproducibility issues. These issues were extensively investigated by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratories Standards Institute (CLSI). Both recommend microbroth dilution MIC determination as the only reliable and reproducible colistin susceptibility test method under the condition that uncoated polystyrene microtiter plates and cation-adjusted Mueller-Hinton culture broth are used without any other additives (i.e. in particular polysorbate 80 and other surfactants). They currently do not recommend susceptibility testing by other methods, including agar dilution, disk diffusion and gradient diffusion until historical data have been reviewed or new study data have been generated. Work on validation of these methods is ongoing [20].

A rapid screening test for the detection of colistin resistance in Enterobacteriaceae in less than 2 hours has recently been developed, based on the detection of glucose metabolisation associated with bacterial growth in a defined concentration of colistin [21]. A new growth medium for screening polymyxin-resistant gram-negative isolates (SyperPolyxin) has also been developed [22]. However, these tests have not yet been prospectively validated on a large scale. They could be used in the near future as a first-step screening for colistin-resistant Enterobacteriaceae before undertaking MIC determination and molecular investigations.

The direct detection of the *mcr-1* gene can be achieved either by means of polymerase-chain reaction (PCR) assay on genomic DNA or by whole-genome sequencing (WGS) and sequence homology search using dedicated antimicrobial resistance gene identification applications such as ResFinder [23]. Protocols for PCR amplification and sequencing have been developed for the detection, confirmation and molecular characterisation of the *mcr-1* gene [4].

Prevalence of colistin resistance among human and animal Enterobacteriaceae isolates in the EU

Surveillance of antimicrobial resistance among gram-negative bacilli causing human infection in the EU is based on data on the bacterial resistance phenotypes among invasive clinical isolates for selected species, including *E. coli* and *K. pneumoniae*, but does not monitor the frequency of molecular resistance mechanisms. The European Antimicrobial Resistance Surveillance Network (EARS-Net) collects data based on routine clinical laboratory antimicrobial susceptibility testing (AST) results. The availability of AST results for specific antimicrobial agents depends on local testing protocols. These protocols are primarily designed to advise clinicians on patient therapy and not for public health surveillance, i.e. many laboratories serving populations with low prevalence of colistin resistance in clinical isolates do not routinely test isolates for colistin susceptibility. Although EARS-Net data on colistin resistance are not complete, some countries, especially countries with an already high prevalence of carbapenem resistance, report large numbers of colistin-resistant isolates [24]. Indeed, colistin resistance is increasing in carbapenemase-producing Enterobacteriaceae (CPE) in Europe. In Italy, 43% of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* isolates collected during a period of six months from November 2013 to April 2014 [25] and 13% of carbapenem-resistant *K. pneumoniae* isolates from blood cultures reported to EARS-Net in 2014 were also resistant to colistin [24]. In Romania and Greece, approximately 20% of carbapenem-resistant *K. pneumoniae* isolates from blood cultures reported to EARS-Net in 2014 were resistant to colistin [24]. This recent increase in colistin resistance follows the increasing trend in consumption of colistin in human medicine, which has almost doubled between 2010 and 2014 in Europe [3] and is the highest in countries with high prevalence rates of carbapenem and multidrug resistance in gram-negative bacilli. EARS-Net does not collect molecular data on the presence of *mcr-1* gene or other colistin resistance mechanism.

The latest available data on colistin resistance in *Salmonella* from humans, animals and food are provided in the European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from 2014 [26]. Mandatory EU reporting for colistin resistance in *Salmonella* and indicator *E. coli* from animals and meat thereof was launched in 2014 [27]. The overall percentage of colistin resistance in *E. coli* and *Salmonella* from poultry and poultry meat is 2.6% and 8.5%, respectively [5].

Recent molecular studies of case reports and case series have determined the mechanisms of colistin resistance in extensively drug-resistant (XDR) and PDR strains of Enterobacteriaceae. Investigation of a large collection of colistin-resistant carbapenemase-producing *K. pneumoniae* isolates from Greece and Italy revealed that inactivation of the chromosomal *mgrB* regulatory gene is a common mechanism of colistin resistance [28], and this resistance mechanism was found to be prevalent also among colistin-resistant strains from other sources [29]. In 2015, the first complete genome sequence of a PDR strain of *K. pneumoniae* was described. This strain was resistant to all commercially available antibiotics, and whole-genome analysis elucidated the insertion of a mobile genetic element carrying the resistance to carbapenems into the chromosomal *mgrB* gene, making the bacterium simultaneously resistant to carbapenem and colistin [18]. In 2016, a clinical isolate of ESBL-producing and colistin-resistant *K. pneumoniae* was recovered from a patient treated with cefotaxime. The strain harboured an ESBL gene

associated with an IS element which transposed into the *mgrB* gene, again making it simultaneously resistant to carbapenem and colistin [19]. These anecdotal findings suggest that, in addition to spread of *mcr-1*, acquisition of other multidrug resistance determinants increases the likelihood of outbreaks of nosocomial infections by XDR or PDR strains of Enterobacteriaceae, for which no effective treatment is available.

The prevalence of *mcr-1* carrying isolates among colistin-resistant Enterobacteriaceae from humans and animals in the EU is not known due to the lack of surveillance data at EU level. The rapid publication of cases of retrospectively identified *mcr-1* positive isolates from human, animals and environmental samples in several EU/EEA countries is a testimony to the powerful information source provided by WGS analysis and public access to sequence databases.

The available information so far suggests that the prevalence of transferable colistin resistance mediated by *mcr-1* gene is still low among human isolates and among food-producing animal isolates of Enterobacteriaceae in Europe.

ECDC threat assessment for the EU

There are still major information gaps relating to the current prevalence of colistin resistance in human clinical isolates in the EU/EEA, as the data on colistin resistance collected through EARS-Net are not complete and due to inherent issues with methods for colistin susceptibility testing. In addition, there is also a general lack of information on the historical and current prevalence of colistin resistance due to the *mcr-1* gene and its evolution in gram-negative bacteria that form part of animal, human and food microbiomes.

Clinical hazard of *mcr-1*: limited treatment options and adverse prognosis

Despite its nephro- and neurotoxicity, colistin is increasingly used as one of the last available treatment options for patients with severe infections caused by carbapenem-resistant Enterobacteriaceae and carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. As parenteral colistin therapy is used mostly for infections with carbapenem-resistant bacteria, it can function as a surrogate marker for carbapenem resistance for surveillance purposes [30]. Whereas colistin resistance alone is not clinically relevant as long as other effective antimicrobial agents such as cephalosporins, carbapenems or fluoroquinolones are active, it can shift an MDR strain to an XDR or PDR phenotype when, as seen with several *mcr-1*-positive isolates, it is found in association with multiple other resistance genes [5,6]. Enterobacteriaceae isolates that produce different carbapenemases such as New Delhi Metallo-beta-lactamase (NDM)-5, NDM-9, Oxacillinase (OXA)-48, and *Klebsiella pneumoniae* carbapenemase (KPC)-2 in association with colistin resistance mediated by *mcr-1* [11,31-34] illustrate the diverse combinations of resistance genes that can render these bacteria XDR.

Systemic infections with CRE are in general associated with fatality rates above 50% [35]. Colistin resistance further limits the already scarce treatment options for patients with infections with CRE to the point where no effective treatment is available and has been associated with an increased risk of fatal outcome [36].

Public health hazard: *mcr-1* gene spread in the human microbiome

Spread in healthcare settings

The *mcr-1* gene has been detected in Enterobacteriaceae isolates from humans with severe infections [6]. Enterobacteriaceae such as *E. coli* and *K. pneumoniae* are frequent causes of healthcare-associated infections, and the incidence of MDR *E. coli* and *K. pneumoniae* isolates is increasing steadily in the EU/EEA [24]. *E. coli* is frequently exchanged between the environment, animals and humans and is part of the normal human intestinal microbiome, but also a common cause of urinary tract infections as well as bloodstream infections [37]. *Klebsiella* spp. and *Enterobacter* spp. are also associated with healthcare-associated infections and MDR outbreaks in acute care settings. In the ECDC point prevalence survey of healthcare-associated infections and antimicrobial use in acute care hospitals 2011–2012, *Klebsiella* spp. were isolated as the fifth most frequent organism in 8.7% of the documented health-care associated infections, while *Enterobacter* spp. were isolated as the as the ninth most frequent organism in 4.2% of the documented health-care associated infections [38].

The detection of *mcr-1* in invasive Enterobacteriaceae isolates highlights the risk of increasing co-resistance in pathogens that are already causing outbreaks of difficult-to-treat infections in hospitals. The *mcr-1* gene has also been detected in residents of long-term-care facilities [39]. The spread of *mcr-1* might go unnoticed in healthcare settings as colistin testing is not routinely performed in clinical laboratories, where testing is only carried out for isolates already resistant to first-line antibiotics.

Spread in the community

The use of colistin is much higher in animals than in humans. Estimates cite an average that is more than 600 times higher in food-producing animals than in humans (19 EU/EEA Member States included in the ECDC–EFSA–EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals) [40]. There is a larger number of publications that have reported the detection of *mcr-1* in isolates in animals than in humans. These findings might be biased by the fact that there are more data available for animals and food.

The authors of the original publication had already hypothesised that *mcr-1* first arose in animals and was transmitted to humans [4]. There is additional evidence for the link between *mcr-1* and animals such as the association of *mcr-1* with resistance genes widespread in animals and an IS originating from a common animal pathogen [41]. Detection of the *mcr-1* gene in farmers was shown to be associated with colistin use in Vietnamese poultry farms. Farmers exposed to chickens – and especially *mcr-1*-positive chickens – had a higher risk of faecal colonisation than individuals not exposed to poultry [42]. The multiple reports of *mcr-1* from animal samples raise the possibility that there is already a reservoir of *mcr-1* in animals [5,6,41], indicating that measures directed only at nosocomial transmission may not be sufficient.

The presence of *mcr-1* in food-producing animals such as pigs, chickens and veal calves and various food products, predominantly meat but also vegetables, has been described in several EU Member States including France, Germany, the Netherlands, Belgium, Italy, Portugal and the UK [5,6]. The presence of the *mcr-1* gene represents a potential food hazard with possible subsequent incorporation into the human intestinal microbiome. However, the extent of the risk cannot be quantified until further data are generated. Intestinal carriage of the *mcr-1* gene could be amplified in hospitalised patients by further genetic mobilisation and plasmid transfer to MDR strains under the selective pressure of high antibiotic use in EU/EEA hospitals, e.g. carbapenems and colistin.

There are very limited data on the duration of human carriage of bacteria harbouring the *mcr-1* gene, and further studies investigating the longitudinal carriage are needed. In a study on acquisition of faecal colonisation of MDR Enterobacteriaceae, the *mcr-1* gene was detected in isolates from six Dutch travellers shortly after returning from North Africa, Asia and South America. Subsequent samples from these travellers were negative after one month [43]. However, the duration of carriage of *mcr-1*-positive bacteria might be different in immunocompromised patients and patients exposed to antibiotics.

The *mcr-1* gene was first detected in China in isolates from food-producing animals and several subsequent reports document the detection of *mcr-1* in Asia (Figure 1), although these data may be biased by the lack of testing in other regions. In any event, it is likely that migration and travel can result in further spread of the *mcr-1* gene between regions and countries.

Conclusions and options for response

The recently recognised global distribution of a self-transferable plasmid-borne colistin resistance determinant (*mcr-1* gene) poses a substantial public health risk to the EU/EEA. This specific mode of molecular dissemination of drug resistance is an example of a so-called plasmid-mediated gene epidemic [44,45].

This plasmid-mediated gene epidemic is of exceptional public health concern because it further limits treatment options in patients with infections caused by multidrug-resistant (MDR) gram-negative bacteria and can spread colistin resistance more easily between bacteria and humans than colistin resistance resulting from chromosomal mutation. MDR gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae strains that acquire the *mcr-1* gene, remain susceptible to only a few antimicrobial agents, which means that infections caused by these strains are very difficult to treat and result in excess mortality. As the limited development of new antimicrobials is unlikely to provide a solution anytime soon, it is crucial to take measures to control the spread of *mcr-1* and thus protect the activity of colistin.

Options for actions to reduce identified risks

Improved laboratory methods for colistin resistance and *mcr-1* detection

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratories Standards Institute (CLSI) recommend MIC determination by microbroth dilution as the reference method for colistin susceptibility testing. For the time being, these organisations do not recommend other methods (e.g. agar dilution, disk diffusion or gradient diffusion) for colistin susceptibility testing until historical data have been reviewed or new study data have been generated [20]. ECDC has added colistin to the priority panel of antimicrobial agents to test for as part of the EU surveillance of antimicrobial-resistant *Salmonella* infections (revised EU surveillance protocol

to be published June 2016) and initiated a project with EUCAST to study the gradient strip MIC and disk diffusion methods as an alternative.

PCR for *mcr-1* detection could be conveniently combined with the detection of other resistance gene targets in multiplex PCR-based assays used for the detection of multi-drug resistance determinants of epidemiological and clinical importance in gram-negative bacilli, such as extended-spectrum beta-lactamases and carbapenemases. Whole-genome sequencing (WGS) can be used for the detection or confirmation of the presence of the *mcr-1* gene and offers additional information about the associated plasmid vector, additional resistance genes, and strain type.

Improved surveillance

To gather more information on the extent of the spread and the prevalence of gram-negative bacteria carrying the *mcr-1* gene in the human and animal microbiomes, it is advisable to perform sentinel testing surveys of food, animal, environmental and human isolates to determine the presence of the *mcr-1* gene and characterise the associated mobile genetic vectors. The compulsory monitoring of colistin resistance in zoonotic and commensal bacteria isolated from food-producing animals and their meat is already in place in accordance with EU legislation [27].

At the local level, healthcare facilities affected by sporadic cases or epidemic outbreaks of carbapenem-resistant and other MDR Enterobacteriaceae should consider prospective, at least sentinel, colistin susceptibility testing followed by PCR detection of the *mcr-1* gene in colistin-resistant isolates to ascertain the introduction of the *mcr-1* gene into their facility and ensure its containment as part of their efforts to prevent and control MDR bacteria. Testing of MDR Enterobacteriaceae isolates from patients with recent contact with healthcare facilities in foreign countries for colistin susceptibility and for presence of the *mcr-1* gene could also be considered, based on available laboratory resources and following current national guidelines.

At the national level, there is a need for performing retrospective and prospective structured molecular testing surveys of the prevalence of the *mcr-1* gene in colistin-resistant isolates of carbapenem-resistant Enterobacteriaceae (CRE) and other MDR Enterobacteriaceae. In the *European Survey on Carbapenemase-Producing Enterobacteriaceae* (EuSCAPE) project, all EU/EEA Member States reported having a national surveillance system for CRE in place supported by national reference identification of carbapenemase genes. Performing additional phenotypic testing for co-resistance to colistin and molecular detection of the *mcr-1* gene on isolates collected within those sentinel, survey-based systems would be therefore valuable and feasible.

ECDC plans to initiate a new EU sentinel WGS-based surveillance module for CRE and extensively drug-resistant Enterobacteriaceae (XDRE) infections, by means of periodically repeating structured, pan-EU surveys following the sampling design of the EuSCAPE project. Analysis of WGS data from collected isolates will determine the genetic complement and predict the phenotype by mobilome/resistome/virulome profiling and the identification of genetic markers of high-risk clones and plasmids. This approach would enable identification of internationally disseminated high-risk colistin-resistant CRE clones that carry the *mcr-1* gene and monitor their geographical distribution across EU/EEA countries.

Clinical management

Timely and reliable laboratory investigation of microbiological samples and timely reporting of the results is essential to avoid a delay in the administration of appropriate antimicrobial treatment, which has been found to be associated with increased morbidity and mortality. Colistin treatment should be accompanied by colistin susceptibility testing. Patients with infections due to colistin-resistant bacteria are likely to benefit from consultations with experts in infectious diseases or clinical microbiology, which ensures the best possible therapeutic regimen considering the limited treatment options.

Actions to prevent transmission in healthcare settings

Infection control precautions

Adherence to standard infection control practices, including hand hygiene, environmental cleaning and adequate reprocessing of medical devices, and adequate capacity of microbiological laboratories are the basis for prevention of transmission of MDR bacteria.

Surveillance and detection of *mcr-1* in healthcare facilities should be conducted as outlined above. Prompt notification of patients who were identified as carriers or infected with colistin-resistant MDR gram-negative bacteria to the clinical team and to the infection prevention and control/hospital hygiene team is essential.

The need for enhanced control measures such as contact isolation precautions, single-room isolation or cohorting, and dedicated nursing for patients who tested positive, should be determined based on the antibiotic resistance pattern of the detected *mcr-1*-carrying Enterobacteriaceae isolates. Such enhanced control measures should be considered in particular for patients colonised or infected with isolates carrying both the *mcr-1* gene and carbapenemase genes. For patients colonised or infected with isolates carrying the *mcr-1* gene in combination with

other resistance genes, individual decisions should be taken based on the resistance profile, the likelihood of nosocomial transmission, and previous evidence of association with outbreaks. This decision should take into account evidence on the spread of the strain and on control measures as soon as they become available.

Antimicrobial stewardship

Antimicrobial stewardship refers to coordinated programmes that implement interventions to ensure appropriate antimicrobial prescribing with the aim to improve clinical efficacy of antimicrobial treatment and limit antimicrobial resistance through reducing selective pressure. Although evidence for a specific beneficial effect of antimicrobial stewardship on the emergence and spread of colistin resistance is currently lacking, reduction of the use of broad-spectrum antimicrobials can reduce the emergence of carbapenem-resistant bacteria and therefore obviate the need for colistin treatment. Implementation of comprehensive antimicrobial stewardship programmes is therefore important. Caution should be exercised in using colistin-containing selective digestive tract decontamination as it can promote selection and spread of colistin resistance, especially if plasmid-based.

Nevertheless, targeted and appropriate use of antimicrobial agents is not likely to fully reverse the current resistance trends, and there is an urgent public health need to promote the development of new antibacterial agents active against MDR gram-negative bacteria.

Actions to prevent spread of plasmid-based colistin resistance in the community

One Health approach

It is important to carefully monitor the presence of *mcr-1* and to try to avoid the potential transmission of *mcr-1* via contaminated food. Close cooperation between veterinary medicine and human medicine, combined with regular monitoring in domestically produced and imported foodstuffs, is needed to assess whether consumers may be exposed to *mcr-1*-positive gram-negative bacteria via food.

Reduction of colistin use in animals

Prudent use of antimicrobials, and especially the reduction of colistin use in food-producing animals, would be effective in minimising the further emergence and spread of MDR bacteria, including those carrying *mcr-1*, via the food chain. Detailed guidance on the use of colistin in animals is available in a draft advice from the European Medicines Agency (EMA) dated 26 May 2016 [5]. As indicated by EMA, emphasis should also be placed on the improvement of the conditions of animal husbandry (e.g. biosecurity, hygienic conditions) and the implementation of alternative measures that would reduce the need for antimicrobial agents and therefore also reduce the rise in antimicrobial-resistant bacteria in food-producing animals.

References

1. Woerther PL, Angebault C, Jacquier H, Clermont O, El Mniai A, Moreau B, et al. Characterization of fecal extended-spectrum-beta-lactamase-producing *Escherichia coli* in a remote community during a long time period. Antimicrob Agents Chemother. 2013 Oct;57(10):5060-6.

2. Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect. 2014 Sep;20(9):821-30.

3. European Centre for Disease Prevention and Control. Antimicrobial consumption interactive database [Internet]. Stockholm: ECDC. Available from: http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/esac-net-database/Pages/database.aspx.

4. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, 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):161-8.

5. European Medicines Agency. Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health, draft: 26 May 2016 [Internet]. London: EMA; 2016. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/05/WC500207233.pdf.

6. Skov RL, Monnet DL. Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. Euro Surveill [Internet]. 2016; 21(9):[pii=30155 p.]. Available from: http://www.eurosurveillance.org/images/dynamic/EE/V21N09/art21403.pdf.

7. Yu CY, Ang GY, Chin PS, Ngeow YF, Yin WF, Chan KG. Emergence of *mcr-1*-mediated colistin resistance in *Escherichia coli* in Malaysia. Int J Antimicrob Agents. 2016 Jun;47(6):504-5.

8. Coetzee J, Corcoran C, Prentice E, Moodley M, Mendelson M, Poirel L, et al. Emergence of plasmid-mediated colistin resistance (*mcr*-1) among *Escherichia coli* isolated from South African patients. S Afr Med J. 2016;106(5):449-50.

9. Elnahriry SS, Khalifa HO, Soliman AM, Ahmed AM, Hussein AM, Shimamoto T, et al. Emergence of plasmid-mediated colistin resistance gene *mcr*-1 in a clinical *Escherichia coli* isolate from Egypt. Antimicrob Agents Chemother. 2016 May;60(5):3249-50.

10. McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, et al. Escherichia coli harboring *mcr*-1 and *bla*CTX-M on a novel IncF plasmid: first report of *mcr-1* in the USA. Antimicrob Agents Chemother. 2016 May 26.

11. Yu H, Qu F, Shan B, Huang B, Jia W, Chen C, et al. Detection of mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae (CRE) from different hospitals in China. Antimicrob Agents Chemother. 2016 May 23.

12. Zhang XF, Doi Y, Huang X, Li HY, Zhong LL, Zeng KJ, et al. Possible transmission of *mcr*-1-harboring *Escherichia coli* between companion animals and human. Emerg Infect Dis. 2016 Sep 15;22(9).

13. Fernandes MR, Moura Q, Sartori L, Silva KC, Cunha MP, Esposito F, et al. Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr*-1 gene. Euro Surveill [Internet]. 2016 Apr 28; 21(17):[pii=30214 p.]. Available from: http://www.eurosurveillance.org/images/dynamic/EE/V21N17/art22458.pdf.

14. Anjum MF, Duggett NA, AbuOun M, Randall L, Nunez-Garcia J, Ellis RJ, et al. Colistin resistance in *Salmonella* and *Escherichia coli* isolates from a pig farm in Great Britain. J Antimicrob Chemother. 2016 May 4.

15. European Medicines Agency. Use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health: 19 July 2013 [Internet]. London: EMA; 2013. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2013/07/WC500146813.pdf.

16. Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski A, 3rd, et al. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. Pharmacotherapy. 2010 Dec;30(12):1279-91.

17. Sekyere JO, Govinden U, Bester LA, Essack SY. Colistin and Tigecycline resistance in carbapenemase-producing gram-negative bacteria: emerging resistance mechanisms and detection methods. J Applied Microbiol [Internet]. 2016 Forthcoming. Available from: http://onlinelibrary.wiley.com/doi/10.1111/jam.13169/abstract.

18. Zowawi HM, Forde BM, Alfaresi M, Alzarouni A, Farahat Y, Chong TM, et al. Stepwise evolution of pandrug-resistance in *Klebsiella pneumoniae*. Sci Rep. 2015;5:15082.

19. Jayol A, Nordmann P, Desroches M, Decousser JW, Poirel L. Acquisition of broad-spectrum cephalosporin resistance leading to colistin resistance in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2016 May;60(5):3199-201.

20. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST polymyxin breakpoints working group: 22 May 2016 [Internet]. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf.

21. Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in Enterobacteriaceae . Emerg Infect Dis. 2016 Jun;22(6):1038-43.

22. Nordmann P, Jayol A, Poirel L. A universal culture medium for screening polymyxin-resistant gram-negative isolates. J Clin Microbiol. 2016 May;54(5):1395-9.

23. Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agerso Y, et al. Detection of *mcr*-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. Euro Surveill. 2015;20(49).

24. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2014. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) [Internet]. Stockholm: ECDC; 2014. Available from: http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-europe-2014.pdf.

25. Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S, et al. Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. Euro Surveill [Internet]. 2014; 19(42). Available from: http://www.eurosurveillance.org/images/dynamic/EE/V19N42/art20939.pdf.

26. European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014 [Internet]: EFSA & ECDC; 2016. Available from: https://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4380.pdf.

27. European Commission. Commission implementing decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) [Internet]: European Commission; 2013. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013D0652&from=EN.

28. Cannatelli A, Giani T, Antonelli A, Principe L, Luzzaro F, Rossolini GM. First Detection of the *mcr*-1 colistin resistance gene in *Escherichia coli* in Italy. Antimicrob Agents Chemother. 2016 May;60(5):3257-8.

29. Poirel L, Jayol A, Bontron S, Villegas MV, Ozdamar M, Turkoglu S, et al. The mgrB gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*. J Antimicrob Chemother. 2015 Jan;70(1):75-80.

30. Kadri SS, Hohmann SF, Orav EJ, Bonne SL, Moffa MA, Timpone JG, et al. Tracking colistin-treated patients to monitor the incidence and outcome of carbapenem-resistant gram-negative infections. Clin Infect Dis. 2015 Jan 1;60(1):79-87.

31. Yao X, Doi Y, Zeng L, Lv L, Liu JH. Carbapenem-resistant and colistin-resistant *Escherichia coli* co-producing NDM-9 and MCR-1. Lancet Infect Dis. 2016 Mar;16(3):288-9.

32. Mulvey MR, Mataseje LF, Robertson J, Nash JH, Boerlin P, Toye B, et al. Dissemination of the *mcr*-1 colistin resistance gene. Lancet Infect Dis. 2016 Mar;16(3):289-90.

33. Falgenhauer L, Waezsada SE, Yao Y, Imirzalioglu C, Kasbohrer A, Roesler U, et al. Colistin resistance gene *mcr*-1 in extended-spectrum beta-lactamase-producing and carbapenemase-producing gram-negative bacteria in Germany. Lancet Infect Dis. 2016 Mar;16(3):282-3.

34. Du H, Chen L, Tang YW, Kreiswirth BN. Emergence of the *mcr*-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae . Lancet Infect Dis. 2016 Mar;16(3):287-8.

35. Tumbarello M, Trecarichi EM, De Rosa FG, Giannella M, Giacobbe DR, Bassetti M, et al. Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentre study. J Antimicrob Chemother. 2015 Jul;70(7):2133-43.

36. Capone A, Giannella M, Fortini D, Giordano A, Meledandri M, Ballardini M, 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):E23-30.

37. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med. 2010 May 13;362(19):1804-13.

38. European Centre for Disease Prevention and Control. Microorganisms isolated in HAIs (all HAI types) in acute care hospitals in EU/EEA, ECDC-PPS 2011-2012 [Internet]. Stockholm: ECDC. Available from: http://ecdc.europa.eu/en/healthtopics/Healthcare-associated_infections/database/Pages/hai-pps-database-microorganisms-antimicrobial-resistance.aspx.

39. Giufre M, Monaco M, Accogli M, Pantosti A, Cerquetti M. Emergence of the colistin resistance mcr-1 determinant in commensal Escherichia coli from residents of long-term-care facilities in Italy. J Antimicrob Chemother. 2016 Jun 3.

40. European Food Safety Authority. ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals [Internet]: ECDC, EFSA & EMA; 2015. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/4006.

41. Nordmann P, Poirel L. Plasmid-mediated colistin resistance: an additional antibiotic resistance menace. Clin Microbiol Infect. 2016 May;22(5):398-400.

42. Nguyen VT. Prevalence and risk factors for faecal colonization with mcr-1 carrying baceria in backyard poultry and humans in Vietnam: e-poster at the 26th European Congress of Clinical Microbiology and Infectious Diseases [Internet]. Amsterdam: ESCMID; 2016. Available from: http://eccmidlive.org/#resources/prevalence-and-risk-factors-for-fecal-colonization-with-mcr-1-carrying-bacteria-in-backyard-poultry-and-humans-in-vietnam.

43. Arcilla MS, van Hattem JM, Matamoros S, Melles DC, Penders J, de Jong MD, et al. Dissemination of the *mcr*-1 colistin resistance gene. Lancet Infect Dis. 2016 Feb;16(2):147-9.

44. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae . Clin Microbiol Rev. 2015 Jul;28(3):565-91.

45. Struelens MJ. Multiple-resistant enterococci and gram-negative bacteria: tracking gene dissemination. Microbial drug resistance (Larchmont, NY). 1995 Fall;1(3):193-4.