Journal of Food Protection, Vol. 82, No. 8, 2019, Pages 1440–1448
https://doi.org/10.4315/0362-028X.JFP-19-116

Review

Global Emergence of Colistin-Resistant *Escherichia coli* in Food Chains and Associated Food Safety Implications: A Review

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MS 19-116: Received 8 March 2019/Accepted 12 April 2019/Published Online 24 July 2019

ABSTRACT

Antimicrobial resistance in bacteria represents one of the most important challenges for public health worldwide. Human infections from antimicrobial-resistant bacteria can be transmitted from person to person, via the environment (especially in the hospital environment), or via handling or eating contaminated foods. Colistin is well known as a last-resort antibiotic for the treatment of human infections; a recent study performed in the People's Republic of China has revealed that colistin resistance is also conferred by the plasmid-mediated *mcr-1* gene in *Escherichia coli*. After that discovery, further plasmid-mediated, colistin resistance genes have been detected. However, to date, only reports on *E. coli* carrying the *mcr-1* gene (*E. coli mcr-1*$^+$) in foodstuff are available. *E. coli mcr-1*$^+$ has been isolated from food of animal origin and vegetables; this discovery has opened a debate among food safety experts. This review aims to provide a critical overview of the currently available scientific literature on the presence of the plasmid-mediated, colistin resistance gene *E. coli mcr-1* in foodstuffs, focusing on the main implications and future perspectives for food safety.

HIGHLIGHTS

- Antimicrobial resistance in the food chain: a One Health perspective.
- *Escherichia coli* carries the *mcr-1* gene in food-producing animals.
- *Escherichia coli* carrying the *mcr-1* gene in food from animals and vegetables is significant.

Key words: Colistin resistance; *Escherichia coli*; Food safety; *mcr-1*

Escherichia coli is one of the most studied microorganisms worldwide *(7)*. *E. coli* is a gram-negative bacterium that belongs to the family *Enterobacteriaceae,* commonly found in the gastrointestinal tract of humans and warm-blooded animals as part of the normal flora of the gut *(70)*. Most *E. coli* strains are harmless, whereas some pathogenic strains cause serious foodborne diseases and represent a significant public health problem *(76)*. Pathogenic *E. coli* is mainly transmitted to humans through consumption of contaminated foods, such as raw or undercooked ground meat products, raw or unpasteurized milk and dairy products, unpasteurized fruit juices, and contaminated raw vegetables and sprouts *(24, 76)*.

The importance of *E. coli* in the food industry is demonstrated by its inclusion in food safety regulations worldwide. In particular, the European Union (EU) legislation defines the microbiological limit for *E. coli,* which is used in the food industry as indicators of fecal contamination in the assessment of food hygiene and safety *(18)*.

Antimicrobial resistance (AMR) is defined by the World Health Organization as the "ability of a microorganism to stop an antimicrobial from working against it" *(74)*. AMR occurring in a wide range of infectious agents represents an ongoing public health threat. In fact, new resistance mechanisms, which compromise the usefulness of antimicrobials, are emerging and spreading globally. These dynamics and related challenges might lead us to a postantimicrobial era in which common infections and minor injuries, as well as more-serious diseases and health conditions, will no longer be treatable *(69)*.

AMR rates in *E. coli* are rising rapidly; according to the latest data on antibiotic resistance in the EU, published by the European Centre for Disease Prevention and Control, antibiotic resistance in *E. coli* requires close attention because the percentage of isolates resistant to commonly used antibiotics continues to increase throughout Europe *(16)*. In fact, several studies have reported colonization of food animals and food product contamination by antibiotic-resistant *E. coli* strains *(11, 54),* including extended

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spectrum β-lactamase–producing *E. coli* isolated from food samples, such as vegetables and fresh meat products made from beef, poultry, and pork *(51),* and VIM-1 carbapenemase–producing *E. coli* isolated from swine and poultry farms *(23)*.

Antimicrobials in food-producing animals. Antimicrobials are largely used in the animal production sector for treating many infections or diseases caused by bacteria as well as for metaphylaxis purposes and growth promotion worldwide *(65)*. It is estimated that there is more-extensive antibiotic use in livestock and poultry than in human medicine; in fact, in some countries, i.e., United States, approximately 80% of the total consumption of antibiotics is in the animal sector, largely for growth promotion in healthy animals *(64, 75)*. The use of antimicrobials in animal production is associated with the emergence and spread of AMR in food-related bacteria and leads to the selection of antimicrobial resistance among pathogenic and commensal bacteria in the intestinal tract of food animals. Therefore, resistant bacteria can contaminate food products and colonize the human microbiota via the food chain by the handling and/or consumption of contaminated foods *(31, 66, 73)*. In light of this, on 1 January 2006, an EU-wide ban was placed on the use of antibiotics as growth promoters in animal feed *(17)*. In addition, on 12 November 2013, Commission Implementing Decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria came into force *(19)*. This program includes colistin susceptibility testing in *E. coli*; cecal-content samples are collected at slaughter in poultry, fattening pigs, and bovines younger than 12 months old.

AMR and gut microbiota. The human gastrointestinal tract harbors a large bacterial population, collectively termed gut microbiota *(2, 63),* which represents a reservoir for antibiotic resistance genes and an ideal environment for the horizontal transfer of genetic material *(58)*. In fact, resistance to antibiotics may arise in a population of susceptible bacteria by accumulation of spontaneous mutations or by the horizontal transfer of antibiotic resistance genes *(47)*. The three major mechanisms by which bacteria transfer antibiotic resistance genes horizontally are conjugation, natural transformation, and transduction, of which conjugation and transduction mostly contribute to the horizontal spread of antibiotic resistance genes in the gut microbiota *(71)*. Antibiotic administration in humans and animals can lead to an increase in resistant bacteria because of the selective pressure exerted by antibiotics and increased interbacterial gene transfer *(69)*. Some bacteria, although only transient in the intestines, are capable of exchanging AMR genes to the commensal microbiota when they pass through the gut *(1)*. The transmission of antibiotic resistance genes mostly involves nonpathogenic gut commensal bacteria *(55)*; however, those genes can potentially be transferred to opportunistic pathogens, which may remain for months in the gut of the carrier without causing any symptoms, potentially spreading human-to-human transmission, or translocate across the intestinal barrier and potentially cause infection *(9, 67)*.

Rediscovery of colistin. Within the past 2 decades, the spread of multidrug-resistant gram-negative bacteria associated with the shortage of new antimicrobial agents has led to the reconsideration of colistin as a valuable treatment and last-resort therapy used against infections caused by these bacteria *(22, 41)*. In the 1970s, there was a significant reduction in the clinical use of colistin because of its renal and neurologic side effects after parenteral administration *(59)*. This molecule was almost completely abandoned in the 1980s and was largely replaced by less-toxic antibiotics, such as aminoglycosides, that have a comparable or broader antibacterial spectrum *(6)*. In 2016, the first plasmid-mediated, colistin resistance mechanism, mediated by the *mcr-1* gene, was reported in *E. coli* isolated from food animals and raw meat. Before that discovery, resistance to colistin was only described as chromosomally mediated and could only be inherited from a mother bacterial cell by its own daughter cells upon cell division. In contrast, plasmid-mediated resistance enables a much more efficient horizontal transfer of colistin-resistant genes to other bacteria *(42),* raising concerns about the spread of colistin resistance in the food chain.

Colistin and the *mcr-1* gene. Colistin, also known as polymyxin E, is a polypeptide antibiotic discovered in the 1940s and produced by *Paenibacillus polymyxa* var. *colistinus* with significant concentration-dependent bactericidal activity against gram-negative bacteria *(39, 60)*. Antibiotics in the polymyxin family include five different compounds (polymyxins A, B, C, D, and E), of which polymyxin E and B are the only two used clinically and commercialized for both human and veterinary use *(13, 22)*. They are pentacationic polypeptides consisting of a cyclic heptapeptide, a linear tripeptide, and a hydrophobic acid tail linked to the N-terminal of the tripeptide *(35)*.

Colistin has a narrow antibacterial spectrum against gram-negative bacteria *(82)* (Table 1). The target of antimicrobial activity of colistin is the bacterial cell membrane. In more detail, this molecule binds to the lipopolysaccharide (LPS) component of the outer membrane *(29),* and it is not active against gram-positive bacteria, all cocci, or anaerobes *(22, 41)*. Colistin specifically targets lipid A, which has a major role in the control of cell permeability. This takes place via an electrostatic interaction between positively charged diaminobutyric acid residues of colistin and negatively charged phosphate groups of lipid A *(68)*. Because of that interaction, colistin competitively displaces divalent cations (Ca^{2+} and Mg^{2+}), causing an alteration in the LPS structure and the formation of destabilized areas, through which colistin crosses the outer membrane and eventually damages the structure of the phospholipid bilayer of the inner membrane. This whole process leads to inner membrane lysis and cell death *(29, 49)*. The chromosomal mechanism of colistin resistance is the result of specific mutations leading to an overexpression of LPS-modifying genes *(52)*.

TABLE 1. *Colistin antibacterial spectrum*[a]

Susceptible	Resistant	Variably resistant
Gram-negative bacilli	Gram-negative cocci	Gram-negative bacilli
Pseudomonas aeruginosa	*Neisseria gonorrheae*	*Stenotrophomonas maltophilia*
Acinetobacter spp.	*Neisseria meningitides*	*Aeromonas* spp.
Escherichia coli	*Moraxella catarrhalis*	*Vibrio* spp.
Klebsiella spp.	*Brucella* spp.	
Enterobacter spp.	Gram-negative bacilli	
Salmonella spp.	*Proteus* spp.	
Shigella spp.	*Providencia* spp.	
Haemophilus influenzae	*Morganella morgani*	
Bordetella pertussis	*Serratia* spp.	
Anaerobic gram-negative bacilli	*Edwardsiella tarda*	
Prevotella spp.	*Burkholderia* spp.	
Fusobacterium spp.	All gram-positive organisms	
	Other anaerobic gram-negative bacilli	

[a] Data from Balaji et al. *(3)*.

Two forms of colistin are commercially available: colistin methanesulfonate sodium administered parenterally because it is less toxic than the other form, colistin sulfate *(4)*. The latter is administered either orally for bowel decontamination (without absorption) or topically as a powder for skin infections *(22)*. Both can be inhaled *(41)*.

In November 2015, a novel plasmid-mediated colistin resistance gene, *mcr-1,* was identified in *E. coli* isolates in the People's Republic of China from pigs at slaughter and from retail meats *(42)*. After its first isolation, the colistin resistance gene *mcr-1* has attracted global attention and several reports have documented its presence in *Enterobacteriaceae* in many countries in Europe and worldwide *(14, 43)*. The *mcr-1* gene has been found in gram-negative bacteria, mostly *E. coli,* isolated from food animals, the environment, various types of meat and vegetables, and humans, who are either symptomatic patients or asymptomatic carriers *(27, 30, 53, 83)*.

In addition to *mcr-1,* further plasmid-mediated colistin resistance genes have been detected. A second colistin resistance gene, *mcr*-2, was reported in June 2016 by Xavier et al. *(78)* in porcine and bovine *E. coli* in Belgium. In a porcine *E. coli* isolate from Malaysia and in two human isolates of *Klebsiella pneumoniae* and *Salmonella* from Asia and the United States, respectively, the recently discovered *mcr-3* gene was identified *(81)*. The *mcr-4* gene has been identified for the first time in *E. coli* of swine origin isolated from various European countries, i.e., Italy, Spain, and Belgium, between 2015 and 2016 *(8)*. In addition, the *mcr-5* gene was detected for the first time in *E. coli* from food-producing animals in Germany *(28)*. The latest *mcr* genes to be identified were *mcr-6* and *mcr-7,* two *mcr* homologs very recently deposited into GenBank *(80),* and the *mcr-8* in *K. pneumoniae* isolates from both animals and humans *(72)*. The *mcr-1* gene encodes for a phosphoethanolamine transferase responsible for a modification of the lipid A portion of the LPS with the addition of phosphoethanolamine, which reduces its binding affinity to colistin and leads to bacterial resistance. Both *mcr-1* and *mcr-2* are classified as lipid A–phosphoethanolamine transferases that promote the addition of a phosphoethanolamine group to lipid A, leading to a decreased affinity for colistin by the LPS *(42, 61, 78)*. The mechanisms of action for some novel *mcr* genes are largely unknown *(79)*.

These findings have raised serious concerns about the possible loss of effectiveness of colistin, the "last-line" therapy to treat infections caused by multidrug-resistant, gram-negative bacteria, when essentially no other options are available *(22, 41)*. For this reason, the World Health Organization, in the 5th edition of *Critically Important Antimicrobials for Human Medicine* states that

> Polymyxins were moved to the "Highest Priority Critically Important Antimicrobials" classification because of the increasing usage of colistin to treat serious infections in humans in many parts of the world, the discovery of the *mcr1* and *mcr2* genes that confer transmissible resistance to colistin and the spread of colistin-resistant bacteria via the food chain. . . *(75)*.

Colistin is mostly used in human medicine for treating health care–associated infections from carbapenemase-producing, gram-negative bacteria *(15)*. In this context, in accordance with article 31 of the Directive 2001/83/EC of the European Parliament, colistin can only be used for the treatment of infections with limited treatment options *(21)*. With regard to veterinary medicine, colistin should only be administered if no effective, alternative antimicrobials, authorized for the respective target species and indication, are available *(20)*.

Role of food in the spread of *E. coli* harboring the plasmid-mediated colistin resistance gene *mcr-1*. The human diet and the consumption of contaminated food and water have a major role in the acquisition of AMR-resistant *E. coli*. In fact, Corpet *(12)* investigated the effect of a sterile diet on the excretion of resistant enterobacteria and showed that, in people eating sterile food, there is a rapid and substantial fall in the numbers of drug-resistant *E. coli* bacteria these people carry. In addition, effective government policies aimed at monitoring antibiotic use in human

TABLE 2. Escherichia coli *harboring the plasmid-mediated colistin resistance gene* mcr-1 *isolated from foodstuffs*

Food	No. of positive samples/ total samples examined (%)	Year(s) of isolation	Country	Origin	Reference
Chicken meat	5 isolates[a]	2012–2014	Denmark	Germany	*30*
Chicken meat	35/297 (11.78)	2011–2014	China		*42*
Pork meat	43/226 (19.03)				
Chicken meat	3/196 (1.53)	2009–2015	Netherlands	2009: unknown 2014: European	*38*
Vegetables	2/60 (3.33)	2014	Switzerland	Thailand and Vietnam	*83*
Chicken meat	25/580 (4.31)	2010–2015	Germany		*34*
Turkey meat	57/676 (8.43)				
Veal	1/70 (1.42)				
Beef	0/152				
Pork	0/95				
Eggs	0/90				
Bulk tank milk	0/253				
Cheese	0/76				
Ground Beef	2 isolates[a]	2010	Canada		*46*
Ground beef, chicken and pork	18/306 (5.88)	2012–2015	Taiwan		*40*
Chicken meat	8/41 (19.5)	2016	Brazil		*44*
Chicken meat	1/70 (1.42)	2015–2016	Japan		*50*
Mutton meat	1/71 (1.41)	2017	India		*26*
Poultry meat	2/71 (2.82)				

[a] No. of positive samples; total no. of samples not reported.

and veterinary medicine have produced excellent results in reducing antimicrobial resistance, e.g., in Denmark *(25, 32)*.

E. coli harboring the plasmid-mediated, colistin resistance gene *mcr-1* was isolated for the first time in food products in China by Liu et al. *(42),* where between 2011 and 2014, pork and chicken meat samples were collected from open markets and supermarkets. A high prevalence of *E. coli* isolates carrying the *mcr-1* gene was found in retail meat: 35 (11.78%) of 297 chicken samples and 43 (19.03%) of 226 pork samples were found positive.

After that discovery, several authors reported the presence of the colistin resistance *mcr-1* gene in *E. coli* in food worldwide (Table 2). The *mcr-1* gene was reported in The Netherlands by Kluytmans-van den Bergh et al. *(38),* who documented the presence of this gene in *E. coli* isolates from retail chicken meat collected in Dutch supermarkets between 2009 and 2015. The *mcr-1* gene was detected in 3 (1.5%) of 196 of the chicken meat–derived *E. coli* strains. Interestingly, the labeling of the samples did not indicate the country in which the animals were raised, whereas the origin of the meat was only available for the positive samples collected in 2014 (labeled as "non-Dutch" or "European"); this might indicate widespread dissemination of the *mcr-1* strains of *E. coli* in the EU *(38)*.

Five *E. coli* isolates carrying the *mcr-1* gene were detected in chicken meat of European origin imported to Denmark in 2012, 2013, and 2014 *(30)*. Furthermore, 8 (19.5%) of 41 of the colistin-resistant *E. coli* isolates from chicken meat sampled from different markets in Sãn Paulo, Brazil, tested positive for *mcr-1* in the summer of 2016 *(44),* and two *mcr-1*$^+$ *E. coli* isolates were detected in ground beef purchased in 2010 in Ontario, Canada *(46)*.

Another retrospective study investigated the presence of *E. coli mcr-1*$^+$ in retail meats purchased from traditional markets and supermarkets in 2012, 2013, and 2015, in Taiwan *(40)*. In that report, 18 colistin-resistant *E. coli* isolates positive for the *mcr-1* gene were identified.

The isolation rate of *mcr-1* among the meat *E. coli* isolates was significantly higher in 2015 (11 of 126, 8.7%) than in 2012 (1 of 89, 1.1%) and 2013 (6 of 91, 6.6%). In line with the available literature, among chicken, beef, and pork samples, the chicken samples were the ones in which the *mcr-1* gene was found.

In Germany, between 2010 and 2015, 10,600 *E. coli* isolates from the national monitoring of zoonotic agents were screened for phenotypic colistin resistance *(34)*. Afterward, the 505 resistant isolates were tested by real-time PCR for the presence of the *mcr-1* gene; the gene was found in *E. coli* isolated from different food matrices. The highest prevalence was found in turkey meat (30 of 307, 9.8%), followed by chicken meat (14 of 172, 8.1%) collected in 2012 and 2011, respectively, whereas a low prevalence (below 1 in 70, 1.4%), was detected in veal at retail. No *mcr-1*$^+$ strain was detected in isolates from beef, pork, dairy products, or eggs.

Raw food samples, such as poultry meat, fish, mutton meat, fruits, and vegetables, were collected from 22 sources, 14 shops and 8 households, in the Chennai metropolitan city in India in 2017. *E. coli* carrying the *mcr-1* gene was detected by PCR in three (one mutton and two poultry meat samples) of the 71 (4.2%) screened isolates *(26)*.

E. coli mcr-1$^+$ was also detected in retail domestic chicken meat (1 of 70, 1.4%) in Japan *(50)*.

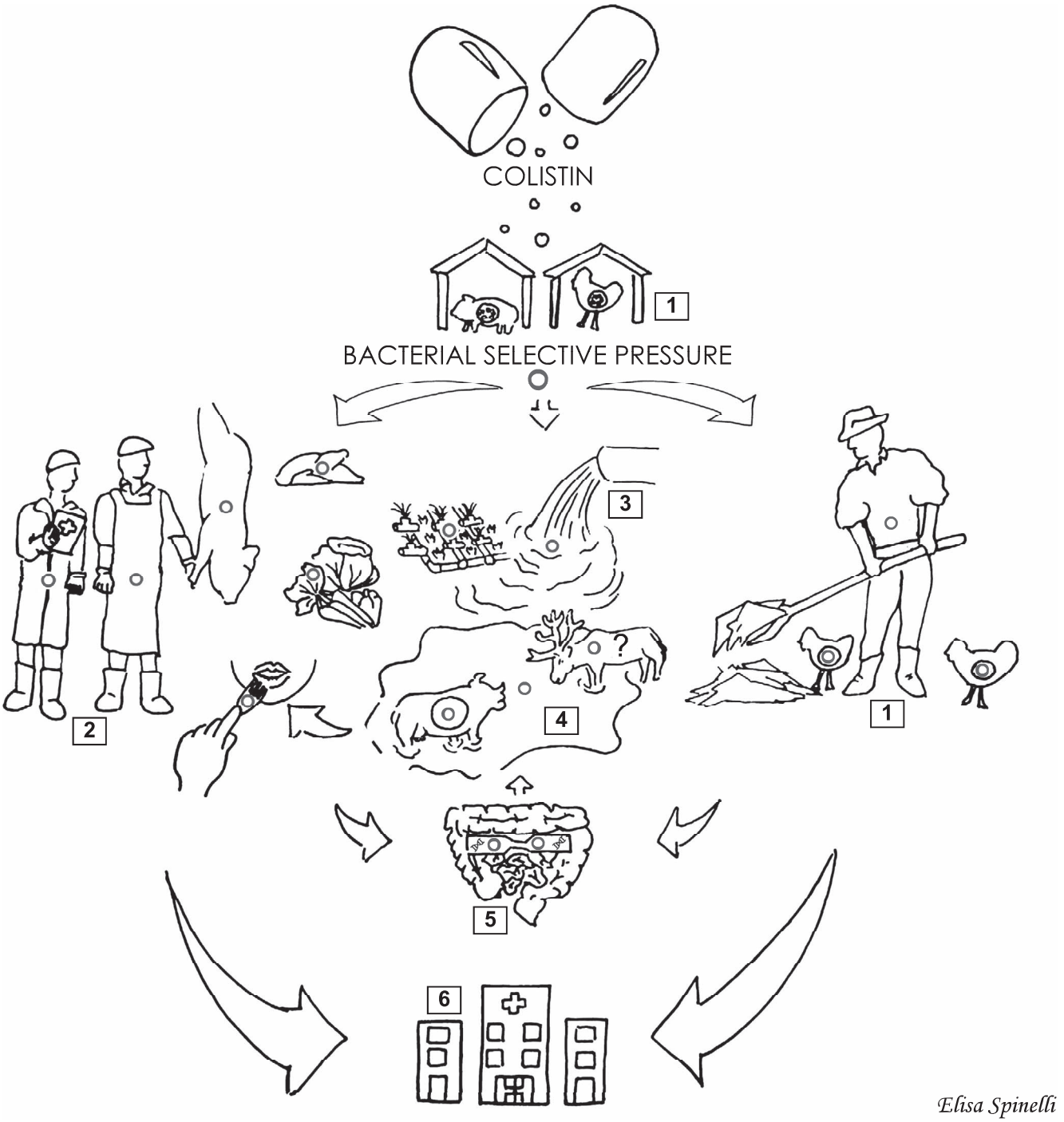

FIGURE 1. *Main pathways of selection and spread of* Escherichia coli *carrying the* mcr-1 *gene (*E. coli mcr-1^{+}*) in animals, humans, and the environment. Administration of colistin to intensively reared animals could lead to selective pressure of* E. coli mcr-1^{+} *in their gut microbiota; in addition, unprocessed colistin eliminated in these animals feces can contaminate the wastewater and reach the aquatic environment, contributing to the selective pressure.* E. coli mcr-1^{+} *during production could reach the carcasses and, therefore, the meat. Humans could acquire* E. coli mcr-1^{+} *by handling or consuming raw or undercooked meat, as well as on farm. As a consequence, in humans (i) clinical infections could develop, (ii)* E. coli mcr-1^{+} *could be transferred from* E. coli *to other commensal bacteria in the gut microbiota, and (iii)* E. coli mcr-1^{+} *could reach the aquatic environment via human waste. When this water is used for agricultural purposes, it may contaminate the vegetables and reach consumers. In addition, livestock could potentially drink contaminated freshwater and the contaminated products thereof; these products could reach the consumers as well. The role of wild animals in the selection and spread of* E. coli mcr-1^{+} *has not yet been investigated. Circles:* E. coli mcr1^{+}. *1, Farm; 2, slaughterhouse (veterinarians and slaughterhouse operators); 3, wastewater; 4, environment (domestic and wild animals); 5, intestine; 6, hospital settings.*

Retail meat is the main food from which *E. coli* carrying the *mcr-1* gene has been isolated. In fact, to date, only Zurfuh et al. *(83)* has succeeded in isolating *E. coli* carrying the gene in vegetable samples. The *Enterobacteriaceae* isolated from 42 vegetables imported from Asia were screened for the presence of the *mcr-1* gene, which was detected in 2 (3.3%) of 60 vegetable strains. The involvement of vegetables as well as meat products in the spread of the gene is a public health concern because the production and trade of fresh vegetables may constitute an additional route for the dissemination of the plasmid-mediated, colistin resistance *mcr-1* gene *(83)*. The possible routes of transmission of *E. coli mcr-1*$^+$, are summarized in Figure 1.

In line with the available literature, it is possible to assume that the intestinal carriage of antimicrobial-resistant *E. coli* in livestock and food thereof, i.e., retail meat, might increase the occurrence of infections with antimicrobial-resistant *E. coli* in humans *(62)*. Moreover, the presence of *E. coli* carrying the *mcr-1* gene in foodstuffs is likely to be underestimated because, to date, only strains carrying the *mcr-1* gene have been searched for. It must be emphasized that *mcr* genes, other than *mcr-1,* have been detected, and it is likely that they also circulate in food-related bacteria.

CONTROL OF *MCR-1*$^+$ *E. COLI*

The transmission of AMR *E. coli* via food animals and food products has clearly been demonstrated *(45)*. However, it is not possible to make a complete risk assessment on the role of food in the transfer of the human infections caused by *E. coli mcr-1*$^+$ because some pieces of the puzzle are still missing. Undoubtedly, the significance of this organism in food animals, in the environment, and in foodstuffs must be carefully assessed from a food safety perspective. The optimal actions for better defining the human health risks associated with the spread of *E. coli mcr-1*$^+$ via the food chain can be summarized as follows.

Humans and food. The following are crucial.

1. Prevent the use of colistin as a growth promoter worldwide; moreover, its prescription in veterinary and human medicine should be drastically reduced and only used as a last-resort molecule. Unfortunately, the use of colistin as a growth promoter in 2016 was reported in 13 (21.7%) of the 60 countries included in the second World Organization for Animal Health (OIE) annual report on antimicrobial agents intended for use in animals *(77)*.
2. Evaluate the prevalence of human carriers of *E. coli mcr-1*$^+$, especially in food manufacturers and food handlers, to reduce the risk of contamination. This is crucial for health care operators, who could contaminate food intended for immunosuppressed patients. In fact, it is well known that this kind of transmission (via food handlers) was the cause of an outbreak of methicillin-resistant *Staphylococcus aureus* that affected 27 patients, 5 (18.5%) of whom died, at the Rotterdam hospital (Netherlands) *(37)*.
3. Reduce the spread of *E. coli mcr-1*$^+$ in the hospital environment. During the admission process, doctors should ask the patients whether they work in the food chain, especially in primary animal production. In the case of an affirmative answer, it is important to ensure that the patient is not a carrier of the organism.
4. Evaluate the efficacy of the sanitization protocols in the food industry against *E. coli mcr-1*$^+$. In fact, some resistant bacteria are also resistant to disinfectants that would be effective against their antimicrobial-susceptible homologues *(56)*. To date, no specific studies are available on the resistance of *E. coli mcr-1*$^+$ against disinfectants used in the food industry.
5. Study the viability of *E. coli mcr-1*$^+$ in raw and processed food, along with its interaction with the microflora of different foods, especially fermented foods, such as cheeses, salami, etc., in which the possibility of exchanging genetic material, including plasmids, exists *(10, 48)*.
6. Evaluate the prevalence of *E. coli mcr-1*$^+$ in ready-to-eat foods, such as cut fruit and salads, which represent a well-known route for the transmission of foodborne pathogens.
7. Develop specific protocols for monitoring *E. coli mcr-1*$^+$ during food processing and for its rapid and specific detection in food products; for example, it would be useful to implement a screening test based on molecular detection of the *mcr-1* gene and subsequent isolation of the positive samples using selective and differential solid media.

Animals and environment. The presence of *E. coli mcr-1*$^+$ in bred animals and foods thereof represents a challenge for both food operators (farmers, livestock transporters, slaughterhouse workers, food handlers, etc.) and consumers. Various reports have documented dissemination of *E. coli* carrying the *mcr-1* gene in healthy food-producing animals in several countries *(5, 36, 33)*. However, studies on the prevalence of this organism in food are still scarce and fragmentary and, for certain food products, totally absent. For example, the milk sector has been poorly investigated, and nothing is known about the prevalence of *E. coli mcr-1*$^+$ in fishery products and wild game meat. The investigation of bivalve molluscs, e.g., mussels, clams, oysters, could be of interest from a food safety point of view, considering that these foods are often consumed raw worldwide. In addition, game meat, especially from wild boar, might represent a challenge for consumers, considering the rapid diffusion that this species is having in some European countries (e.g., Italy) and the habit of making ready-to-eat *salami* from it.

Thus, the following are important.

1. Plan and intensify the surveillance on the prevalence of *E. coli mcr-1*$^+$ in reared food animals to evaluate the effects of the policy measures adopted by the government aimed at reducing the use of colistin in veterinary medicine.
2. Evaluate the viability of *E. coli mcr-1*$^+$ in animal waste to establish the correct procedures for using it in agriculture. It is crucial that animal manure does not

contaminate the environment, i.e., soil, water, and vegetables with viable *E. coli mcr-1*$^{+}$.

3. Evaluate the possible foodborne risks linked to the internalization of *E. coli mcr-1*$^{+}$ into the edible parts of vegetables, as reported for other foodborne pathogens *(57),* with a focus on its persistence after the sanitization process during the production of packaged salads.

CONCLUSIONS

More research, using the One-Health approach, is needed if we want to define the food safety significance of *E. coli mcr-1*$^{+}$. However, it is important to underscore that the presence of *mcr* in food-related bacteria represents a challenge for human health associated with its potential to transmit infections that are difficult to treat.

In addition, food has an important role in the spread of *E. coli mcr-1*$^{+}$ worldwide, especially considering globalization and international trade. Hence, there is the need to alert food business operators along the entire food chain about this specific risk and to adopt and follow good hygienic practices when producing and handling foods.

The competent authorities need to be aware of the spread of AMR bacteria via food and implement official controls on primary production (correct use of antimicrobials in bred animals) and postprimary production (strict controls to establish the food business operator is in compliance with good hygienic practices during slaughtering, cutting, handling, and selling of meats and during milking).

Finally, consumers should be informed about the presence of this novel risk linked to foods and should be trained via simple tools (fliers, Web pages, etc.), to apply good hygienic practices in their own kitchen, to avoid cross-contamination between raw meat and other foods, to wash salads and other vegetables properly, and to cook meat thoroughly.

ACKNOWLEDGMENTS

The work was supported by the University of Foggia, PRA 2018. Antimicrobial resistant bacteria in ready to eat salads: a still unexplored threat for public health.

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