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Infections with *Salmonella* and Shiga-toxin producing *E. coli* harboring a new mobile colistin resistance gene, *mcr-9* – United States, 1999-2019

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Abstract

Infections with *Salmonella* and Shiga-toxin producing *E. coli* harboring a new mobile colistin resistance gene, *mcr-9* – United States, 1999-2019
By Matthew T. Ryan

Background: Colistin is an antibiotic in the polymyxin class that is important for the treatment of patients with certain severe multidrug-resistant (MDR) infections such as carbapenamase resistant *Enterobacteriaceae* and is referred to as an antibiotic of last resort. In May of 2019, a new mobile (plasmid-mediated) colistin resistance gene, *mcr-9*, was reported in a *Salmonella* Typhimurium isolate from a U.S. resident of Washington who had no international travel. Nine *mcr* genes that confer transmissible colistin resistance have been identified since 2015, however most have been associated with international travel. We describe the timeline, geographic distribution, and exposure history of *mcr-9* isolates.

Methods: In this ongoing retrospective analysis of surveillance data (n=141), all ~24,000 whole genome assemblies from enteric pathogen isolates in CDC's surveillance databases were screened for resistance determinants, including *mcr-9*. Additional *mcr-9*(+) isolates were identified through the NCBI pathogen detection pipeline and were rescreened for confirmation. State health departments submitted de-identified patient interviews containing pertinent epidemiologic information. Due to variance in questionnaire language by state, variables of interest were standardized before data compilation.

Results: As of December 31st, 2019, 137 patients with *mcr-9*(+) nontyphoidal *Salmonella* (NTS) infections and four with *mcr-9*(+) Shiga toxin-producing *E. coli* infections in 36 states were identified. Isolates were collected from 1999 to 2019. Twenty-three states had three or more cases of *mcr-9*(+) enteric infections. The median age of cases was 21 years (IQR=47.5, 25th percentile=5, 75th percentile=52.5); 50% were male. Of those with information on hospitalization and travel, 27% were hospitalized and 13% traveled internationally in the seven days before their illness. Of those with exposure information, 51% reported poultry consumption, 58% reported consuming unbottled water, and 34% reported dog exposure, which were all the highest in their respective categories (food, water, animal). The most common NTS serotypes were I 4,[5],12:i- (50%), Typhimurium (11%), Heidelberg (10%).

Conclusions: We identified *mcr-9* isolates in the U.S. from as early as 1999; however, it is likely that *mcr-9* was circulating domestically before that. Most patients acquired these infections domestically, likely via consumption of poultry or contact with untreated water or through animal exposure. A study between these patients and a comparable general population is needed to assess *mcr-9* risk factors for the acquisition of *mcr-9*.

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This thesis is dedicated to my mom, Kim, who I know would have loved to see the finished product.

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Introduction

Polymyxins are a class of antibiotics whose use in recent years is increasing due to limited available options in the treatment of carbapenem-resistant gram-negative bacteria, especially *Enterobacteriaceae*.[1] Colistin (polymyxin E) is an antibiotic in the polymyxin class that is important for the treatment of patients with certain severe multidrug-resistant (MDR) infections such as carbapenamase resistant *Enterobacteriaceae* (CRE) and has been called an antibiotic of last resort as it is generally only used when all other antibiotic options have been exhausted.

Chromosomal mediated polymyxin resistance has been thoroughly described in gram-negative bacteria. However, in 2015, plasmid-mediated polymyxin resistance was first reported when the *mcr*-1 gene was found in bacteria from food animals in China.[2] Mobile colistin resistance (*mcr*) genes are located on plasmids, which are mobile genetic elements that can pass easily between different species of bacteria and are able to confer resistance to colistin.[3] These genes have been identified in several species of Enterobacteriaceae, including pathogenic strains of *Escherichia coli* (*E. coli*) and several serotypes of *Salmonella enterica*, bacteria which are commonly transmitted through food or water, and in which chromosomal colistin resistance is rare.[4]

Clinical demand for colistin has increased in recent years due to the rise in antimicrobial resistance among many hospital-acquired gram-negative pathogens. Nine *mcr* genes (*mcr-1* through *mcr-9*) that confer transmissible colistin resistance have been identified since 2015. Due to the emergence of CREs and the slow development of new antibiotics, colistin has become an important drug in human medicine.[5]

In May of 2019, *mcr-9* was isolated from a *Salmonella enterica* Typhimurium isolate from a U.S. resident of Washington state who did not travel internationally and was reported to the Centers for Disease Control and Prevention (CDC). Soon after this initial finding, many more clinical isolates were found in persons who did not travel internationally, and isolates from food animals were also reported.[6] It has been noted that there is an increasing number of mobilized genes that are able to confer colistin resistance. When comparing *mcr-9* to previously described *mcr* genes, it most closely resembles *mcr-3*; however, *mcr-9* is less able to confer colistin resistance than *mcr-3*.[6]

Published reports of *mcr-9* exposures and sources are scarce and thus far, only case reports that had *mcr-9*(+) isolates related to their *Enterobacteriaceae* infection have been described. Of the *mcr-9* isolates that have been identified to date, most are found in a variety of nontyphoidal *Salmonella* serotypes, such as I 4,[5],12:I:-, Typhimurium, and Heidelberg, with a lesser amount of Shiga toxin-producing *E. coli* isolates. Various other *mcr* genes such as *mcr-1* have been found in bacteria in highly trafficked water sources as well as in soil, manure, and city effluent so there is a possibility that *mcr-9* may have a niche found in domestic water sources such as tap water, swimming pools, or natural water sources such as lakes and oceans.[7-9]

In an effort to determine when, where, and how *mcr* genes entered the United States and to identify risk factors for acquiring these genes, CDC and other federal partners have begun screening for *mcr* in bacteria from retail meats, food animal sources, and humans as part of the U.S. The National Antimicrobial Resistance Monitoring System (NARMS). The NARMS surveillance system was set up over 20 years ago to detect and track emerging resistance in enteric bacteria, such as *Salmonella, Shigella, E.*

coli and *Campylobacter* in clinical samples, retail meats and food animals. Nontyphoidal *Salmonella* is responsible for the greatest number of hospitalizations, illnesses, and deaths.[10]

In this ongoing retrospective analysis of surveillance data, we compiled all available case reports and laboratory information from the respective state health departments in which the *mcr-9*(+) isolate was detected. To our knowledge, this is the first report of all available epidemiological information from patients with *mcr-9*(+) isolates in the United States. We analyzed the demographics, timeline of infection, geographic distribution of cases and exposures such as international travel of *mcr-9*(+) cases to determine factors associated with *mcr-9*.

We reviewed surveillance reports of all *mcr-9*(+) cases from 1999-2019 in an attempt to address the following study aims: To describe the distribution of mcr-9(+) cases with respect to person (case demographics, travel, exposures, outcomes), place (state), time, and isolate type. To determine if a specific year or state has a higher number of *mcr-9*(+) enteric disease cases than expected which may indicate a potential source of domestic introduction of the gene. To determine exposures such as international travel, food, water, animal, healthcare that were associated with *mcr-9*.

Methods

Study Design

This study is an ongoing retrospective analysis of surveillance data of human patients from the United States with enteric pathogens harboring the *mcr-9* resistance gene. The study is retrospective in that it collects available epidemiological information for cases that occurred before the identification of *mcr-9,* and prospective as the standardized data collection questionnaire is shared with new cases following the identification of *mcr-9* which will be added to the ongoing *mcr-9* database.

Case Identification

State public health laboratories routinely receive patient isolates for nationally notifiable enteric diseases, such as *Salmonella, Shigella,* Shiga toxin-producing *E. coli* and *Campylobacter*. In 2019, whole genome sequencing became the primary subtyping method for enteric pathogens nationwide; some state laboratories began sequencing isolates routinely as early as 2014, and many have since sequenced older isolates. Sequence data are submitted to CDC via PulseNet, a national laboratory network, and usually uploaded to the National Center for Biotechnology Information's (NCBI's) pathogen detection pipeline.

NARMS is designed to detect emerging resistance in certain types of enteric bacteria that are found in ill patients as in both retail meats and food animals. NARMS is composed of CDC and state and local public health departments (human isolates), the U.S. Food and Drug Administration (FDA; retail meats), and the U.S. Department of Agriculture (USDA; food animals).[11] CDC-NARMS receives a subset of surveillance isolates for five

enteric bacterial pathogens including *Salmonella* and Shiga toxin-producing *E. coli*; all NARMS isolates have undergone whole genome sequencing since 2015.

To date, CDC-NARMS has screened a total of approximately 24,000 whole genome assemblies from clinical enteric pathogen isolates in CDC's surveillance databases of enteric bacterial isolates for resistance genes, including *mcr-9*.

The vast majority of these assemblies were from isolates collected after 2014, when state health departments began implementing whole genome sequencing, although isolates were identified from as early as 1999 to 2019 through retrospective screening.[12]

Additional *mcr-9*(+) isolates were identified through the NCBI pathogen detection pipeline and were then rescreened by CDC for confirmation. Once an *mcr-9*(+) isolate was identified, the state health department submitted epidemiologic information from patient interviews to CDC. All *mcr-9*(+) isolates included in this study were from U.S. patients.

Data Collection

The epidemiological data in this study was provided to CDC through case reports sent from state and local health departments. Copies of de-identified patient interview information were sent to CDC from state health departments where the *mcr-9*(+) isolate was identified and compiled in an Epi Info™ database. The database included all questions from CDC's standardized epidemiological questionnaire; however most patient interview information was reported through individual state questionnaires. All *mcr*-9(+) isolates were included regardless of the completeness of the patient interview.

Incomplete and Missing Information

The questionnaires were not standardized across states; therefore, we standardized variables of interest based on CDC's standardized epidemiological questionnaire before data was compiled to minimize biases. Since many isolates were identified retrospectively, years after the initial report from the state, several patients were lost-to-follow-up and could not be re-interviewed or had incomplete patient interview forms. All completed forms were recorded and incomplete data fields were noted as missing.

Variables of Interest

Demographic variables such as gender, age, and race/ethnicity were summarized along with patient state of residence and year of infection and number of infections by enteric pathogens. Since the source of enteric pathogens with *mcr-9*(+) was unknown, many candidate exposures were entered into the database from the patient interviews. All exposure questions pertained to the two weeks before illness onset. Since it is hypothesized that the gene is present in the environment, we analyzed data on food, water, animal, and food-source exposures. Food questions asked about consumption of poultry, beef, dairy, pork, fish, egg and other meats; food source questions included grocery store, local market, restaurant, and whether foods were eaten raw or undercooked. Other food items such as fruits and vegetables were tracked variably by state, and were not included in CDC's standardized questionnaire, so these were not systematically tracked. Water exposures included drinking unbottled water, swimming in natural water sources such as lakes, ponds, rivers, and oceans, and swimming in a pool. Animal exposures were contact with amphibians, reptiles, cats, dogs, birds, and livestock. All exposure category (food, water, animal) responses were dichotomous, and multiple exposures within each category was possible.

Descriptive Analyses

All analyses were performed in RStudio Version 1.2.1335.[13] Demographic information was summarized as the percentage of patients with data for a particular group. An epidemic curve was generated to outline the number of *mcr*-9(+) enteric cases identified by year to track the distribution of cases, along with the percentage of cases with each organism serotype. The geographic distribution of the number of cases across the U.S. was displayed using a choropleth map.

Results

Demographic characteristics of the 141 identified cases are in Table 1. As of December 31st, 2019, there were 130 cases with information on gender of which 50% were men. There were approximately equal numbers of children under age 5 years (n=31) and ages 5-17 years (n=32); collectively, children represented nearly half of total cases (47%). The median age of cases was 21 years, with an IQR of 47.5 (25th percentile=5 years, 75th percentile=52.5 years). Most cases were white (74%). Of the 84 cases with international travel information, 13% had traveled internationally in the seven days before illness onset. Travel destinations include Mexico (n=5), Morocco (n=2), Belize (n=1), Dominican Republic (n=1), England (n=1), Ethiopia (n=1), Honduras (n=1), Japan (n=1), Norway (n=1), Spain (n=1), and Taiwan (n=1).

Table 1. Demographic characteristics of patients with *mcr-9* (n=141)

	n / total with data	Percent
Male sex	65/130	50%
Age in years		
< 5 years	31/133	23%
5-17 years	32/133	24%
18-64 years	53/133	40%
≥ 65 years	17/133	13%
Race		
White	35/47	74%
Black/African American	8/47	17%
Asian/Pacific Islander	4/47	9%
Hispanic ethnicity	7/45	16%
Hospitalization	23/86	27%
International travel		
Yes	11/84	13%
No	73/84	87%

The earliest case identified was from 1999, and the most recently reported case occurred in November of 2019. The majority of cases were from 2014-2019, with 2017 having the greatest number of reported cases (n=34) (Figure 1).

Most *mcr-9* genes (n=137, 97%) were found in nontyphoidal *Salmonella*, and four *mcr-9* genes were found in Shiga toxin-producing *E. coli*. *Salmonella* I 4,[5],12:i- was the most common serotype of *Salmonella* (n=70, 50%). *Salmonella* Typhimurium (n=16, 11%) and *Salmonella* Heidelberg (n=14, 10%) were the next most common. Several other *Salmonella* serotypes accounted for 29 cases, and these serotypes were Agona, Alachua, Albany, Braenderup, Concord, GP. Monophasic B;i:-, Grumpensis, I 13,23:b:-, Infantis, Mbandaka, Montevideo, Oranienburg, Saintpaul, Thompson, and Worthington. For eight isolates, the specific serotype of *Salmonella* or *E. coli* was unable to be determined (Figure 1).

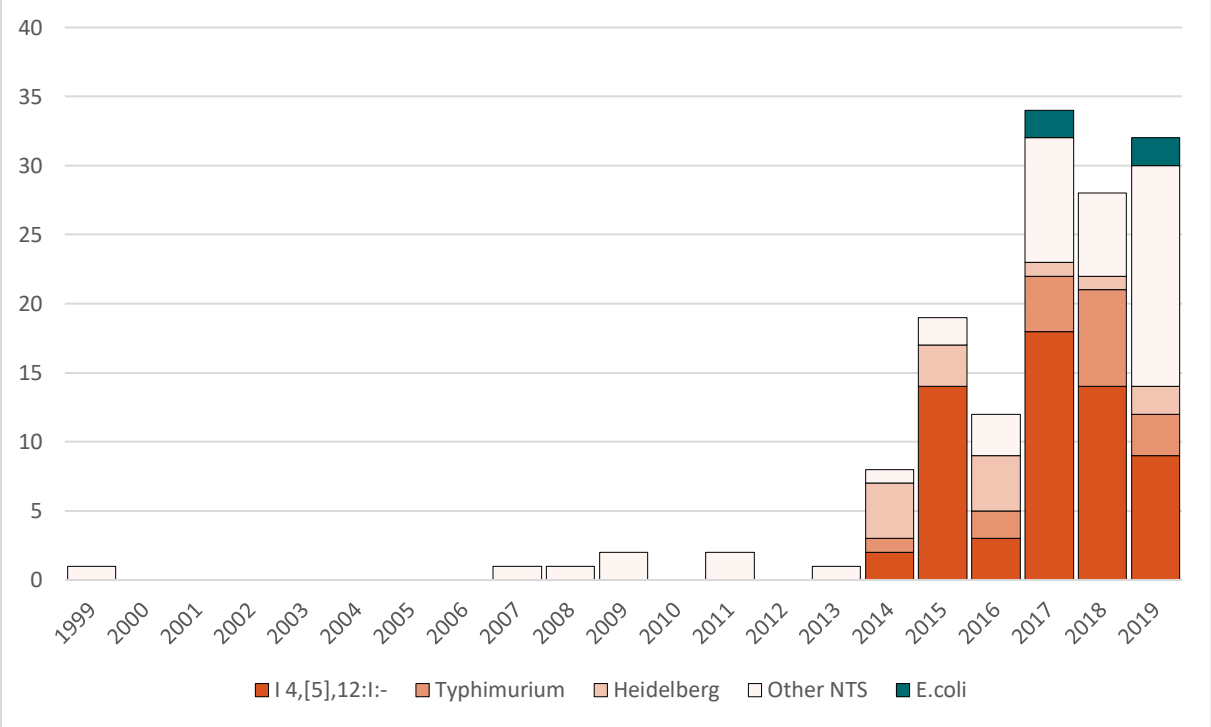

Figure 1. Epidemic curve with number of *mcr-9* in nontyphoidal *Salmonella* and Shiga toxin-producing *E. coli* isolates by year of isolation in the U.S., 1999-2019

Figure Credit: Meseret G. Birhane

As of December 2019, the state with the most cases of enteric *mcr*-9(+) infections was Wisconsin (n=17, 12%). Most of these cases were associated with a single 2015 outbreak. States which border Wisconsin also had high numbers of cases; Iowa had eight cases and Minnesota had seven. There were 36 states with at least one case, and 23 of those had three or more cases (Figure 2).

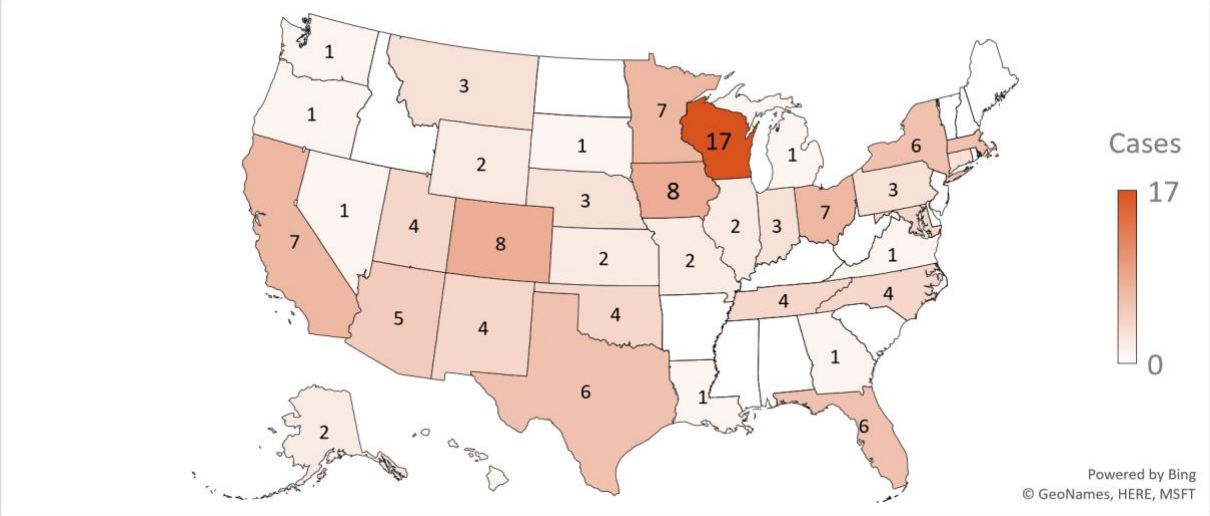

Figure 2. States with *Salmonella* and Shiga toxin-producing *E. coli* isolates containing *mcr-9* were detected in the U.S., 1999-2019

Figure Credit: Meseret G. Birhane

The distribution of possible exposures is shown in Table 2. Food consumption from a grocery store (69%) and from a restaurant (67%) in the two weeks prior to illness were the most common food sources among cases; many cases consumed food from both grocery stores and restaurants. Of those with food type history available, 51% reported poultry (chicken, turkey, duck) consumption in the two weeks before illness onset. Fish was the least common food type eaten (33%). Consumption levels were similar for dairy (44%), eggs (44%), beef (43%), and pork (41%) (Table 2).

Of all individuals with water exposure information, 58% reported drinking unbottled water, 12% had exposure to pool water, and 9% had exposure to natural waters such as lakes and oceans. Dog exposure (34%) was the most common type of animal exposure, followed by bird exposure (22%) and livestock exposure (19%). Livestock exposure includes animals such as cattle, swine, poultry, and sheep.

Table 2. Exposures of *mcr-9* Cases (n=141)

	n / total with data	Percent
2 Week Food Sources		
Grocery Store	44/64	69%
Restaurant	43/64	67%
Local Market	5/64	8%
Raw/Undercooked Food	2/64	3%
2 Week Food Type		
Poultry	36/70	51%
Dairy	31/70	44%
Eggs	31/70	44%
Beef	30/70	43%
Pork	29/70	41%
Fish	23/70	33%
Other Meat	3/70	4%
2 Week Water Exposures		
Unbottled water	33/57	58%
Pool water swimming	7/57	12%
Natural water swimming[1]	5/57	9%
2 Week Animal Exposures		
Dog	25/73	34%
Bird	16/73	22%
Livestock[2]	14/73	19%
Cat	8/73	11%
Reptile	1/73	1%

[1] Denotes water sources such as lakes, ponds, rivers, and oceans.

[2] Denotes livestock animals such as cattle, swine, poultry, and sheep.

Discussion

This is the first compilation of *mcr*-9(+) isolates that summarized all available case reports for NARMS reportable enteric bacteria isolates regarding demographics, geographic distribution, year of case isolate identification, infectious organism and exposure history. This study assembled all currently known *mcr-9*(+) isolates identified in patients in the United States through December 31st, 2019. The majority of cases with travel information did not report international travel in the seven days before their illness, with travelers comprising only 13% of cases. There were 36 states with at least one *mcr-9*(+) isolate, and the states with the highest number of isolates were in the upper Midwest. The most prevalent food, water, and animal exposures were poultry consumption, unbottled water consumption, and dog exposure. The majority of *mcr-9* genes were found in nontyphoidal *Salmonella* isolates, with I 4,[5],12:i- being the most common serotype. The source of the *mcr-9* gene, and all other *mcr* genes, is not definitively known, nor is the method by which U.S. domestic non-travelers are acquiring these genes.

The widespread geographic distribution of *mcr*-9(+) infections across the United States as well as the identification of cases as far back as 1999 demonstrates that *mcr-9* gene has been circulating in the U.S. long before *mcr-1* was first identified in 2015.[2] The U.S. has not used colistin in agriculture, so these genes may have been introduced domestically over a period of time, possibly through imported food products such as beef, pork, poultry, or fruits and vegetables from countries who still use colistin in agriculture.[14] Most *mcr*-9(+) cases were identified after 2014, with a large increase in cases in 2017, which is most likely an artifact of expanded whole genome sequencing (WGS) for routine surveillance *E.* coli and *Salmonella* in 2016.[15] WGS of 100% of

Salmonella and *E. coli* has not been achieved; therefore, these preliminary findings suggest that *mcr-9* is likely present in either *Salmonella* or *E. coli* isolates from all 50 states, even though some states have not yet reported a case. The broad geographic distribution of cases suggests that there is a common domestic source of *mcr-9* among enteric pathogens such as *Salmonella.*

Only 11 of the 84 *mcr-9*(+) cases with known travel status traveled internationally within seven days of illness onset. The low rate of international travel among cases before illness onset supports the hypothesis that *mcr-9* is already in circulation in the United States, either from genetic selection or importation from another country. This is in contrast to *mcr-1,* which was isolated primarily from healthcare settings and those from the United States who had traveled to countries in South America, Asia, and northern Africa.[16] Additionally, *mcr-1* has been detected in chicken isolates outside of the United States since the 1980s, but the earliest detection of *mcr-1* in a human isolate was from a 2008 isolate of *mcr-1* carrying *Shigella sonnei* in Vietnam.[17] This history suggests that *mcr-1* may have initially been present in food animals and eventually spread to humans by the food chain. If the same assumptions regarding the introduction of *mcr-1* into humans via the food chain hold true for *mcr-9*, then it is possible that food animal exposure could be a legitimate route of the gene becoming widespread in the United States

This study found that over half (51%) of cases had consumed poultry in the two weeks before illness onset, the most common food exposure. Per the United States Department of Agriculture, as of 2017, chicken is the most consumed meat product in the United States. [18] This is consistent with our findings and the prevalence of poultry

consumption relative to other meat products among cases. If *mcr-9* is capable of being transmitted through food products such as chicken, then the risk of widespread colistin resistance is increased. Furthermore, a systematic review and meta-analysis estimated the prevalence of *mcr* genes in bacteria, animals, animal products, and the environment and it was found that pathogenic *E.* coli had a higher prevalence of *mcr-1* in food-animals.[19] If this result is consistent for *mcr-9* in food animals, this suggests a role for foodborne or animal contact transmission of colistin resistance genes as 19% of *mcr-9* cases had some type of livestock exposure. Urban areas are home to more than 80% of the U.S. population who would likely not be in regular contact with livestock animals, and amongst the much smaller rural population, it is unlikely that most those individuals have regular contact with livestock animals.[20] According to the Central Intelligence Agency, less than 2% of the total U.S. population works in agriculture, farming, forestry, and fishing, occupations where livestock exposure would be most common.[21] The livestock exposure seen in *mcr-9*(+) cases is much higher than what would be expected, which may be related to the number of cases seen in states with a higher percentage of rural populations.

There were several limitations in our study. There may have been incomplete case finding data since we relied on WGS to identify *mcr-9*(+) cases and WGS was not fully implemented in all states. Accuracy of food exposures may have been limited by the time delay between illness and interview date. If patients weren't interviewed soon after their illness onset, information about food exposures might reflect dietary preference rather than true exposures. The lack of implementation of WGS in states before 2016 limited our ability to identify isolates before 2016. Many cases had incomplete patient interview

information because most illnesses occurred months or years prior to the identification of the *mcr-9* gene and patients were not re-interviewed. The lack of a standardized questionnaire limited generalizability between states, and we were unable to analyze all exposures (i.e., fruit and vegetable consumption) because not all state questionnaires abstracted the same information.

The *mcr-9* gene is capable of spreading between bacteria under the proper conditions and can spread colistin resistance via plasmid-mediated transfer.[6] If *mcr-9* were to spread into and be expressed in an already MDR pathogen, it is possible that it could create a "superbug" that is untreatable with antibiotics, given that colistin is generally the antibiotic of last resort. The circumstances in which *mcr-9* are expressed vary by pathogen and require a two-component regulatory system. In a colistin-resistant human fecal *E. coli* strain, it has been shown that subinhibitory concentrations of colistin induced the expression of *mcr-9*, which led to increased minimum inhibitory concentration (MIC) levels for colistin. This expression was mediated by a two-component regulatory system encoded by genes located downstream of *mcr-9*, and further analysis showed *mcr-9* was carried on an IncH12 plasmid. While the circumstances in which this gene transfer can occur are still be elucidated across various Enterobacter species, this may be an indication that *mcr* genes are not activated or detected until they are induced by the presence of colistin.[22]

In 2018, a report from the WHO was published regarding the progress of 194 WHO Member States in their development of a multisectoral national action plan in response to the 2015 Global Action plan on antimicrobial resistance. This report revealed that only 41.6% of members had limited their usage of critically important antimicrobials

for growth promotion in agriculture.[23] This highlights that colistin-resistant infections will be problematic on an international scale, as it does not appear the necessary steps to limit the spread of antimicrobial resistance genes have been effectively implemented. By having complete information from WGS and case reports from the years 2017-2019, this study also helps to establish a baseline for humans infected with enteric pathogens harboring *mcr-9* so that it is possible to monitor changes in the incidence of these cases on a yearly basis. It is imperative that we fully understand the epidemiology of *mcr*-9 in the United States in order to develop timely and effective intervention to cull the spread of colistin resistance in response to the ongoing usage of colistin in agriculture on an international level. This is necessary to protect the most vulnerable members of the population from developing an untreatable infection: patients in healthcare settings, particularly those in the ICU who are immunocompromised following transplants, chemotherapy, and other lifesaving procedures.

Additional research is needed to fully understand the source of *mcr-9* carrying bacteria. Future studies should include case-control studies to identify key health, lifestyle, and dietary risk factors, which may provide insight into which domestic sources may harbor the *mcr-9* gene. The identification of domestic sources may also help with determining appropriate prevention measures to limit the spread of *mcr-9*. Similar studies could be conducted in other countries with *mcr-9*(+) isolates, as this study was limited to isolates found in the United States. Compiling case reports for *mcr-1*(+), *mcr-3*(+), and *mcr-4*(+) isolates may provide additional insight, as there have been documented cases of these genes in isolates from U.S. residents.

In the United States, *mcr-9* is likely being transmitted domestically through food and animal exposures and appears to have domestic sources in most states dating back at least two decades. Colistin is an antibiotic of last resort, and with the increasing rate of antimicrobial resistance worldwide, there is an undeniable danger associated with multidrug-resistant bacteria expressing colistin resistance as a result of plasmid-mediated transfer of *mcr* genes. It is necessary to continue to monitor the presence of *mcr* genes both domestically and internationally to prevent superbugs that are unable to be treated by antibiotics. This would greatly hinder our ability to treat individuals who become infected with a highly resistant strain of bacteria and have a devastating impact on some of the most vulnerable members of our society: the immunocompromised and the elderly.

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