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Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds

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On 18 November 2015, Liu et al. reported the first description of plasmid-mediated colistin resistance (*mcr-1* gene) in food animals, food and humans in China [1]. In this issue, Kluytmans-van den Bergh et al. report on their finding of the *mcr-1* gene in *Escherichia coli* isolates from three (1.5%) of 196 samples of chicken meat collected at Dutch supermarkets, one in 2009 and two in 2014 [2]. This was done by whole genome sequencing of all *E. coli* isolates and then screening for the presence of the *mcr-1* gene by comparing the assembled sequences with sequence data from two databases. The same study did not find any *mcr-1*-positive isolate among 2,275 extended-spectrum beta-lactamase-positive *Escherichia coli* (screening and clinical isolates) sampled in humans between 2009 and 2015. The exact origin of the sampled chicken meat was not known, with the two samples from 2014 being labelled 'non-Dutch, European'. The fact that the genomes of the two isolates from 2014 differed by only three loci and were from the same lot of chicken meat strongly suggest cross-contamination from a common source.

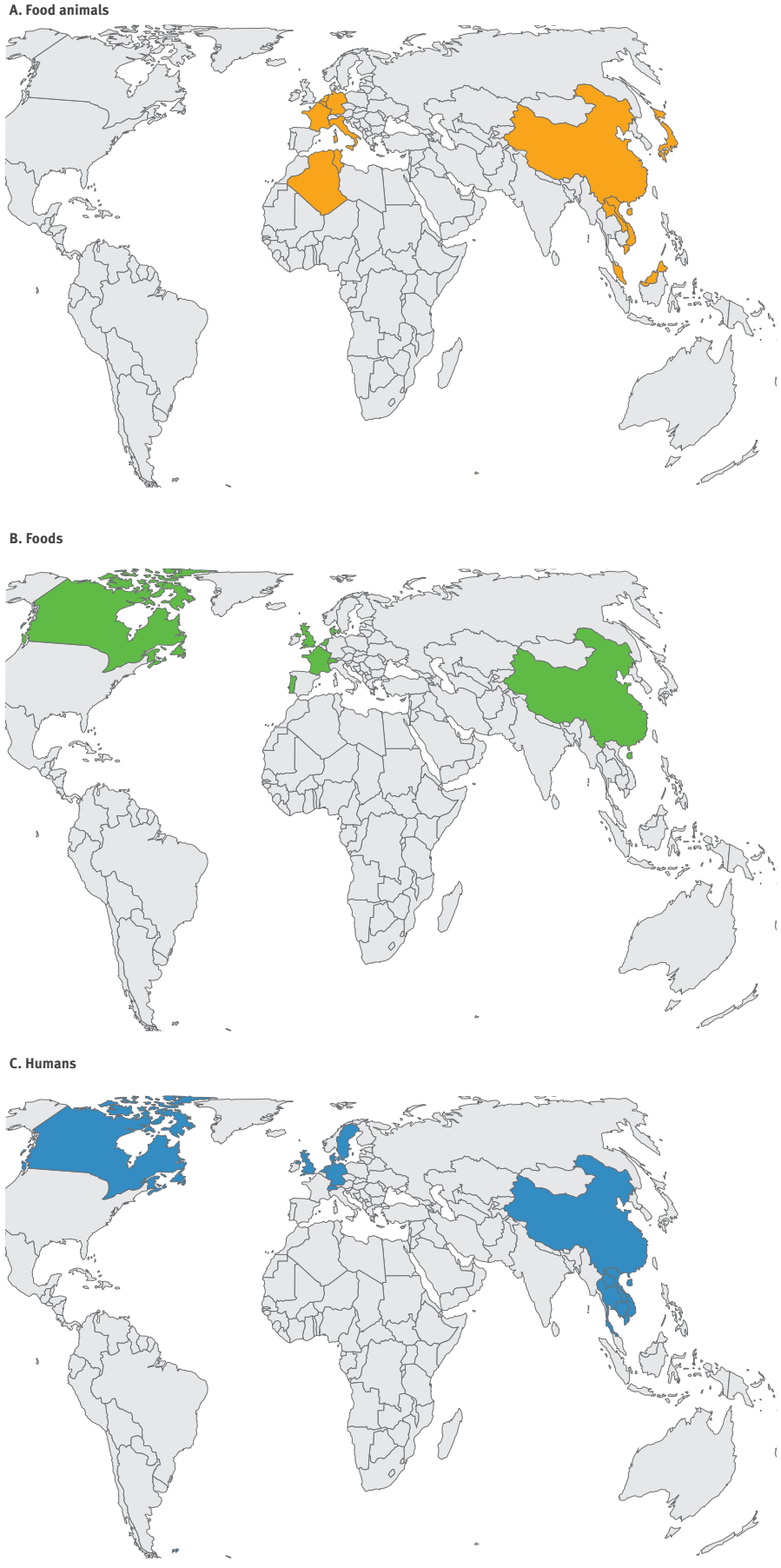
This study adds to the already long list of articles on plasmid-mediated colistin resistance published in this and other journals [3-30] (Figure and Table). Within just three months of the first description, we learned that the *mcr-1* gene (i) had spread to most continents (Figure), (ii) had been found in bacteria isolated from various food animals, from the environment including river water, from various types of meat and vegetables, and from infected patients and asymptomatic human carriers including international travellers, (iii) had been found in various bacterial species, mostly *E. coli*, and on several different plasmids, and (iv) was highly transferrable with in vitro transfer rates as high as 10^{-1}. The fact that we have gained much additional information in such a short time highlights the strength of whole genome sequencing and publicly available sequence databases.

Another important piece of information is that the *mcr-1* gene has been present, though not detected, for a long time. Shen et al. reported an *mcr-1*-positive isolate from chickens in China dating back to the 1980s [21]. In Europe, the oldest isolate reported so far is an *E. coli* from a diarrhoeic veal calf in France in 2005 [10]. The earliest reported isolate from humans is a *Shigella sonnei* from Vietnam in 2008. Trends are available in one study from China and show that the proportion of *mcr-1*-positive isolates in *E. coli* from chickens has been increasing sharply since 2009 [21]. For most studies, it is impossible to calculate the prevalence of *mcr-1*-positive isolates because detection of the *mcr-1* gene was only performed on colistin-resistant isolates. In France, systematic screening of all isolates from the routine European Union surveillance of antimicrobial resistance in zoonotic commensal bacteria showed that prevalence of the *mcr-1* gene ranged from 0.5% in *E. coli* from pigs to 5.9% in *E. coli* from turkeys in the period 2013 and 2014 [16].

Plasmid-mediated colistin resistance lies at the interface between animal health and human health. Polymyxins, and in particular colistin, have been used, both in human and veterinary medicine, for more than 50 years, although their parenteral usage in humans has been limited because of concerns about nephrotoxicity and neurotoxicity. In veterinary medicine, colistin is widely used, especially for controlling diarrhoeal diseases in pig and poultry production [31]. However, its use varies widely between countries; in Europe, from 0 mg (Finland, Iceland, Norway) to more than 20 mg (Italy, Spain) per kg animal biomass were used in 2013 [32]. Data from other parts of the world are more scarce, however Liu et al. reported that the market value for colistin for veterinary usage increased from USD 8.7 billion (EUR 8.0 billion) in 1992 to a projected USD 43 billion (EUR 39.6 billion) in 2018, with China being the largest user of a projected 12,000 tonnes in 2015 [1]. The Committee for Medicinal Products for Veterinary

Figure

Geographic distribution of the *mcr-1* gene (as of 1st March 2016)



Countries shown in colour have reported at least one isolate with the *mcr-1* gene [1-30].

Table a

Characteristics of *mcr-1*-positive isolates from food animals, the environment, food and humans, 1980s–2015 (as of 1st March 2016)

Source	Year	Country	Type of specimen/ animal /infection	Origin/ travelled region	Isolates n (%)	Species	Extended-spectrum beta-lactamase (ESBL)	Carbapenemase	Reference
Food animals	1980s–2014	China	Chickens	[a]	104	*E. coli*	NA	NA	[21]
	2005–2014	France	Veal calves	[a]	106	*E. coli*	CTX-M-1 (n = 7)	No	[10]
	2008–10	Japan	Pigs	[a]	2	*E. coli*	NA	NA	[23]
	2010–2011	Germany	Pigs	[a]	3	*E. coli*	CTX-M-1 (n = 3)	No	[7]
	2010-2015	The Netherlands	Chickens, veal calves, turkeys	[a]	4 (< 1%)	*E. coli*	NA	NA	[5]
	2011	France	Pigs	[a]	1 (< 1%)	*E. coli*	NA	NA	[16]
	2011–12	Belgium	Pigs	[a]	6	*E. coli*	No	No	[13]
	2011–12	Belgium	Veal calves	[a]	7	*E. coli*	No	No	[13]
	2012	Laos	Pigs	[a]	3	*E. coli*	NA	NA	[30]
	2012	China	Pigs	[a]	31 (14%)	*E. coli*	NA	NA	[1]
	2012–13	Japan	Cattle	[a]	4	*E. coli*	CTX-M-27	No	[23]
	2013	Japan	Pigs	[a]	1	*Salmonella* Typhimurium	NA	NA	[23]
	2013	China	Pigs	[a]	68 (25%)	*E. coli*	NA	NA	[1]
	2013	Malaysia	Chickens	[a]	3	*E. coli*	NA	NA	[17]
	2013	Malaysia	Pigs	[a]	1	*E. coli*	NA	NA	[17]
	2013	France	Pigs	[a]	1 (< 1%)	*E. coli*	No	No	[16]
	2013	France	Chickens	[a]	3 (2%)	*E. coli*	No	No	[16]
	2013	France	Chickens (farm)	[a]	1	*Salmonella* 1,4 [5],12:i:-	NA	NA	[26]
	2014	France	Broilers	[a]	4 (2%)	*E. coli*	No	No	[16]
	2014	France	Turkeys	[a]	14 (6%)	*E. coli*	CMY-2	No	[16]
	2014	Italy	Turkeys	[a]	1	*E. coli*	No	No	[4]
	2014	China	Pigs	[a]	67 (21%)	*E. coli*	NA	NA	[1]
	2014	China	Chickens	[a]	1	*E. coli*	CTX-M-65	NDM-9	[27]
	2014–15	Vietnam	Pigs	[a]	9 (38%)	*E. coli*	CTXM-55	No	[14]
	2015	Tunisia	Chickens	France /Tunisia	37 (67%)	*E.coli*	CTX-M-1	NA	[9]
	2015	Algeria	Chickens	[a]	1	*E. coli*	NA	NA	[30]
Environment	2012	Switzerland	River water	[a]	1	*E. coli*	SHV-12	NA	[29]
	2013	Malaysia	Water	[a]	1	*E. coli*	NA	NA	[17]
Food	2009	The Netherlands	Chicken meat	Unknown	1	*E. coli*	CTX-M-1	No	[2]
	2009-2016	The Netherlands	Retail meat (mostly chicken and turkey)	Dutch fresh meat and imported frozen meat	47 (2%)	*E. coli*	NA	NA	[5]
	2010	Canada	Ground beef	Unknown	2	*E. coli*	No	No	[15]
	2011	Portugal	Food product	NA	1	*Salmonella* Typhimurium	CTX-M-32	No	[25]
	2011	China	Chicken meat	[a]	10 (5%)	*E. coli*	NA	NA	[1]
	2011	China	Pork meat	[a]	3 (6%)	*E. coli*	NA	NA	[1]
	2012–2014	Denmark	Chicken meat	Germany	5	*E. coli*	CMY-2, SHV-12	No	[11]
	2012	France	Chicken meat, guinea fowl pie	NA	2	*Salmonella* Paratyphi B	NA	NA	[26]
	2013	France	Pork sausage	NA	1	*Salmonella* Derby	NA	NA	[26]
	2013	China	Chicken meat	[a]	4 (25%)	*E. coli*	NA	NA	[1]
	2013	China	Pork meat	[a]	11 (23%)	*E. coli*	NA	NA	[1]
	2014	China	Chicken meat	[a]	21 (28%)	*E. coli*	NA	NA	[1]
	2014	China	Pork meat	[a]	29 (22%)	*E. coli*	NA	NA	[1]
	2014	The Netherlands	Chicken meat	Europe, non-Dutch (n = 1), origin unknown (n = 1)	2	*E. coli*	SHV-12	No	[2]
	2014	Switzerland	Vegetables	Thailand, Vietnam	2	*E. coli*	CTX-M-55, CTX-M-65	No	[29]
	2012–2015	United Kingdom	Poultry meat	European Union, non-United Kingdom	2	*Salmonella* Paratyphi B var Java	NA	NA	[19]

NA: not available; *E. coli*: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*.

[a] Same as reporting country.

Table b

Characteristics of *mcr-1*-positive isolates from food animals, the environment, food and humans, 1980s–2015 (as of 1st March 2016)

Source	Year	Country	Type of specimen/ animal /infection	Origin/ travelled region	Isolates n (%)	Species	Extended-spectrum beta-lactamase (ESBL)	Carbapenemase	Reference
Humans	2008	Vietnam	Dysentery	Vietnam	1	*Shigella sonnei*	NA	NA	[24]
	Before 2010	China	Faecal carriage	[a]	27 (7%)	NA	NA	NA	[12,20]
	2011	Canada	Gastrostomy tube	Egypt (previous healthcare)	1	*E. coli*	NA	OXA-48	[15]
	2011	The Netherlands	Bloodstream infection	[a]	1 (0.08%)	*E. coli*	NA	NA	[5]
	2012–2013	The Netherlands	Faecal carriage	China (n = 2), South America (n = 2), Tunisia, South-East Asia	6	*E. coli*	CTX-M-1, CTX-M-14, CTX-M-15, CTX-M-55 (2), CTX-M-65	No	[3]
	NA	Sweden	Faecal carriage	Asia	2	*E. coli*	NA	NA	[8]
	2012	Thailand	Faecal carriage	[a]	2	*E. coli*	NA	NA	[30]
	2012	Laos	Faecal carriage	[a]	6	*E. coli*	NA	NA	[30]
	2012	Cambodia	Faecal carriage	[a]	1	*E. coli*	CTX-M-55	No	[22]
	2012–2015	United Kingdom	Salmonellosis	Asia (n = 2)	8	*Salmonella* Typhimurium	No	No	[19]
	2012–2015	United Kingdom	Salmonellosis	Asia	1	*Salmonella* Paratyphi B var Java	No	No	[19]
	2012–2015	United Kingdom	Salmonellosis	[a]	1	*Salmonella* Virchow	No	No	[19]
	2012–2015	United Kingdom	NA	NA	3	*E. coli*	CTX-M-type	No	[19]
	2014	Germany	Wound infection (foot)	NA	1	*E. coli*	No	KPC-2	[7]
	2014	China	Inpatient	[a]	13 (1%)	*E. coli*	NA	NA	[1]
	2014–2015	China	Bloodstream infection	[a]	2	*E. coli*	CTX-M-1	No	[6]
	2015	Denmark	Bloodstream infection	[a]	1	*E. coli*	CTX-M-55, CMY-2	No	[11]
	2015	Switzerland	Urinary tract infection	NA	1	*E. coli*	No	VIM	[18]
	2015	China	Inpatient	[a]	3 (< 1%)	K. *pneumoniae*	NA	NA	[1]
	2015	China	Surgical site infection, peritoneal fluid	[a]	2	K. *pneumoniae*	CTX-M-1	NDM-5	[6]
	2015	China	Faecal carriage (children)	[a]	5 (2%)	*E. coli*	CTX-M-15	No	[28]

NA: not available; *E. coli*: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*.

[a] Same as reporting country.

Use (CVMP) of the European Medicines Agency (EMA) reviewed all veterinary medicinal products containing colistin oral use and recommended variations to the terms of their marketing authorisations, for example that the indication is restricted to enteric infections caused by non-invasive *E. coli* susceptible to colistin and that presence of the disease in the herd should be established before metaphylactic treatment [33]. This opinion of the CVMP was converted into a Decision by the European Commission on 16 March 2015 [34], and a similar review is currently being performed for combination products containing colistin. In addition, in view of the recent developments with plasmid-mediated colistin resistance and at the request of the European Commission, the Antimicrobial Advice ad hoc Expert Group of the EMA is currently working on an update of 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" [35].

In human medicine, colistin is increasingly used parenterally for the treatment of patients infected with highly resistant bacteria such as carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter* spp. for which other treatment options are limited. In addition it is used topically by inhalation, especially in cystic fibrosis patients, as well as part of the regimen for selective decontamination of the digestive tract and of the oropharynx. As a result, consumption of polymyxins, mainly colistin, in European healthcare increased by 50% between 2010 and 2014, although with wide variation in the consumption rate depending on the country [36]. In some European countries, this has resulted in increasing percentages of isolates and outbreaks of *Enterobacteriaceae*, mainly *Klebsiella pneumoniae*, that

are resistant to both carbapenems and colistin, the latter because of chromosomal point mutations [37,38].

In 2012, consumption of polymyxins, mainly colistin, was on average more than 600 times higher in food animals than in humans for those 19 Member States in the European Union and European Economic Area that reported complete data both for food animals and for humans and after controlling for biomass (analysis of data from the first joint report by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Agency (EFSA) and EMA on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals [39], data not shown). The fact that plasmid-mediated colistin resistance originated from animals combined with the much larger use of colistin in animals than in humans, has contributed to the perception that the problem needs to be tackled first in veterinary medicine. As documented by Kluytmans-van den Bergh et al., *mcr-1*-positive isolates have so far only been found sporadically in humans in Europe [2]. This could be due to absence of selection in a non-favourable environment as indicated by the fact that all travellers that were tested positive for *mcr-1* upon return were negative after one month [3]. However, the presence of plasmid-mediated colistin resistance in foods and asymptomatic human carriers combined with increasing colistin use in European hospitals may be a game changer. In addition, *mcr-1*-positive isolates often carry multiple resistance genes, including genes encoding for an extended-spectrum beta-lactamase or a carbapenemase (Table), and may thus be selected by usage of most antibiotics. Ultimately, if index cases are not detected early and proper control measures are not implemented, Europe may face hospital outbreaks of infections for which there will be little, and possibly no, antibiotic treatment options.

Hospitals must be aware of this new threat to patient safety and may want to consider a few practicable and proportionate preparedness options. Clinical microbiology laboratories should consider testing for colistin susceptibility more frequently, within their available resources, for example in situations involving multidrug-resistant Gram-negative bacteria, isolates from patients that receive or have received colistin, or isolates from patients transferred from or recently hospitalised in a foreign country. It should be noted that disk diffusion is not a reliable test for colistin susceptibility, which should rather be assessed by a method measuring the minimum inhibitory concentration [40]. Enhanced infection control precautions, including patient isolation, should be considered already at the suspicion of colistin resistance and not await confirmation from a reference laboratory. Finally, measures aiming at strengthening infection prevention and control (hospital hygiene) as well as a more prudent use of antibiotics are essential to prevent and control antimicrobial resistance in general, and should be considered for plasmid-mediated colistin resistance.

There is no doubt that more information will surface in the coming months. In the meantime, increased awareness and preparedness may prevent spread of *mcr-1*-positive *Enterobacteriaceae* in hospitals and other healthcare settings in Europe and elsewhere.

Conflict of interest

None declared.

Authors' contributions

RSK and DLM both compiled the data and wrote the manuscript.

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Zika virus detection in urine from patients with Guillain-Barré syndrome on Martinique, January 2016

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We report two cases of Guillain–Barré syndrome who had concomitant Zika virus viruria. This viruria persisted for longer than 15 days after symptom onset. The cases occurred on Martinique in January 2016, at the beginning of the Zika virus outbreak. Awareness of this possible neurological complication of ZikV infection is needed.

Two cases of Guillain–Barré syndrome (GBS) were diagnosed in January 2016 on Martinique, a French West Indies island of 390,000 inhabitants. Both patients were found to have ZikV in their urine at hospital admission. An outbreak of Zika virus (ZikV) infections has been ongoing on the island since December 2015 [1] and spread rapidly, with more than 1,000 estimated cases per week in 2016 [2].

ZikV infection is usually benign, when symptomatic. The disease is a dengue-like syndrome, characterised by fever, headache, retro-orbital pain, non-purulent conjunctivitis, maculopapular rash, arthralgia, and myalgia. The symptoms last for four to seven days and are self-limiting. Recent ZikV epidemics in French Polynesia, Brazil and Central America have been associated with Guillain–Barré syndrome (GBS) outbreaks, the probable link between these two diseases was made based on serological and anamnestic data [3-6].

Case description

Case 1

In the first week of January, four weeks after the detection of the first ZikV-positive cases on Martinique, a young adult complaining of gait disturbance was admitted to the University Hospital of Martinique. In the week before admission, the patient had felt numbness in their four extremities followed by constipation. There was no history of infectious respiratory symptoms, diarrhoea or recent arboviral infection. At first clinical evaluation, the patient had a flaccid tetraparesis, with asymmetric peripheral facial palsy and deglutition disorders (without oculomotor disturbance or ataxia). Intravenous polyvalent immunoglobulin (IVIg) (0.4 g/kg/day for five days) was administered, starting on the first day of admission. However, the neurological disorders worsened and two days later, due to paralysis of the respiratory musculature leading to respiratory failure, the patient needed mechanical ventilation. The patient recovered autonomous ventilation after seven days of intensive care, where they were hospitalised for a total of 10 days. The patient is currently in the rehabilitation unit of the University Hospital.

Electromyography (EMG) and nerve conduction studies were performed on day 15 after onset of neurological symptoms. There were abnormal sensory nerve action potentials with sural sparing pattern. Motor abnormalities were consistent with demyelination (delayed distal latencies, slowed nerve conduction velocity, temporal dispersion of waveforms, some conduction blocks and absent F waves) and the spontaneous needle EMG was normal. Total spinal cord magnetic resonance imaging (MRI) was normal. We did not perform any brain MRI. Blood, cerebrospinal fluid (CSF) and urine samples were taken before the administration of IVIg. Blood analysis showed no biochemical disorders. Blood count was normal except for a white blood cell count of 11,440 cells/mL (norm: 4–10). The analysis of CSF

Table

Microbiological data for two patients with Guillain-Barré syndrome, Martinique, January 2016

Microorganism	Detection	Case 1	Case 2
Campylobacter jejuni	Serology	Ratio < 8 (N < 128)	Ratio < 8 (N < 128)
Campylobacter fetus	Serology	Ratio < 8 (N < 128)	Ratio < 8 (N < 128)
Mycoplasma pneumoniae	Serology	IgM < 0.8 (N < 0.8) IgG < 10 (N < 10)	IgM < 0.8 (N < 0.8) IgG < 10 (N < 10)
Epstein–Barr virus	Serology	IgM anti-VCA: 0.14 IU/mL (N < 0.9 UI/ml) IgG anti-VCA: 2.42 IU/mL (N < 0.9 UI/ml) IgG anti-EBNA: 2.42 IU/mL (N < 0.9 UI/ml)	IgM anti-VCA: 0.12 IU/mL (N < 0.9 UI/ml) IgG anti-VCA: 2.66 IU/mL (N < 0.9 UI/ml) IgG anti-EBNA: 2.42 IU/mL (N < 0.9 UI/ml)
Human immunodeficiency virus	Serology	Ratio: 0.23 (N < 0.9)	Ratio: 0.15 (N < 0.9)
Herpes simplex virus	CSF (PCR)	Negative	Negative
Cytomegalovirus	Serology	Ratio IgM: 0.22 (N < 0.7) Ratio IgG: 270 (N < 0.5)	Ratio IgM: 0.19 (N < 0.7) Ratio IgG: 233 (N < 0.5)
	CSF (PCR)	Negative	Negative
Varicella zoster virus	CSF (PCR)	Negative	Negative
Enterovirus, incl poliovirus	CSF (RT-PCR)	Negative	Negative
Dengue virus	Serology	Ratio IgM: 0.63 (N < 0.9) Ratio IgG: 6.28 (N < 1.8)	Ratio IgM: 1.24 (N < 0.9) Ratio IgG: 3.74 (N < 1.8)
	Plasma (RT-PCR)	Negative	Negative
	CSF (RT-PCR)	Negative	Negative
Chikungunya virus	Serology	Ratio IgM: 0.079 (N < 0.8) Ratio IgG: 0.136 (N < 0.8)	Ratio IgM: 0.304 (N < 0.8) Ratio IgG: 2.669 (N < 0.8)
	Plasma (RT-PCR)	Negative	Negative
	CSF (RT-PCR)	Negative	Negative
Zika virus	Plasma (RT-PCR)	Negative	Negative
	CSF (RT-PCR)	Negative	Negative
	Urine D[a]5 (RT-PCR)	Not tested	Positive
	Urine D15 (RT-PCR)	Positive	Positive
	Urine D21 (RT-PCR)	Negative	Positive

CSF: cerebrospinal fluid; EBNA: Eppstein–Barr nuclear antigen; IgG: immunoglobuline G; IgM: immunoglobuline M; IU: international unit; N: normal value; PCR: polymerase chain reaction; RT-PCR: reverse transcription polymerase chain reaction; VCA: viral capsid antigen;

[a] Day after the onset of the neurological symptoms.

showed an albuminocytological dissociation with 1.50 g/L proteins (norm: 0.15–0.40) and a white blood cell count of 4/mL (norm < 10). The glycorachia/glycaemia ratio was normal (norm > 0.5).

The patient was screened for the common aetiologies of GBS: serological tests for *Campylobacter jejuni, C. fetus, Mycoplasma pneumoniae* and human immunodeficiency virus (HIV) were negative. Direct detection in CSF of herpes simplex virus, varicella zoster virus and cytomegalovirus by PCR were negative. Direct detection of ZikV by RT-PCR in urine gave a positive result on day 15 after onset of neurological symptoms (Table) but was negative in plasma and CSF.

Case 2

In the third week of January 2016, a person in their 50s was admitted to the University Hospital of Martinique complaining of gait disturbance. Numbness of the four extremities and constipation had started three days before admission. The patient had no history of infectious respiratory symptoms, diarrhoea or recent arboviral infection. At first clinical evaluation, flaccid tetraparesis with bilateral asymmetric peripheral facial palsy and signs of respiratory distress were present. IVIg (0.4 g/kg/day for five days) was promptly administered. On the day after admission, the patient was tetraplegic and paralysis of the respiratory musculature, leading to respiratory failure, necessitated support by mechanical ventilation. The patient was hospitalised in intensive care unit for one month.

EMG and nerve conduction studies were performed on day 10 after onset of neurological symptoms. The results were similar as for Case 1. There were abnormal sensory nerve action potentials with sural sparing pattern. Motor abnormalities were consistent with demyelination (delayed distal latencies, slowed nerve conduction velocities, temporal dispersion of waveforms, some conduction blocks and absent F waves) and the spontaneous needle EMG was normal. Total spinal cord MRI was normal. We did not perform any

brain MRI. Blood, CSF and urine samples were taken before the administration of intravenous immunoglobulin. The analysis of CSF showed an albuminocytological dissociation with 0.79 g/L protein (norm: 0.15–0.40) and a white blood cell count of 1/mL (norm < 10). The glycorachia/glycaemia ratio (norm > 0.5) was normal. The patient was screened for the common aetiologies of GBS: serological tests for *Campylobacter jejuni, C. fetus, Mycoplasma pneumonia* and HIV were negative. Direct detection of herpes simplex virus, varicella zoster virus and cytomegalovirus by PCR in CSF were negative. Direct detection of ZikV by RT-PCR in urine was positive on days 5, 15 and 21 after onset of neurological symptoms. Detailed microbiological results are shown in Table.

Discussion

We present two typical cases of GBS according to clinical, electrophysiological and lab findings. Laboratory confirmation of ZikV infections is based on the detection of viral RNA in serum by RT-PCR and of IgM against ZikV by enzyme-linked immunosorbent assay (ELISA). This is challenging because viraemia in ZikV-infected patients is short. Furthermore, there is cross-reactivity between ZikV antibodies and antibodies against other flaviviruses (including dengue virus (DENV)) [7]. ZikV antibody specificity can be determined by plaque reduction neutralisation tests [3]; these tests are done by the French National Reference Laboratory for arboviruses in Marseille, France, which is 7,800 km away from Martinique. All ZikV serology for the University Hospital of Martinique is done in that reference laboratory. The serological results for the two patients are currently pending, which limits this report in that ZikV infection has not yet been confirmed, although we consider the diagnosis to be likely.

Case 2 had a DENV IgM ratio of 1.24 (normal < 0.9). DENV-specific IgM ELISA is an appropriate test for serum specimens collected from day 5 of illness. However, its positive predictive value is limited by potential cross-reactivity with other flaviviruses and false positivity due to other pathogens causing acute febrile illness such as leptospirosis or due to past DENV infection [8]. Martinique has experienced several outbreaks of dengue fever since 2001 and, in 2011, a prospective study in adult blood donors reported a 93% seroprevalence for DENV antibodies [9]. The IgM result for Case 2 could be explained by persistent seropositivity from an older DENV infection or a cross-reaction to a recent ZikV infection.

For dengue, Zika and West Nile virus infections, several authors have demonstrated that RNA is detectable in urine at higher load and for a longer time than in plasma, and proposed that detection of RNA in urine could be used for the diagnosis of these infections [7,10,11]. For these reasons, direct RNA identification by RT-PCR in plasma and urine may be a good way to confirm flavivirus infections in populations exposed to *Aedes* spp. mosquitos.

Conclusion

This report has introduced two patients with GBS who had concomitant ZikV viruria. However, the detected asymptomatic prolonged excretion may not be related to the neurological symptoms. The average annual incidence of GBS on Martinique is close to eight cases per 390,000 inhabitants (data not shown). An association with ZikV infection has to be confirmed on further cases. Potential physiopathological mechanisms of ZikV-related GBS should be explored.

Viral excretion in urine was longer than 15 days in our patients, whereas RNA detection in blood was negative. We think that ZikV viruria needs to be investigated for a period longer than 15 days after onset of the neurological symptoms in GBS cases. We have to investigate if the genito-urinary compartment is a sanctuary for persistent replication.

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Conflict of interest

None declared.

Authors' contributions

BR, FN, PH, AC Wrote the manuscript. AS, JF, KA, JJ, SJ, MS, YB, HM, RV and the Zika Working Group took part in the clinical management of the patients. FN, RC Collaborated in molecular biology techniques. LF, RC, FN collaborated on the serological techniques. All authors participated in the outbreak investigation. All authors read and approved the final manuscript.

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Research article

Presence of *mcr-1*-positive *Enterobacteriaceae* in retail chicken meat but not in humans in the Netherlands since 2009

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Recently, the plasmid-mediated colistin resistance gene *mcr-1* was found in *Enterobacteriaceae* from humans, pigs and retail meat in China. Several reports have documented global presence of the gene in *Enterobacteriaceae* from humans, food animals and food since. We screened several well-characterised strain collections of *Enterobacteriaceae*, obtained from retail chicken meat and hospitalised patients in the Netherlands between 2009 and 2015, for presence of colistin resistance and the *mcr-1* gene. A total of 2,471 *Enterobacteriaceae* isolates, from surveys in retail chicken meat (196 isolates), prevalence surveys in hospitalised patients (1,247 isolates), clinical cultures (813 isolates) and outbreaks in healthcare settings (215 isolates), were analysed. The *mcr-1* gene was identified in three (1.5%) of 196 extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* isolates from retail chicken meat samples in 2009 and 2014. Two isolates were obtained from the same batch of meat samples, most likely representing contamination from a common source. No *mcr-1*-positive isolates were identified among 2,275 human isolates tested. All *mcr*-1-positive isolates were colistin-resistant (minimum inhibitory concentration (MIC)›2 mg/L). Our findings indicate that *mcr*-1-based colistin-resistance currently poses no threat to healthcare in the Netherlands. They indicate however that continued monitoring of colistin resistance and its underlying mechanisms in humans, livestock and food is needed.

Introduction

The worldwide emergence of extended-spectrum beta-lactamases (ESBL) and carbapenemases has limited the available treatment options for infections with Gram-negative bacteria [1]. Colistin is considered to be an antibiotic of last resort for the treatment of infections with carbapenem-resistant bacteria, and its use in humans is increasing [1].

In November 2015, the presence of a plasmid-mediated colistin-resistance gene, *mcr-1*, in *Enterobacteriaceae* from food animals, food and patients in China was reported [2]. The *mcr-1* gene was detected in 21% of *Escherichia coli* isolates cultured from pigs at slaughter and in 15% of *E. coli* isolates cultured from retail meat between 2011 and 2014. In addition, the *mcr*-1 gene was present in 1.4% of *E. coli* isolates and 0.7% of *Klebsiella pneumoniae* isolates from clinical cultures from patients in two Chinese hospitals in 2014. Directly following this publication, the *mcr-1* gene was reported to be present in 0.2% of ESBL- and AmpC-producing *E. coli* isolates from human bloodstream infections, and in 2% of *E. coli* isolates cultured from imported chicken meat in Denmark since 2012 [3].Hereafter, several reports have documented the global presence of the *mcr-1* gene in *Enterobacteriaceae* cultured from humans, food animals and food [4-13].

Traditionally, colistin resistance was thought to be mediated by chromosomal mutations only, and to spread exclusively via clonal transmission of resistant isolates [14]. The emergence of plasmid-mediated

Table 1

Enterobacteriaceae isolates from retail chicken meat, rectal samples, clinical cultures and outbreaks by year of culture, type of isolate, and colistin-resistance, analysed by whole genome sequencing for the presence of the mcr-1 gene, the Netherlands, 2009–2015 (n = 2,471)

Isolate origin	Year	Type of isolate	Number of isolates	Number of colistin-resistant isolates	Number of *mcr-1*-positive isolates
Retail chicken meat (n = 196)					
Prevalence survey (n = 74)	2009	ESBL-producing *Escherichia coli*	68	NA[a]	1
		ESBL-producing *Klebsiella pneumoniae*	6	NA	0
Prevalence survey (n = 122)	2014	ESBL-producing *E. coli*	119	2	2
		ESBL-producing *K. pneumoniae*	3	0	0
Hospitalised patients, rectal samples (n = 1,247)					
Prevalence survey, 4 hospitals (n = 50)	2009	ESBL-producing *E. coli*	39	NA	0
		ESBL-producing *K. pneumoniae*	11	NA	0
Prevalence surveys, 1 hospital (n = 63)	2013–2014	ESBL-producing *E. coli*	54	0	0
		ESBL-producing *K. pneumoniae*	8	0	0
		ESBL-producing *K. oxytoca*	1	0	0
Prevalence surveys, 14 hospitals (n = 1,134)	2011–2014	ESBL-producing *E. coli*	821	2	0
		ESBL-producing *K. pneumoniae*	172	3	0
		ESBL-producing *K. oxytoca*	13	0	0
		ESBLproducing *Enterobacter cloacae*	77	2	0
		ESBL-producing *Citrobacter* spp.	38	1	0
		ESBL-producing *Morganella morganii*	6	6[b]	0
		Other ESBLproducing *Enterobacteriaceae*	7	0	0
Hospitalised patients, clinical cultures (n = 813)					
Blood cultures, 4 hospitals (n = 25)	2009	ESBL-producing *E. coli*	16	NA	0
		ESBL-producing *K. pneumoniae*	7	NA	0
		ESBL-producing *K. oxytoca*	2	NA	0
Blood cultures, 4 hospitals (n = 77)	2013–2014	ESBL-producing *E. coli*	67[c]	0	0
		ESBL-producing *K. pneumoniae*	8[c]	0	0
		ESBL-producing *K. oxytoca*	2	0	0
Clinical cultures, 14 hospitals (n = 711)	2011–2014	ESBL-producing *E. coli*	546	4	0
		ESBL-producing *K. pneumoniae*	101	2	0
		ESBL-producing *K. oxytoca*	5	0	0
		ESBL-producing *E. cloacae*	46	3	0
		ESBL-producing *Citrobacter* spp.	4	0	0
		ESBL-producing *M. morganii*	3	3[b]	0
		ESBL-producing *Proteus mirabilis*	2	2[b]	0
		ESBL-producing *P. vulgaris* group	1	1[b]	0
		Other ESBL-producing *Enterobacteriaceae*	3	0	0
Outbreaks in healthcare settings (n = 215)					
Several wards, including rehabilitation centre (n = 29)[d]	2012–2015	CTX-M-15 producing *K. pneumoniae*	29	0	0
Surgical ward (n = 14)	2014	*E. cloacae*	14	0	0
Intensive care unit (n = 86)	2009–2014	Colistin-resistant *E. cloacae*	86	86	0
Nursing home (n = 10)	2012	Colistin-resistant KPC-producing *K. pneumoniae*	10	10	0
ERCP related procedures (n = 50)	2014–2015	Colistin-resistant *K. pneumoniae*	50	43	0
Neonatal intensive care unit (n = 26)[d]	2014–2015	Colistin-resistant *Serratia marcescens*	26	26[b]	0

ERCP: endoscopic-retrograde cholangio-pancreaticography; ESBL: extended-spectrum beta-lactamase; KPC: *Klebsiella pneumoniae* carbapenemase; NA: not available.

[a] The *mcr-1*-positive isolate was tested colistin-resistant with Etest.

[b] Intrinsic resistance.

[c] Two *E. coli* isolates and one *K. pneumoniae* isolate were not available for whole genome sequencing.

[d] Outbreak and subsequent surveillance.

Colistin resistance was defined as a colistin minimum inhibitory concentration (MIC) > 2 mg/L, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [26].

Table 2

Characteristics of the *mcr-1*-positive *Escherichia coli* isolates from retail chicken meat, the Netherlands, 2009–2015

Isolate	Origin	Date of purchase	Supermarket	MLST	Serotype	Resistance genes	Plasmid replicons
213	Chicken meat	14 October 2009	A	ST2079	O8:H19	*aadA1, aadA2, aadA3, aph(3')-Ia, aph(3")-Ib, aph(3')-Ic, aph(6)-Id,* $bla_{CTX-M-1}$, bla_{TEM-1B}, *tet*(A), *mcr-1, lnu*(F), *cmlA1, catA1, sul2, sul3, dfrA5*	FIB, FII, HI2, HI2A, I1, I2, P, p0111
14M009386[a]	Chicken meat	29 January 2014	B	ST117	O159:H4	*aadA1,* bla_{SHV-12}, *mcr-1, sul1, sul3*	FIB, FII
14M009387[a]	Chicken meat	29 January 2014	B	ST117	O159:H4	*aadA1,* bla_{SHV-12}, *mcr-1, sul1, sul3*	FIB, FII

MLST: multilocus sequence typing.

[a] Isolate 14M009386 and 14M009387 were cultured from different meat samples with the same lot number.

colistin resistance enables the much more efficient horizontal transfer of colistin resistance genes to other bacteria, making *mcr-1* a potential threat to public health. The aim of this study was to screen several well-documented strain collections of *Enterobacteriaceae*, obtained from retail chicken meat and hospitalised patients in the Netherlands since 2009, for the presence of colistin resistance and the *mcr-1* gene.

Methods

Strain collections

A total number of 2,471 *Enterobacteriaceae* isolates were analysed for the presence of colistin resistance and the *mcr-1* gene. Isolates originated from prevalence surveys in retail chicken meat (196 isolates), prevalence surveys in hospitalised patients (1,247 isolates), clinical cultures (813 isolates) and several outbreaks in healthcare settings (215 isolates), all collected in the Netherlands between 2009 and 2015.

Retail chicken meat

Two ESBL-producing *Enterobacteriaceae* (ESBL-E) prevalence surveys in Dutch retail chicken meat were performed in 2009 and in 2014 [15,16]. A total number of 196 ESBL-E isolates were obtained, 74 isolates from 71 ESBL-E-positive meat samples in 2009 (89 samples cultured), and 122 isolates from 86 ESBL-E-positive meat samples in 2014 (101 meat samples cultured).

Hospitalised patients, rectal samples

The retail chicken meat surveys in 2009 and 2014 were accompanied by hospital-wide prevalence surveys in patients who were admitted to four hospitals in the region where the chicken meat was bought [15,16]. In 2009, ESBL-E rectal carriage was detected in 45 (5.1%) of 876 patients, who carried 50 ESBL-E isolates. Two repeated prevalence surveys in one of the four hospitals in 2013 and 2014, yielded 63 ESBL-E isolates obtained from 63 (5.9%) ESBL-E carriers among 1,065 patients cultured [17].

A multi-centre cluster-randomised study comparing contact isolation strategies for known ESBL-E carriers was performed in 14 Dutch hospitals between 2011 and 2014 (SoM study) [18]. All consecutive adult patients with a routine clinical culture with ESBL-E were placed on contact precautions and enrolled in the study (= index patient). Ward-based ESBL-E prevalence surveys were performed one week after enrolment of the index patient. Perianal swabs were obtained from 10,691 patients and identified 992 (9.3%) ESBL-E carriers, from whom 1,134 ESBL-E isolates were cultured.

Hospitalised patients, clinical cultures

In 2009, 2013 and 2014, all consecutive ESBL-E isolates from blood cultures were prospectively collected in the four hospitals that participated in the ESBL-E rectal carriage prevalence surveys [15,16]. A total number of 102 ESBL-isolates from blood cultures were obtained, 25 isolates from 23 patients with an ESBL-E-positive blood culture in 2009, and 77 isolates from 76 patients in 2013 and 2014. Three isolates that were collected in 2014 were not available for whole genome sequencing. In the SoM study, a total number of 711 clinical ESBL-E isolates were obtained from 654 ESBL-E-positive patients.

Outbreaks in healthcare settings

Since 2009, several outbreaks with antimicrobial-resistant bacteria in Dutch hospitals and nursing homes have been documented. Six outbreaks, comprising 215 isolates, for which whole genome sequence data were available, were included in this analysis: (i) an outbreak of CTX-M-15-producing *K. pneumoniae* in several wards of a hospital and an associated rehabilitation centre in 2012–2015 (29 isolates) [19]; (ii) an outbreak of *Enterobacter cloacae* in a surgical ward in 2014 (14 isolates); (iii) an outbreak of colistin-resistant *E. cloacae* in an intensive care unit between 2009 and 2014 (86 isolates); (iv) an outbreak of colistin-resistant KPC-producing *K. pneumoniae* in a nursing home in 2012 (10 isolates) [20]; (v) an outbreak of colistin-resistant *K. pneumoniae* in patients after endoscopic retrograde cholangio-pancreaticography (ERCP) procedures in

Table 3

Antimicrobial susceptibility of *mcr-1*-positive *Escherichia coli* isolates from retail chicken meat, the Netherlands, 2009–2015

Antimicrobial agent	Isolate					
	213		14M009386		14M009387	
	MIC (mg/L)	S/I/R	MIC (mg/L)	S/I/R	MIC (mg/L)	S/I/R[a]
Polymyxins						
Colistin	3[b]	R	≥16	R	≥16	R
Penicillins						
Ampicillin	≥32	R	≥32	R	≥32	R
Amoxicillin/clavulanic acid	8	S	≤2	S	4	S
Piperacillin/tazobactam	≤4	S	≤4	S	≤4	S
Cephalosporins						
Cefuroxime	≥64	R	16	R	16	R
Cefotaxime	8	R	4	R	4	R
Ceftazidime	≤1	S	16	R	16	R
Cefepime	2	I	≤1	S	≤1	S
Cefoxitin	≤4	S[c]	≤4	S[c]	≤4	S[c]
Carbapenems						
Meropenem	≤0.25	S	≤0.25	S	≤0.25	S
Imipenem	≤0.25	S	≤0.25	S	≤0.25	S
Aminoglycosides						
Gentamicin	≤1.0	S	≤1	S	≤1	S
Tobramycin	≤1.0	S	≤1	S	≤1	S
Fluoroquinolones						
Ciprofloxacin	0.5	S	≤0.25	S	≤0.25	S
Norfloxacin	2	R	≤0.5	S	≤0.5	S
Folate pathway inhibitors						
Trimethoprim/sulfamethoxazol	≥16/304	R	≤1/19	S	≤1/19	S

I: intermediate; MIC: minimum inhibitory concentration; R: resistant; S: susceptible.

[a] According to the European Committee on Antimicrobial Susceptibility (EUCAST) clinical breakpoints [26].

[b] Etest: MIC = 3 mg/L; Vitek2: MIC = 2 mg/L.

[c] No clinical breakpoint available; S refers to the screening breakpoint for AmpC Enterobacteriaceae.

2014–2015 (50 isolates); and (vi) an outbreak of (intrinsic) colistin-resistant *Serratia marcescens* in a neonatal intensive care unit in 2014–2015 (26 isolates).

Whole genome sequencing and analysis of sequence data

Whole genome sequencing (WGS) was performed, on either a MiSeq, HiSeq 2500 or NextSeq sequencer (Illumina). De novo assembly was performed using CLC genomics Workbench 7.0.4 (Qiagen) or the open source SPAdes 3.5.0 software (http://bioinf.spbau.ru/spades) [21]. Sequence data were screened for the presence of the *mrc-1* gene by running the assembled sequences against a task template containing the *mcr*-1 gene sequence in Ridom SeqSphere+version 3.0.1 (Ridom, Germany) or by uploading the assembled sequences to the open access bioinformatic webtool ResFinder (updated version 2.1, including the *mcr-1* sequence) of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/) [22]. For isolates from two outbreaks (colistin-resistant *E. cloacae* and ERCP-related colistin-resistant *K. pneumonia*), the thresholds for sequence identity and coverage length were set to 98% and 60%, respectively, while for all other isolates both thresholds were set to 80%. The sequence data of the *mcr-1*-positive isolates were further analysed by using a genotyping plugin that allowed serotyping of the isolates and detection of acquired antibiotic resistance genes and plasmids with a 80% threshold for both sequence identity and coverage length (BioNumerics v7.6 beta software, Applied Maths). Reference data for acquired antimicrobial resistance genes and plasmid replicons were retrieved from the ResFinder and PlasmidFinder databases (version 9 November 2015) of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/) [22,23]. Whole genome multilocus sequence typing (wgMLST) analysis was performed using a pan-genome MLST scheme comprising 9,347 genes, based on 19 well-annotated reference genomes of *E. coli* and *Shigella* spp. (BioNumerics v7.6 beta, Applied Maths). Additionally, single nucleotide polymorphism (SNP) calling was performed by mapping the paired-end reads of isolate 14M009387 and isolate 213 to the de novo assembled genome of isolate 14M009386, using Bowtie 2.5.5 [24] and SAMtools [25]. Resulting Binary Alignment Maps (BAM) files were

Table 4

Whole genome multilocus sequence typing analysis and whole genome single nucleotide polymorphism analysis of *mcr-1*-positive isolates from retail chicken meat, the Netherlands, 2009–2015[a]

Isolate	wgMLST			wgSNP
	Loci shared	Different alleles within shared loci		SNP positions
	n	n	%	n
14M009387	4,243	3	0.07%	8
213	3,791	3,606	95.1%	100,215

MLST: multilocus sequence typing; SNP: single nucleotide polymorphism; wg: whole genome.

[a] Isolate 14M0009386 was used as reference.

used to perform whole genome SNP (wgSNP) analysis (BioNumerics v7.6 beta, Applied Maths).

Antimicrobial susceptibility testing

Isolates for which antimicrobial susceptibility data were available were screened for the presence of colistin resistance. Susceptibility testing of the three *mcr*-1-positive *E. coli* isolates was performed using Vitek2 (bioMérieux, France) and Etest (bioMérieux, France). The breakpoint tables of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used for the interpretation of minimum inhibitory concentrations (MICs) [26]. Isolates with a colistin MIC›2 mg/L were considered colistin-resistant.

Results

An overview of the 2,471 *Enterobacteriaceae* isolates from retail chicken meat, rectal samples, clinical cultures and outbreaks is presented in Table 1. Colistin resistance was found in two (1.6%) of 122 chicken meat-derived ESBL-E isolates, in 14 (1.1%) of 1,247 isolates from ESBL-E rectal carriers, and in 15 (1.8%) of 813 ESBL-E isolates from clinical cultures. The *mcr*-1 gene was detected in three (1.5%) of 196 chicken meat-derived ESBL-producing *E. coli* isolates, one cultured in 2009 and two in 2014. For all three isolates, the *mcr-1* sequence showed 100% similarity to the gene reported in China [2]. None of the 2,275 human isolates harboured the *mcr-1* gene.

Table 2 shows the general and molecular characteristics of the three *mcr-1*-positive *E. coli* isolates. The isolate that was cultured in 2009 had sequence type ST2079, was CTX-M-1-positive and harboured 17 acquired resistance genes. Both isolates from 2014 had sequence type ST117, were SHV-12-positive and harboured five acquired resistance genes. Although these two isolates were cultured from different meat samples of non-Dutch origin, the meat samples had the same lot number and were bought in the same supermarket on the same day. Plasmid replicons were identified in all three isolates, eight in the isolate from 2009 and two in both isolates from 2014. However, none of the plasmid replicons could be linked to the *mcr*-1 gene.

Antimicrobial susceptibilities for the three *mcr*-1-positive *E. coli* isolates are shown in Table 3. All three isolates were colistin-resistant (MIC›2 mg/L). The isolate from 2009 tested colistin-susceptible by Vitek2 (MIC = 2 mg/L), but resistant by Etest (MIC = 3 mg/L). wgMLST analysis showed that the two isolates from 2014 differed by only three (0.07%) of 4,243 shared loci, whereas the isolate from 2009 differed by 3,606 (95.1%) of 3,791 shared loci (Table 4). The two isolates from 2014 differed by only eight SNPs in wgSNP analysis.

Discussion

In our study, covering the period 2009 to 2015, we detected the recently described plasmid-mediated colistin resistance gene, *mcr-1*, in three ESBL-producing *E. coli* isolates from retail chicken meat samples obtained from Dutch supermarkets in 2009 and 2014. All three *mcr*-1-positive isolates were colistin-resistant, and two of them were genetically closely related. No *mcr-1*-positive isolates were detected in a large collection of *Enterobacteriaceae* isolates of human origin that were collected during the same time period and included isolates of four outbreaks with colistin-resistant *Enterobacteriaceae*.

In addition to the recent reports on the global occurrence of the *mcr-1* gene in *Enterobacteriaceae* cultured from humans, food animals and food [2-13], our findings confirm the presence of the *mcr-1* gene in the European setting already since 2009.

The observed 1.5% prevalence of *mcr*-1-positive isolates is comparable with the reported 2% (5/255) prevalence in imported chicken meat in Denmark, and is lower than the 15% (78/523) prevalence in retail meat in China [2,3]. This lower prevalence may be related to the relatively low rates of polymyxin use in livestock in Europe. In 2014, polymyxins constituted only 0.4% (0.34 defined daily dose animal (DDDA)/animal year) of all antibiotics used in broilers in the Netherlands, with a decreasing trend over the last few years [27].

It is noteworthy that the observed 1.5% prevalence of *mcr-1*-positive isolates in ESBL-E isolates from retail chicken meat in this study is similar to the 1.5% phenotypic colistin resistance that was found in *E. coli* isolates cultured from Dutch retail chicken meat in 2014 [27]. Unfortunately, no data are currently available on the resistance mechanisms involved in this phenotypic colistin resistance.

The genetic identity between the two *mcr-1*-positive isolates that were obtained from the same batch of meat samples most likely represents batch contamination from a common source.

The *mcr*-1-positive isolates in this study belong to different sequence types as compared with those that were found to be related to the *mcr*-1 gene in the Chinese and Danish study [2,3]. *E. coli* ST2097 is uncommon in

humans, but has been reported once before in a study on ESBL-producing bacteria in flies from broiler farms in the Netherlands [28]. *E. coli* ST117, on the other hand, is common in both poultry and humans [16,29]. The detection of the *mcr*-1 gene in isolates that belong to different sequence types illustrates the potential for horizontal transfer of this resistance gene.

Although all chicken meat samples were bought in Dutch supermarkets, the labelling of the samples did not provide any clue with respect to the country where animals were raised. Available data on the origin of the chicken meat were limited to the producing country for the samples from 2014 (non-Dutch, European), for the 2009 isolate this information was not available. A non-European origin of the *mcr-1*-positive meat samples can, therefore, neither be confirmed, nor excluded.

The absence of the *mcr*-1 gene in human isolates of various origins is in accordance with observations in previous studies that the presence of the *mcr-1* gene in clinical isolates is still rare. In China, 1.4% (13/902) of clinical *E. coli* isolates and 0.7% (3/420) of clinical *K. pneumoniae* isolates were *mcr-1*-positive, and in Denmark, only 0.2% (1/417) of ESBL- and AmpC-producing *E. coli* isolates from bloodstream infections [2,3]. This absence of the *mcr-1* gene in current Dutch collections of human *Enterobacteriaceae* may in part be due to the low use of colistin and its analogues, the polymyxins, in humans in the Netherlands. In 2014, polymyxins constituted less than 0.1% (0.01 defined daily dose (DDD)/1,000 inhabitant-days) of all systemic antimicrobials used in primary care and ca 0.3% (0.2 DDD/100 patient-days) of systemic antimicrobials used in the hospital setting [30].

Short-read sequence data are not optimal for the assembly of plasmid sequences, which are known to contain multiple repetitive elements. This may explain why the analysis of our sequence data did not reveal a link between the *mcr-1* gene and the plasmid replicons identified.

Although the prevalence of *mcr-1*-positive isolates in meat samples was low, the presence of this colistin resistance gene in food represents a potential public health threat, as it is located on mobile genetic elements that have the potential to spread horizontally to other bacteria. With the increase in carbapenem resistance, the use of colistin is increasing and, herewith, the selective pressure for the spread of *mcr*-1 gene-containing plasmids. As colistin has become one of the last resort antibiotic options to treat severe infections with Gram-negative bacteria, the continued monitoring of colistin resistance and its underlying resistance mechanisms is important, not only in humans, but also in food production animals and food. The emergence of plasmid-mediated colistin resistance underpins the recent proposal of veterinary experts to reconsider the use of colistin and its analogues in food production animals [31].

In conclusion, the plasmid-mediated colistin resistance gene *mcr*-1 was detected in three ESBL-producing *E. coli* isolates that had been cultured from retail chicken meat from Dutch supermarkets in 2009 and 2014. Two isolates were obtained from the same batch of meat samples, which most likely represents contamination from a common source. The *mcr*-1 gene was not present in a large collection of human isolates collected between 2009 and 2015 in the Netherlands. These findings indicate that *mcr*-1-based colistin resistance currently poses no threat to healthcare in the Netherlands, but requires continued monitoring of colistin resistance and its underlying mechanisms in humans, livestock and food.

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Conflict of interest

Katrien De Bruyne is an employee of Applied Maths, a company that develops and sells software for microbiological typing methods. All other authors have no competing interest to declare.

Authors' contributions

MK, MJMB, JR, PH collected the data, MK, MB, JR and KDB performed the molecular analysis, MK, PH, MJMB, MB, KDB, JR, AF, PS and JK participated in drafting the manuscript, MK coordinated and edited the manuscript.

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The measles outbreak in Bulgaria, 2009–2011: An epidemiological assessment and lessons learnt

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Measles re-emerged in a nationwide outbreak in Bulgaria from 2009 to 2011 despite reported high vaccination coverage at national level. This followed an eight-year period since the last indigenous cases of measles were detected. The Bulgarian National Centre of Infectious and Parasitic Diseases collated measles surveillance data for 2009–2011. We analysed data for age group, sex, ethnicity, diagnosis confirmation, vaccination, hospitalisation, disease complications, and death and describe the outbreak control measures taken. The outbreak started in April 2009 following an importation of measles virus and affected 24,364 persons, predominantly Roma. Most cases (73%) were among children < 15 years old. Vaccination status was available for 52% (n = 12,630) of cases. Of children 1–14 years old, 22% (n = 1,769) were unvaccinated and 70% (n = 5,518) had received one dose of a measles-containing vaccine. Twenty-four measles-related deaths were reported. The Roma ethnic group was particularly susceptible to measles. The magnitude of the outbreak resulted primarily from the accumulation of susceptible children over time. This outbreak serves as a reminder that both high vaccination coverage and closing of immunity gaps across all sections of the population are crucial to reach the goal of measles elimination.

Introduction

One of the largest outbreaks of measles in the World Health Organization (WHO) European Region in recent years occurred in Bulgaria from 2009 to 2011 and mostly affected Roma communities. The outbreak was first detected in spring 2009 after an eight-year period since the last indigenous measles cases were reported in 2001 [1]. The last major outbreak in Bulgaria occurred in 1991–1992 affecting over 20,000 persons [2].

By December 2009, two preliminary reports on the outbreak were published in the scientific literature [3,4]. Here we provide an overview of the measles outbreak in Bulgaria by analysing measles surveillance data for the whole outbreak period of 2009–2011. We also describe the control measures taken and discuss lessons learnt in relation to the WHO European Regional goal of eliminating measles by 2015 [5].

The measles vaccine was introduced in Bulgaria in 1969 as a monovalent preparation [6]. A two-dose schedule began in 1983. The combined measles-mumps-rubella (MMR) vaccine has been given as the first dose at 13 months of age since 1993, and as the second dose at 12 years of age since 2001. For 2003–2008, the estimated national vaccine administrative coverage with the first MMR vaccine dose ranged from 94.7% to 96.2%, and for the second dose, from 89.4% to 94.3% [7].

Bulgaria forms part of the Balkan Peninsula in south-eastern Europe and consists of 28 administrative regions. The latest census carried out in 2011 reported a population of 7,364,570, consisting of three main ethnic groups: Bulgarians (84.8%), Turks (8.8%) and Roma (4.9%). According to these official statistics, the Roma ethnic group numbers 325,343 persons distributed in all regions, but mainly in Montana (12.7% of population), Sliven (11.8%), Dobrich (8.8%) and Yambol (8.5%) [8].

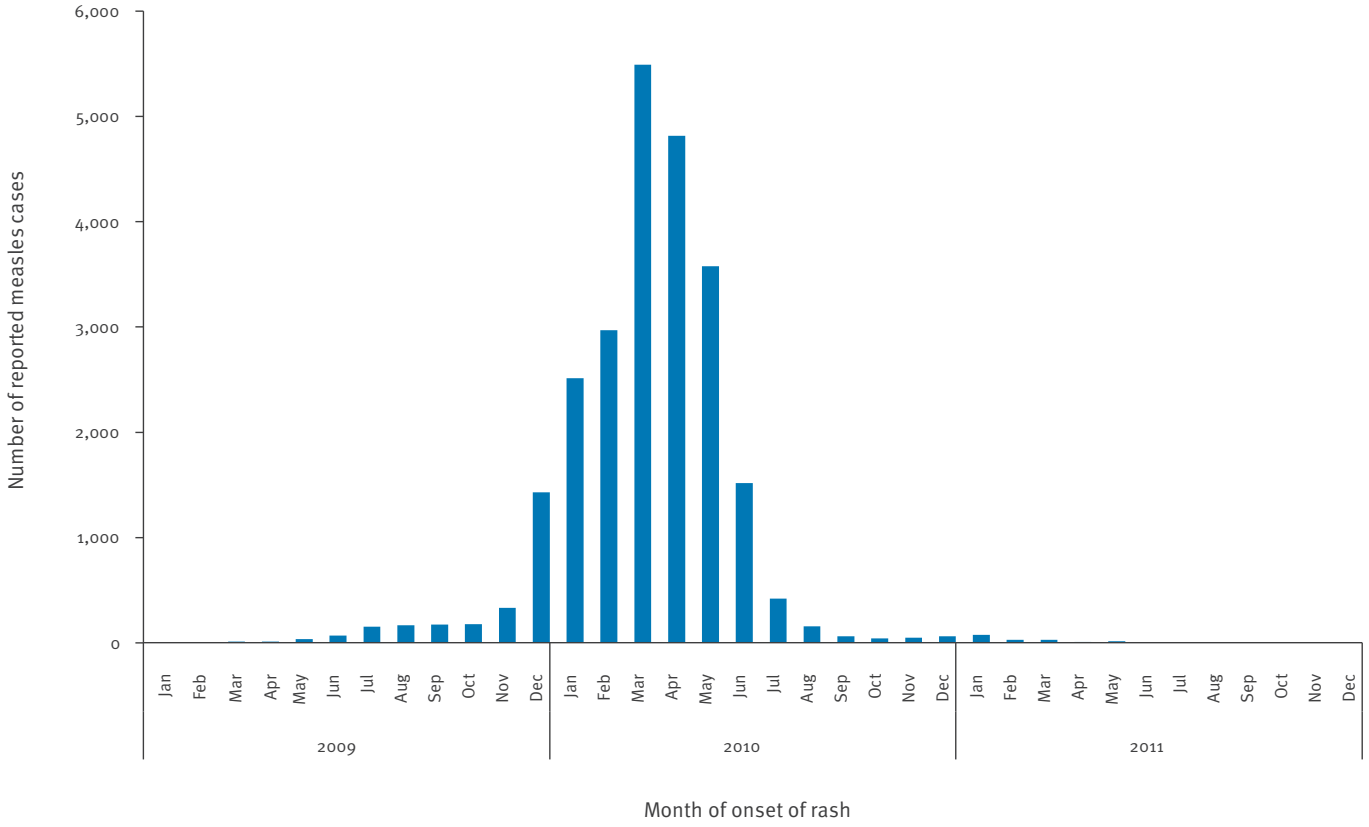
Methods

Epidemiological data

The surveillance of measles in Bulgaria relies on passively reported cases. Measles has been a statutory notifiable disease since 1921 [9], and medical practitioners and medical laboratories are obliged to

Figure 1

Number of reported measles cases by month of onset of rash, Bulgaria, 2009–2011 (n = 24,364)



immediately report suspected measles cases to the respective Regional Health Inspectorate (RHI) [10]. The RHIs are responsible for the epidemiological investigation of cases, tracing contacts of cases, undertaking control measures in affected families and communities and following up cases to register disease outcome. In 2005, the European Union case definition and case classification were adopted for reporting measles surveillance data [11].

During the outbreak period 2009–2011, case-based data were submitted by all 28 RHIs to the Department of Epidemiology and Communicable Disease Surveillance of the National Centre of Infectious and Parasitic Diseases (NCIPD) in Sofia. In October 2009, a web-based system for direct case-based data entry by the RHIs was implemented, gradually replacing previous manual methods of data collection and submission.

Case-based reports provided data for disease onset dates, date of birth, sex, diagnosis confirmation (i.e. laboratory-confirmed, epidemiologically linked and clinically compatible cases), vaccination, hospitalisation, complications and death. Information on vaccination status was obtained from patient immunisation cards whenever such cards were available. The investigators of the outbreak estimated the number of cases in Roma in parallel to routine data collection. We analysed surveillance data of cases with disease onset from 2009 until 2011 and separated the data by specified age groups.

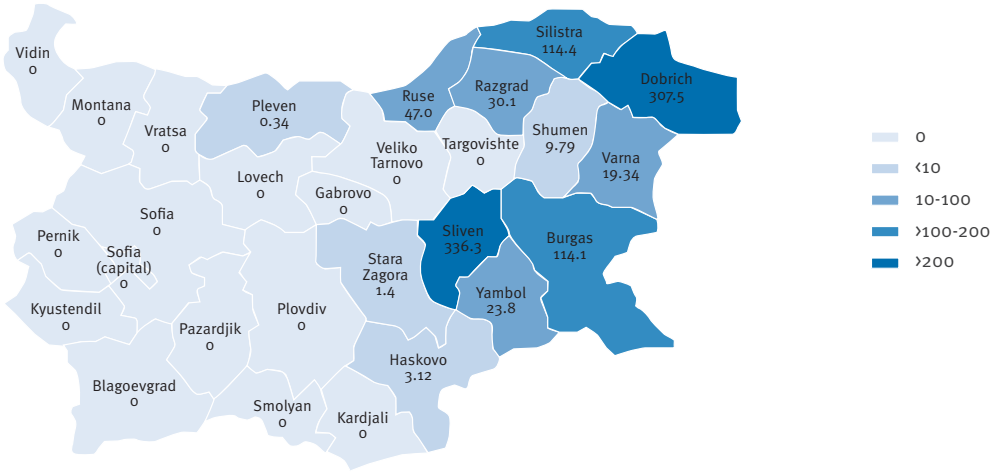
Laboratory data

Laboratory confirmation of cases was carried out by detecting measles IgM antibodies in serum samples submitted mainly to the National Reference Laboratory of the NCIPD and, to a lesser extent, to the laboratories of three regional military hospitals. Clinical specimens of 20 laboratory-confirmed cases were submitted to the WHO European Regional Reference Laboratory for Measles and Rubella at the Robert Koch Institute in Berlin, Germany to determine the genotype of the measles virus (MV) circulating during the outbreak and to identify the likely origin of the virus. The specimens were taken from case-patients in different regions at various points in time (April 2009, May 2009, January 2010, June 2010 and January 2011). Serum was sent for confirmatory testing, and urine specimens and throat swabs were submitted for virus detection, sequencing and genotyping of the MV RNA following standard instructions [12]. IgM and IgG serology tests were carried out as described by Tischer et al. [13] and genotyping was performed according to the WHO recommendation [14]. Sequences were aligned using ClustalW [15] and further analysed using SeqScape 2.5 and MEGA 4.0 DNA analysis software [16]. Phylogenetic trees were constructed using the neighbour-joining method. Genotype assignment was performed by

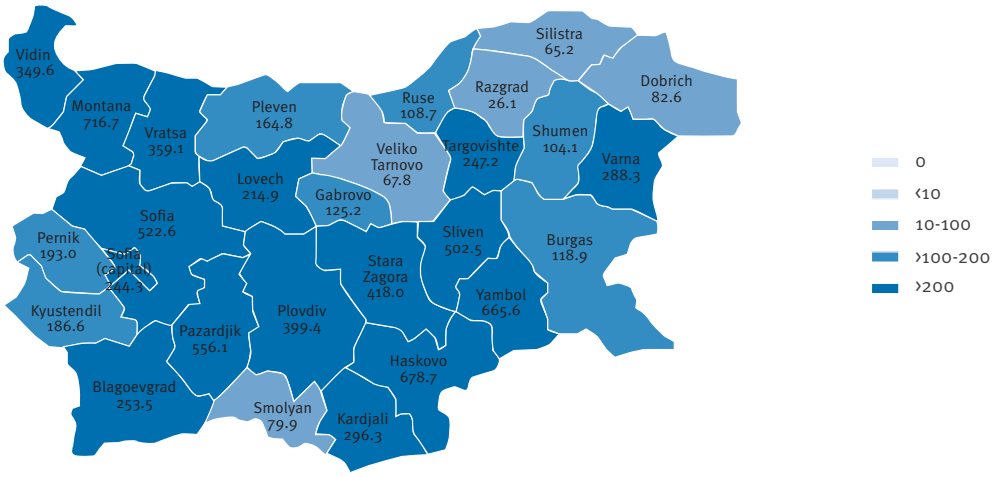
Figure 2

Incidence of measles cases by region in Bulgaria, 2009–2011

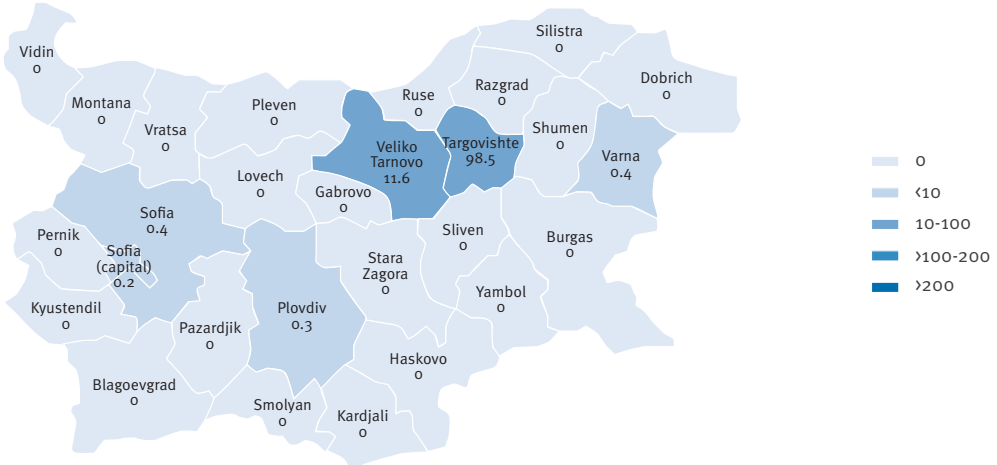
A. 2009 (33.6/100,000 population)



B. 2010 (288.7/100,000 population)



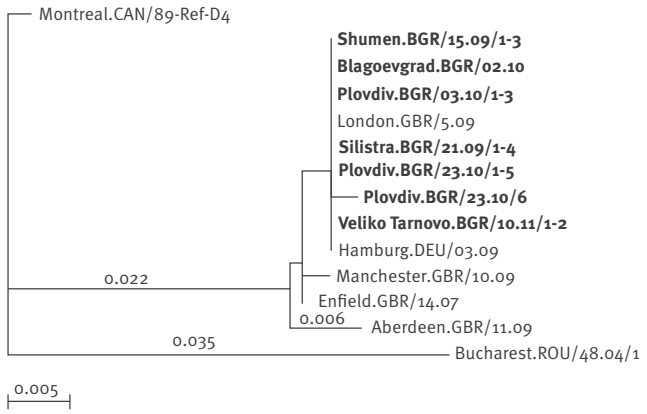
C. 2011 (2.1/100,000 population)



Country incidence per 100,000 inhabitants is indicated in parentheses for each year.

Figure 3

Phylogenetic relationship between the World Health Organization reference strain of measles virus genotype D4 (MVs/Montreal.CAN/89-Ref-[D4]) and MV strains detected in measles outbreak in Bulgaria, 2009–2011



Measles virus (MV) strains detected in Bulgaria are shown in bold. The World Health Organization-named strains of MV genotype D4 (MVs/Enfield.GBR/14.07, MVs/Manchester.GBR/10.09 and MVs/Hamburg.DEU/03.09) circulating in Europe in the same period are also included. The unrooted tree is based on the 456 nt sequence encoding the C-terminus of the MV N gene. The phylogenetic distance scale bar indicates estimated changes per nucleotide.

phylogenetic comparison with the MV reference strains as designated by WHO [17]. The obtained sequence data, genotype information and the official WHO MV sequence name and relevant epidemiologic data were submitted to the WHO database, Measles Nucleotide Surveillance (MeaNS) database [18] and GenBank.

Incidence and case-fatality calculations

Incidence was calculated with the number of measles cases as the numerator and the country and region population, obtained from the National Statistical Institute, as the denominator [19]. Unless otherwise specified, we expressed incidence per 100,000 inhabitants per year, and cumulatively for the three-year period 2009–2011. Case-fatality ratio (CFR) was expressed as the number of measles-related deaths per 100 cases for the three-year period 2009–2011.

Ethical approval or informed consent was not considered to be necessary for this analysis since the data were collated under the Ministry of Health's Regulation 21 on epidemiological surveillance and control of communicable diseases in Bulgaria [10].

Results

Overall, 24,364 cases of measles were recorded between April 2009 and December 2011 corresponding to a cumulative incidence of 326 per 100,000 inhabitants over the three-year period. The outbreak reached its peak by March 2010 (monthly incidence: 73 per 100,000 inhabitants) when all 28 regions of Bulgaria were affected (Figure 1). Figure 2 shows the incidence of measles by region and by year, 2009–2011. During the three-year period of the outbreak, the highest incidence (>500 cases/100,000 inhabitants) was registered in the regions of Sliven (838.8/100,000 inhabitants), Montana (716.7), Yambol (689.5), Haskovo (681.8), Pazardjik (556.1) and the Sofia region (523.0).

Of the total, 3,958 cases (16%) were laboratory-confirmed by detecting measles IgM antibodies in serum samples, 8,233 (34%) cases were epidemiologically linked to a laboratory-confirmed case and 12,173 (50%) cases were classified as clinically compatible cases.

Of the total, 12,472 (51%) were males. The median age of the cases was seven years (range: one day to 71 years). Infants had the highest age-specific incidence per 100,000 inhabitants of 5,457 followed by 2,008 in children aged one to four years. Table 1 shows the age distribution of cases. Data on vaccination status were available for 52% (n=12,630) of all reported cases (Table 2). Of the cases vaccinated with one MMR vaccine dose (n=6,167; 49%), 11% (n=656) were vaccinated within 14 days before onset of disease.

Of the total, 21,821 (89.6%) cases were estimated to occur among Roma. Indeed, the outbreak was first detected in April 2009 among the Roma community in the north-eastern part of the country involving the regions of Razgrad, Shumen, Silistra and Dobrich. The index case was identified as member of the Roma community aged between 20 and 30 years, who fell ill in March 2009, a few days after returning home from Hamburg, Germany. Initial symptoms included high fever, cough, coryza and malaise followed by the development of a rash three days later. The clinical suspicion of measles was confirmed by serological tests. Three of the index case's family members subsequently acquired laboratory-confirmed measles. At the outset, the detection of further cases was delayed.

Mortality, hospitalisation and complications

Measles-related deaths were recorded in 24 patients (14 laboratory-confirmed, five epidemiologically linked, and five clinical cases), corresponding to a CFR of 0.1 per 100 measles cases. The deaths occurred as a consequence of severe complications of measles: 19 cases (79%) suffered acute pneumonia and five cases (21%) suffered acute encephalitis. The median age at death was 1.71 years (range: 32 days–54 years). Infants and cases aged ≥25 years had a higher CFR (0.28% and 0.2%, respectively) compared with cases aged 1–24 years (0–0.09%). All deaths, with the exception of two cases of Bulgarian ethnicity aged between 40 and 49 years of age, occurred in Roma.

Data on hospitalisation status were available for 92% (n=22,296) of cases, of whom 86% (19,167) were hospitalised. Among those hospitalised, 88% (16,854) were aged <19 years. Information on measles-related complications was reported in 86% (21,039) of cases, of whom 38% (8,074) reported complications (Table 3).

Table 1

Age distribution of measles cases (n = 24,364) and measles-related deaths (n = 24), Bulgaria, 2009–2011

Age group (years)	No. of cases (n = 24,364) (% of total reported cases)	Deaths (n = 24)	Case-fatality ratio %
<1	3,891 (16)	11	0.28
1–4	5,858 (24)	5	0.09
5–9	3,473 (14)	2	0.06
10–14	4,706 (19)	1	0.02
15–19	3,167 (13)	1	0.03
20–24	1,246 (5)	0	0
≥25	2,023 (8)	4	0.20

Molecular typing

All three nucleotide (nt) sequences of the variable part of MV N-gene (456 nt) derived from the three household contacts of the index case (MVs/Shumen.BGR/15.09/1–3 [D4]) were identical to D4-Hamburg (MVs/Hamburg.DEU/03.09). D4-Hamburg showed a sequence deviation of one nt from D4-Enfield (MVs/Enfield.GBR/14.07/[D4]), which was endemic in the United Kingdom between 2007 and 2009 [20,21].

The sequences derived from samples collected from four further cases later in 2009 are represented by MVs/Silistra.BGR/21.09/1–4 [D4]. In 2010, specimens from different parts of the country were collected and evaluated: from south-western (MVs/Blagoevgrad.BGR/02.10/1 [D4], central (MVs/Plovdiv.BGR/03.10/1–6 [D4] and northern Bulgaria (MVs/VelikoTarnovo.BGR/10.11/1-2 [D4]). Nineteen out of the 20 laboratory-confirmed cases that submitted clinical specimens for further laboratory analysis showed the sequence variant D4-Hamburg (Figure 3). MVs/Plovdiv.BGR/23.10/6 [D4] was characterised by a sequence deviation of one nt, probably as a result of mutation.

Outbreak control measures

Outbreak management

The local health authorities implemented several control measures in line with the Bulgarian National Programme for the Elimination of Measles and Congenital Rubella Infection (2005–2010) [22]. The same month the outbreak was detected persons of Roma ethnicity living in the first-affected north-eastern regions of the country aged between 13 months and 30 years were targeted for immunisation with one dose of MMR vaccine. In February 2010, the campaign was extended to a national level targeting persons aged 13 months to 20 years who had not received two MMR vaccine doses. From the end of March 2010, the vaccine was available on request to all persons aged 30 years and older who had not received two MMR vaccine doses. Throughout the outbreak period, healthcare workers were offered a dose of MMR vaccine, irrespective of their immunisation status or age. Between April 2009 and December 2010, 188,700 MMR vaccine doses were administered free of charge by the Ministry of Health (MoH) through routine immunisation services.

Special outreach teams composed of local epidemiologists and health inspectors in collaboration with Roma health mediators (RHM) were deployed to vaccinate Roma communities. RHM are usually young adult members of the Roma community who are specially educated in the health field and trained to liaise between the community and healthcare facilities [23]. RHM assisted vaccination teams by improving communication between the team members, and leaders and members of the Roma community; by informing Roma leaders and parents of the benefits of vaccinations and by facilitating the transport of children to immunisation centres.

The MoH recommended that patients with measles living in crowded households be admitted to hospital to ensure better conditions for treatment and care and to minimise the spread of the disease in the poor neighbourhoods.

Outbreak communication

Activities to increase awareness of the outbreak among the public and healthcare professionals were undertaken. When the outbreak started spreading beyond the north-eastern part of Bulgaria, the MoH issued a press release on the emerging outbreak, and provided information on the surveillance and immunisation activities. The MoH website also provided regularly updated information. Information leaflets were also distributed to the general population, and specifically to Roma, via their religious and community leaders. Information packages including a description of measles, updates on the status of the outbreak and a call to the public to be vaccinated were also regularly supplied to the media.

The MoH distributed official circular letters to medical professionals in April 2009, August 2009 and February 2010. Medical professionals were requested to pay special attention to patients presenting with rash and fever, to reach out to parents to explain the benefits of vaccination, and to ensure timely routine MMR vaccination of children.

Additional measures

The MoH regularly informed the WHO Regional Office for Europe and the European Centre for Disease Prevention and Control (ECDC) on the outbreak situation and measures taken to mitigate it. In February 2010, experts from both organisations worked closely with the Bulgarian public health authorities to assess the outbreak and potential risk for further spread beyond the country, to review the current vaccination strategies and MMR vaccine supplies in the country for efficient control measures and to provide guidance on long-term strategies that address vaccination among

Table 2

Measles cases with known vaccination status, Bulgaria, 2009–2011 (n = 12,630)

	<1 year (n = 3,296)		1–4 years (n = 3,549)		5–9 years (n = 2,034		10–14 years (n = 2,327)		15–19 years (n = 1,179)		≥20 years (n = 245)		Total (n = 12,630)	
Unvaccinated	3,274	99.3%	1,357	38.2%	223	11.0%	188	8.1%	120	10.2%	63	25.7%	5,225	41.4%
Vaccinated with single dose	19	0.6%	2,085	58.7%	1,660	81.6%	1,773	76.2%	541	45.9%	89	36.3%	6,167	48.8%a
Vaccinated with at least two doses	3	0.1%	107	3.0%	151	7.4%	366	15.7%	518	43.9%	93	38.0%	1,238	9.8%

[a] Of the cases vaccinated with one MMR vaccine dose (n = 6,167), 11% (n = 656) were vaccinated within 14 days before onset of measles.

vulnerable populations. Timely communication through these organisations alerted other countries to respond to any imported cases. In spring 2010, Bulgaria used the opportunity of the 2010 European Immunisation Week to advocate for and gain high-level political commitment to immunisation.

Discussion

Our assessment of the outbreak relied on data collected though routine surveillance based on passively reported cases. Such systems are notorious for underreporting and incompleteness of data. On the other hand, some over-estimation of cases may have occurred since half of these were not confirmed by laboratory testing or were epidemiologically linked, and patients with other rash- and fever-like illnesses may have been wrongly reported as measles cases. Furthermore, since there are no provisions for data collection by ethnicity, the investigators could only estimate the number of Roma cases based on their observations. Moreover, our analysis on vaccination status was limited to the 48% of cases with data on this variable. Despite these limitations, the data we present strongly indicate that the Roma ethnic group was particularly susceptible to measles. Measles outbreaks have also emerged in Roma communities in other European countries [24-27]. As in Bulgaria, their vulnerability was brought to light when the MV was imported from abroad.

Similar to the measles outbreak in neighbouring Greece in 2005–2006 [25], sub-optimal immunisation coverage among Roma children largely contributed to this outbreak. A cross-sectional survey of coverage with routine immunisations in children born in 2006 in the region of Sofia showed that out of 324 Roma children eligible for immunisation, only 68.8% (n = 223) received the first MMR vaccine dose [28].

According to a seroprevalence survey that included 1,666 individual samples collected in 2001–2004, Bulgaria was one of several European countries that had not met the WHO targets for measles susceptibility [29]. For the 2–4 and 5–9 year-old age groups, 30.4% and 25.9% respectively, were seronegative for measles. The WHO susceptibility targets for these consecutive age groups are <15% and <10% [30]. A seroprevalence survey on 249 hospitalised non-measles patients aged ≤ 65 years conducted in 2008 by NCIPD in Burgas, Bulgaria, revealed that 51 patients (20.5% (95% CI 15.6–27.0%)) were measles IgG-negative [31]. These results suggest that the population susceptibility to measles at national level is probably higher than that indicated by the reported minimum of 94.7% immunisation coverage for the first dose of routine measles vaccination for 2003–2008 [7]. In Bulgaria, immunisation coverage is estimated using the administrative method as a proportion of the number of routinely administered vaccine doses by eligible birth cohorts of the previous year. An overestimation of the coverage may have resulted if the denominator did not include all the population targeted for vaccination. Lack of registration of Roma children with a healthcare facility has, indeed, been documented [32].

The magnitude of the outbreak underlined the substantial number of susceptible children that had accumulated gradually since the last major nationwide outbreak in 1991–1992. During the health reforms of the 1990s there were a number of challenges in ensuring access to quality child health services, including immunisation, to the Roma minority [33]. In addition, since primary vaccine failure is reported to occur in 2–5% of vaccinated children after the first measles-containing vaccine (MCV) dose given at 12 months of age [34], the accumulation of non-responders to the first MCV dose probably also contributed to the pool of susceptible individuals. This also explains, at least in part, the relatively large proportion (49%, n = 6,167) of cases reported having received one MCV dose, since in Bulgaria, the second dose is not given until 12 years of age. Other potential contributing factors may include incorrect documentation on vaccination status and issues with the cold chain. Nonetheless, 11% (n = 656) of these cases developed measles within 14 days of vaccination, which was probably administered as part of the outbreak control measures while they were in the incubation period following infection with MV.

Roma communities are often separated from the mainstream of social and economic life in segregated, often crowded, neighbourhoods; however, there is intensive contact between the different communities.

Table 3

Cases with reported major measles-related complications, Bulgaria, 2009–2011 (n = 8,074)

Complication	No. of cases (n = 8,074) (% of total cases with complications)
Pneumonia	4,704 (58)
Diarrhoea	3,206 (40)
Acute encephalitis	15 (0.20)
Otitis media	21 (0.30)
Pneumonia and diarrhoea	123 (2)
Pneumonia and encephalitis	5 (0.10)

This explains the widespread transmission across the country, and also beyond its borders. Between 2009 and 2011, MV variant D4-Hamburg appeared in several European countries. The spread of the MV was mostly, but not exclusively, associated with travelling members of the Roma ethnic group [35].

Poor maternal education was shown to be a risk factor for the development of measles-related complications [36]. However, the high proportion of hospitalised cases reflects the MoH's recommendation to hospitalise measles patients living in poor conditions. While this measure may have benefitted patients admitted to hospital, its impact in limiting the spread of disease in the Roma community is difficult to estimate. Inadvertently, it probably intensified nosocomial transmission [37]. During this outbreak, MV transmission occurred in several healthcare settings and healthcare workers emerged as a group at risk of acquiring measles. This necessitates clear recommendations to adhere to infection control measures in healthcare settings and to ensure healthcare workers are adequately protected.

The Bulgarian health authorities implemented the necessary control measures with coordination, support and directives from the MoH. Regular communication with the WHO Regional Office for Europe and the ECDC allowed transparency, dialogue and advice to be sought. The large number of reported cases posed a major challenge to the surveillance system that relied on time-consuming manual methods of data collection and submission. With the support of the WHO Regional Office for Europe these methods of data collection were replaced by a web-based system allowing direct and timely case-based data entry by the RHIs.

The RHIs played a key role in executing control measures despite financial and human resource limitations. Supplementary immunisation activities were instigated to first target Roma in the affected regions and later the general population. Concurrently, healthcare professionals were urged to strengthen routine immunisation services. Despite these efforts, the initial control measures were arguably not implemented rapidly and widely enough to curb the outbreak. The clinicians' unfamiliarity with the disease probably contributed to the delay in detecting cases and subsequent response to the first cases. Nevertheless, resources permitting, a nationwide vaccination campaign targeting all infants aged nine months and older, children and young adults would probably have curtailed the outbreak sooner.

The outbreak in Bulgaria has served to further develop national and local programmes in collaboration with Roma organisations with the aim of integrating better the Roma community into the health system. Bulgaria was one of the first countries in the WHO European Region to launch the Guide to Tailoring Immunisation Programmes that resulted in several efforts to be undertaken, such as improving curricula and training of RHM across the country [38-40]. In addition, Bulgaria participated in a European collaborative project, Let's Talk About Protection, which aims to communicate effectively and address patients' concerns on vaccine topics [41]. This has resulted in the publication of a practical guide to vaccination adapted to the context in Bulgaria, and intended for use by healthcare workers and visual aid material in the form of handy flip charts for general practitioners and RHM [42].

The outbreak serves as another reminder to all countries of the WHO European Region of their commitment to eliminate measles [43]. To reach this goal every country needs to ensure that their immunisation programmes achieve and maintain high vaccination coverage (≥ 95%) with two MCV doses, while also identifying and closing immunity gaps across all population segments.

In conclusion, a nationwide outbreak of measles in Bulgaria during 2009–2011 resulted from the accumulation of a large susceptible population despite reported high measles vaccination coverage at national level. The outbreak particularly highlighted the vulnerability of Roma communities in Bulgaria to measles. In addition to low coverage among Roma, accumulation of non-responders to the first MCV dose could have also contributed to the pool of susceptible individuals. The development and implementation of strategies to identify susceptible individuals and close immunity gaps across all segments of the population are of vital importance in relation to reach the measles elimination goal.

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Conflict of interest

None declared

Authors' contributions

LM co-ordinated the measles surveillance activities in Bulgaria, collated data and with MM, analysed data and reviewed literature. MM had the primary responsibility of writing the manuscript. AM and SS performed molecular characterisation of the measles virus and produced the related graphic. NG, ZM, AK and MK provided valuable comments and suggestions at various stages in the preparation of this manuscript.

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WHO publishes viral hepatitis surveillance guide

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On 29 February 2016, the World Health Organization (WHO) released its first surveillance guide for hepatitis, 'Technical considerations and case definitions to improve surveillance for viral hepatitis' [1]. The document outlines key actions for improving hepatitis surveillance systems, and provides case definitions for viral hepatitis surveillance.

Viral hepatitis is a global public health problem of epidemic proportions and according to the Global Burden of Disease study [2], it causes approximately 1.46 million deaths each year. New infections caused by the five known hepatitis viruses – A, B, C, D and E (HAV, HBV, HCV, HDV and HEV) can be prevented; however, this relies on surveillance systems generating epidemiological information which is key in preventing and controlling hepatitis epidemics.

In recognition of the burden of viral hepatitis on global health, the World Health Assembly adopted resolutions in 2010 and 2014 [3,4] that called for a comprehensive approach to the prevention and control of viral hepatitis and mandated WHO to work closely with its Member States to develop the necessary guidelines for the surveillance, prevention and control of viral hepatitis.

In response, WHO developed these technical considerations that aim to help develop or strengthen the collection, analysis and reporting of data related to viral hepatitis.

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