

SHORT REPORT

An outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections linked to the consumption of imported horse meat in France

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SUMMARY

In 2003, 14 cases of multidrug-resistant *Salmonella* Newport infections were reported. This is the first documented foodborne outbreak of multidrug-resistant *S. Newport* in France. The *bla*_{CMY} gene was present in all isolates. All cases reported having eaten horse meat from a common wholesaler. The country of origin of the imported meat could not be identified.

In France, non-typhoidal *Salmonella* is the main known cause of bacterial gastroenteritis and is responsible for 65% of reported foodborne outbreaks [1]. *Salmonella* surveillance is based on a network of 2000 voluntary medical laboratories that send their isolates to the National Reference Centre (NRC) for *Salmonella* for serotyping. In 2000, *Salmonella enterica* serotypes Typhimurium and Enteritidis represented 70% of all *Salmonella* isolates in humans [2]. In France, *S. enterica* serotype Newport (*S. Newport*) infection is uncommon. There were 125 and 66 cases in 2001 and 2002 respectively, ~1% of all reported cases of salmonellosis. Most strains (96–98%) were susceptible to antimicrobial agents.

We report an outbreak of multidrug-resistant (MDR) *S. Newport* that occurred in France in 2003.

On 2 June 2003, the NRC for *Salmonella* identified, through routine surveillance, a cluster of 11 MDR *S. Newport* strains isolated over a 3-week period in the North of France. The strain was resistant to ampicillin, streptomycin, sulphamethoxazole, tetracycline,

chloramphenicol and expanded-spectrum-cephalosporins (except cefepime). An investigation was prompted to determine the extent of the outbreak and the source of infection.

A case was defined as a patient with diarrhoea (at least three loose stools daily) and MDR *S. Newport* isolated from a stool specimen between 1 May and 1 July 2003. Cases, identified through the NRC, were interviewed by phone, using a hypothesis-generating questionnaire designed to enquire about symptoms, exposures (food and drink items consumed, contact with other symptomatic persons, contact with animals, places visited, contact with water, etc.) and retail outlets in the 3 days before onset of illness.

Between 12 May and 4 June 2003, 14 cases of MDR *S. Newport* were identified in three districts in the North of France. Seven patients were female. All age groups (nine children, five adults) were affected (mean 24 years, range 1.5–75 years). The most frequently reported symptoms were diarrhoea (100%, bloody 50%), vomiting (86%), fever (71%) and abdominal cramps (64%). Eleven (79%) patients were hospitalized. None died.

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Table. Oligonucleotide primers used in the study

Gene	Primer	Oligonucleotide sequence (5'→3')	Ref.	PCR product size (bp)
<i>bla</i> _{CMY}	CMY-F	ATGATGAAAAAATCGTTATGC	[4]	1145
	CMY-R	TTGCAGCTTTTCAAGAATGCG		
<i>bla</i> _{TEM}	TEM-F	ATAAAATTCTTGAAGACGAAA	[5]	1080
	TEM-R	GACAGTTACCAATGCTTAATC		
<i>bla</i> _{SHV}	SHV-F	TTATCTCCCTGTTAGCCACC	[6]	795
	SHV-R	GATTTGCTGATTTTCGCTCGG		
Class I integron	5'-CS	GGCATCCAAGCAGCAAGC	[7]	Variable
	3'-CS	AAGCAGACTTGACCTGAT		

The consumption of horse meat was the only reported exposure common to all cases. The meat had been consumed as ground meat (11 cases), consumed raw by at least six cases, or steak (three cases). Concurrent diarrhoea was reported by five family members in two of the 14 families in which a case occurred. In one of these families, the five affected family members (including the confirmed case) had eaten horse meat and the only unaffected family member had not eaten it.

Patients had purchased their horse meat from 12 retail outlets in different towns: five butcheries (seven cases) and seven markets (seven cases). All 12 outlets were supplied by one wholesaler located in the North of France. On 20 June 2003, the wholesaler's facilities were inspected. Operational hygiene and the list of horse-meat suppliers were reviewed. No meat from carcasses sold in May was available for microbiological testing. The company purchased its horse meat abroad from eight different countries: Argentina, Australia, Belgium, Brazil, Canada, Hungary, United Kingdom and Uruguay. Since the origin of the horse meat was not recorded after purchase by the wholesaler, it was impossible to determine the exact origin of the meat supplied to the retail shops.

Information on the outbreak was sent to the health authorities of the European member states and to all participants in the international network for surveillance of human gastrointestinal infections (Enternet), with a request to notify all *S. Newport* isolates with a similar resistance pattern. No MDR *S. Newport* isolates were identified in European countries other than France.

All *S. Newport* isolates were screened for resistance to 32 antimicrobials by the disk diffusion method on Mueller–Hinton agar according to the guidelines of the French National Antibiogram Committee [3].

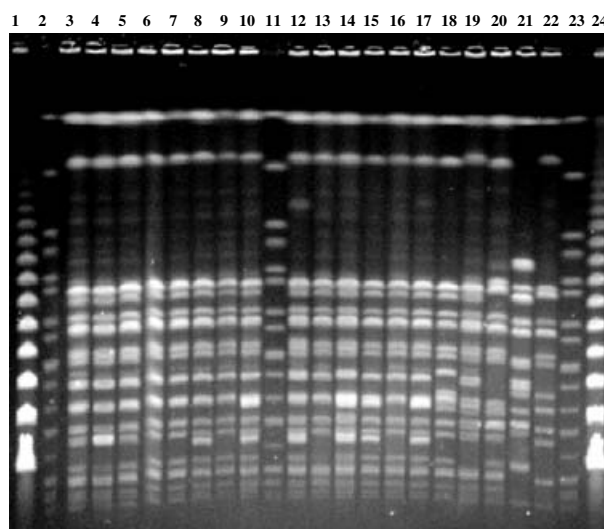


Fig. Pulsed-field gel electrophoresis patterns of *Xba*I-digested genomic DNA from 19 *Salmonella* Newport strains. Lanes 1, 24, bacteriophage lambda DNA concatemers (New England Biolabs, Beverly, MA, USA) which served as molecular size marker; lanes 2, 11, 23, *S. Branderup* H9812; lanes 3–10 and 12–17, MDR *S. Newport* (outbreak strains); lane 18, MDR *S. Newport* (isolated in 2000, France); lanes 19, 20, MDR *S. Newport* (isolated in United States); lane 21, *S. Newport* (isolated in 2003, France); lane 22, *S. Newport* reference strain.

PCR detection of *bla*_{CMY}, *bla*_{TEM}, *bla*_{SHV} genes and class I integrons was performed on all isolates. Total DNA was extracted using the InstaGene matrix kit (BioRad, Marnes la Coquette, France). All amplifications were performed on 50- μ l samples containing DNA (2.5 μ l), primers (50 pmol each) (Table), deoxynucleoside triphosphate (200 μ mol), *Taq* DNA polymerase (1.25 U, Ampli *Taq* Gold, Roche Diagnostics, Mannheim, Germany) and its buffer, MgCl₂ (2 mM), and dimethylsulphoxide (10%). The cycling conditions for *bla*_{CMY}, *bla*_{TEM} and *bla*_{SHV}

genes included 10 min of denaturation at 94 °C (1 cycle); 30 s of denaturation at 94 °C, 30 s of annealing at 50 °C, and 1 min of polymerization at 72 °C (35 cycles), followed by 10 min of extension at 72 °C. The cycling conditions for class I integron were as described previously [7]. The purified PCR fragments were sequenced on both strands by Genome Express (Meylan, France) using an ABI 100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequence was analysed with the Laser-gene software (Dnastar, Madison, WI, USA). The BLASTN program of NCBI [8] was used for databases searches. All *S. Newport* isolates from the outbreak were typed by pulsed-field gel electrophoresis (PFGE) of *Xba*I-restricted genomic DNA according to the CDC PulseNet protocol [9]. Restricted fragments were separated in 1% agarose gel with a CHEF DRIII apparatus (BioRad).

PCR amplification products were obtained in all 14 *S. Newport* isolates only with the *bla*_{CMY} gene primers. Sequencing of PCR products from two isolates showed a β -lactamase gene 100% identical to *bla*_{CMY-2} (GenBank accession no. X91840). Two distinct PFGE patterns were observed among the 14 isolates: pattern A (similar to the most common MDR *S. Newport* PulseNet pattern JJPX01.0014) was seen in eight (57%) isolates and pattern B (similar to PulseNet pattern JJPX01.0176) in six (43%) (Fig.). The two patterns differed by a single band of low molecular weight, possibly corresponding to an additional plasmid.

This is the first documented outbreak of MDR *S. Newport* in France. Previously, MDR *S. Newport* had already been identified in sporadic infections: 15 out of 100 *S. Newport* isolates in 2000, five out of 125 in 2001, and one out of 66 in 2002. No MDR *S. Newport* has been isolated from animals or foods.

In the United States, the increase in *S. Newport* illness in humans observed since the late 1990s has been driven by an increase in a highly resistant strain 'Newport MDR-AmpC' which represented 25% of *S. Newport* strains isolated in 2001 [10]. Illness due to Newport MDR-AmpC is also emerging in cattle. In the United States, risk factors for human illness included contact with cattle and consumption of bovine products [10, 11].

To date, no epidemic phenomena caused by MDR *S. Newport* have been reported in Europe, but a further spread of this highly resistant *S. Newport* strain (or of other serotypes of *Salmonella*), as previously observed for *S. Typhimurium* DT104, is feared.

In our investigation, descriptive epidemiology and trace-back investigations allowed us to incriminate imported horse meat as the most likely source of this outbreak. However, we did not perform a case-control study and were unable to isolate the organism from horse meat. To our knowledge, this is the first MDR *S. Newport* outbreak linked to horse-meat consumption. Unfortunately, meat from horses consumed by cases was not available for microbiological analysis and the origin of the meat and the source of its contamination could not be identified. However, *S. Newport* has previously been isolated from horses [12, 13] and from horse meat and carcasses from Brazil [14] and the United States [15, 16]. The plasmid-mediated CMY-2 AmpC β -lactamases has been identified in strains of *S. Newport* present in horses in 2000 and 2001 in the United States [17].

This report highlights the important role that routine testing for antimicrobial susceptibility plays in surveillance for the early detection of drug resistance among *Salmonella* isolates in a region or a country.

In this outbreak, MDR *S. Newport* infection was associated with a high rate of hospitalization and of bloody diarrhoea, as observed in the United States [10, 11]. Resistance to antimicrobial agents used frequently in the treatment of invasive *Salmonella* infections in humans presents significant therapeutic problems in human health care, hence the importance of closely monitoring further introduction of MDR *S. Newport* to limit its spread in Europe.

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