

as carriers of O157 and O26 strains in Ireland (7,8). In Germany, cattle density has been shown to be significantly associated with human VTEC O157 incidence but only marginally associated with O26 incidence (9); the same study showed no association between cattle density and VTEC O91 infection, indicating that not all serogroups necessarily share the same reservoirs. Alternatively, animals of the same species may be preferentially colonized with different serogroups at different times of the year or at different developmental ages. Other possible explanations could be variation in survival characteristics between the 2 strains, which results in a different seasonal distribution in the environment, or specific human behavior (e.g., seasonal food) resulting in more frequent exposure to sources of VTEC O157 and VTEC O26 at different times of the year.

The consistent differences in seasonality identified here between the 2 most common VTEC serogroups suggest the existence of noteworthy underlying differences in disease etiology between the strains. Further exploration is recommended.

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## New Delhi Metallo- $\beta$ -Lactamase-1-Producing *Klebsiella pneumoniae*, Florida, USA<sup>1</sup>

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**To the Editor:** New Delhi metallo- $\beta$ -lactamase (NDM)-producing *Enterobacteriaceae* have swiftly spread worldwide since an initial report in 2008 from a patient who had been transferred from India back home to Sweden (1). Epidemiologically, the global diffusion of NDM-1 producers has been associated with the Indian subcontinent and the Balkan region, which are considered the primary and secondary reservoirs of these pathogens, respectively (1). However, recent reports suggest that countries in the Middle East may constitute another potential reservoir for NDM-1 producers (1). More than 100 NDM-producing isolates have been reported in the United States, most of

<sup>1</sup>Preliminary results from this study were presented at the 54th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 5–9, 2014, Washington, DC, USA.

which were associated with recent travel from the Indian subcontinent (2,3). We report an NDM-1–producing *Klebsiella pneumoniae* strain that was recovered from a patient who had been transferred from Iran to a hospital in Florida, United States.

The patient was a 72-year-old woman with diabetes who had suffered a hip fracture while residing in Iran. After fixation of the bone failed, the patient underwent hip replacement, which was complicated by dislocation and an infected hematoma. She was transferred to a hospital in Florida in February 2014 for further care. The wound culture collected upon arrival grew *K. pneumoniae* K351. The patient underwent surgical debridement, implant removal, and placement of an antimicrobial spacer for prosthetic joint infection. She was treated with tigecycline; however, the wound did not heal, and she underwent debridement with removal of the spacer and placement of antimicrobial beads.

*K. pneumoniae* K351 from the patient was resistant to all  $\beta$ -lactams tested, including carbapenems, and highly resistant to aminoglycosides and fluoroquinolones, retaining susceptibility only to tigecycline and colistin. PCR and sequencing revealed the presence of  $\beta$ -lactamase genes *bla*<sub>NDM-1</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>SHV-12</sub>, and *bla*<sub>TEM-1</sub> and 16S rRNA methyltransferase genes *rmtC* and *armA*. The strain sequence type (ST) was ST147, which is one of the predominant NDM-producing *K. pneumoniae* lineages and has been reported in many countries (3,4). Conjugation experiments using broth and filter mating methods did not yield any *Escherichia coli* J53 transconjugants with *bla*<sub>NDM-1</sub>, despite repeated attempts. Plasmids of K351 were extracted by using the standard alkaline lysis method and used to transform *E. coli* TOP10-competent cells. An *E. coli* transformant harboring plasmid pK351 grew on Mueller-Hinton agar plates supplemented with 200  $\mu$ g/mL of ampicillin. The transformant exhibited resistance to all  $\beta$ -lactams, including carbapenems and aminoglycosides; this resistance could be attributed to the presence of *bla*<sub>NDM-1</sub> and *rmtC* in plasmid pK351, which was confirmed by PCR.

pK351 was fully sequenced on a PacBio RS II sequencing instrument (Pacific Biosciences, Menlo Park, CA) and annotated (GenBank accession no. KR351290) (5). pK351 is 106,844 bp in length, has an average GC content of 55.4%, and encodes IncFIB and IncFII-like replication proteins, with IncFIB belonging to B36 according to the replicon sequencing typing scheme (6). pK351 is most closely related (98% coverage and 99% identity) to 3 *bla*<sub>NDM-1</sub>-carrying plasmids pKOX\_NDM1, pRJF866, and pNDM-Ec1GN574 (GenBank accession nos. NC\_021501, KF732966, and KJ812998, respectively) (online Technical Appendix Figure, <http://wwwnc.cdc.gov/EID/article/22/4/15-1176-Techapp1.pdf>). Compared with the 3 plasmids, pK351 is missing a 4,086-bp region

between insertion sequence (IS) *IS1* and *IS903*-like mobile elements, probably due to *IS1*-mediated deletion. In addition, the region containing gene *ccdBA* between gene *resD* and an *IS1* remnant is replaced by a region encoding 2 hypothetical proteins in pK351. The remainder of pK351 exhibits 99.95% identity to the 3 related plasmids. The immediate genetic environment of *bla*<sub>NDM-1</sub> in pK351 is identical to that in the 3 related plasmids, encompassing *bla*<sub>NDM-1</sub> itself and the downstream sequence, flanked by 256-bp direct repeats (7).

Plasmids pKOX\_NDM1 and pRJF866 were found in a *K. oxytoca* strain from Taiwan and a *K. pneumoniae* ST11 strain from Shanghai, China, respectively (7,8). *K. oxytoca* (pKOX\_NDM1) was isolated from a patient from Taiwan who underwent surgery in Jiangxi, China. *K. pneumoniae* ST11 (pRJF866) was isolated from a patient in a burn unit in Shanghai just after a highly related NDM-1–producing *K. pneumoniae* ST11 strain was isolated from another patient in the same unit who had traveled to Jiangxi Province (8). pNDM-Ec1GN574 was detected in an *E. coli* strain from a patient previously hospitalized in India before being admitted to a community hospital in Canada (9). Identification of highly similar *bla*<sub>NDM-1</sub>-carrying plasmids in various strain lineages and species in different locales suggests extensive horizontal transfer of these plasmids among broad-range hosts. Acquisition of these plasmids by globally distributed, multidrug-resistant *K. pneumoniae* lineages (ST11 and ST147) is of grave concern.

The epidemiology of NDM-1–producing *Enterobacteriaceae* continues to evolve. The case reported here was imported to the United States upon patient transfer from Iran (10). The unusual path for this NDM-1–producing *K. pneumoniae* supports the hypothesis that the Middle East might be an additional reservoir for NDM producers.

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## Ritual Slaughter as Overlooked Risk Factor for Brucellosis

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**To the Editor:** The current rates of animal and human brucellosis in southern Israel are unacceptably high (1). Unsupervised livestock rearing, smuggling of herds, and the dissolution of Israel's "test, slaughter, and compensate" program for small ruminants in 1997 have generated a large, uncontrolled animal reservoir in the region. The Bedouin Arab inhabitants who live in close proximity to herds and consume unpasteurized dairy products are disproportionately

affected. Outbreaks from parts of Israel that were previously free of brucellosis are reported with increasing frequency. Moreover, 2 decades after supposed elimination of bovine brucellosis, a cattle herd adjacent to Bedouin grazing areas was found to be highly infected with *Brucella melitensis* (2).

We report 5 cases of severe brucellosis in patients from a community whose members typically do not raise herds and do not consume unpasteurized dairy products. The patients were Ethiopian-born Jews who exhibited fever and either respiratory signs or radiologic evidence of new pulmonary findings (Table). The true nature of the infection only became evident when *B. melitensis* was identified from blood cultures. Directed questioning revealed that all patients participated in ceremonial slaughter of sheep that were purchased from Bedouin owners in southern Israel.

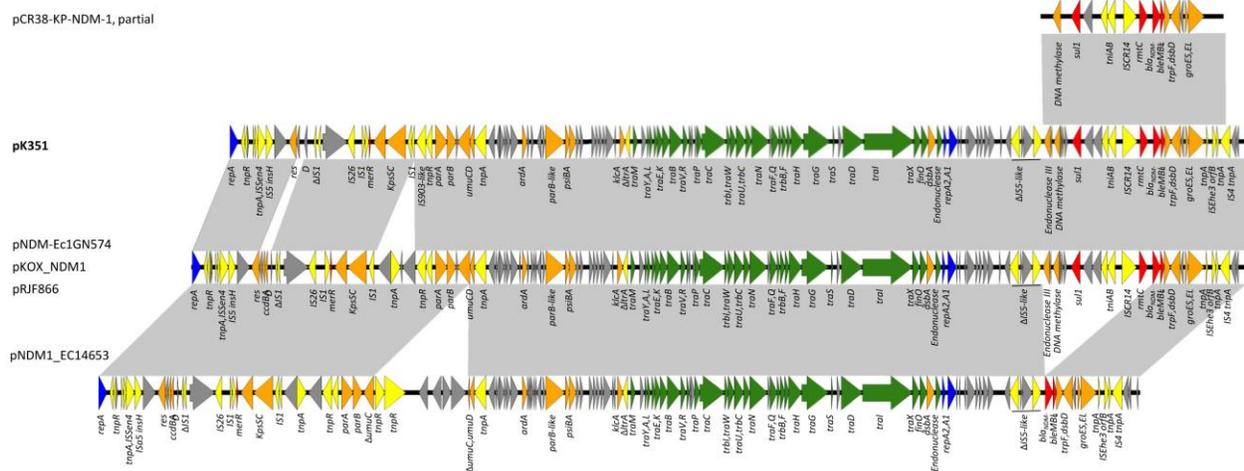
The diagnostic pitfalls encountered by the medical staff are exemplified in the case of patient 1 (Table). In May 2014, this 68-year-old man was admitted to a hospital in southern Israel, with a 14-day history of fever, cough, and night sweats. His medical history was notable for asthma and previously treated pulmonary tuberculosis (TB). Computed tomography scan of his chest showed a new 1.5-cm<sup>2</sup> apical lung lesion with irregular borders. Laboratory evaluations were negative for rickettsiae, *Coxiella burnetii*, HIV, *Plasmodium* spp., and *Mycobacterium tuberculosis*. Four days after admission, gram-negative coccobacilli grew from his blood culture. *Brucella* IgM titer was 1:1,920. The patient received appropriate treatment for 6 weeks (Table) and recovered. *Brucella* IgM titers dropped to 1:40. Four months later, he returned to the infectious diseases clinic exhibiting fever, chills, and clinical and sonographic evidence of epididymo-orchitis. *Brucella* IgM titers rose to 1:1,240. Treatment was resumed for 3 months. In addition, a thoracoscopic biopsy of the lung lesion was performed to rule out malignancy. Pathologic examination of the biopsy specimen revealed a focus of fibrosis, with giant cells surrounded by lymphocytes (online Technical Appendix Figure, <http://wwwnc.cdc.gov/EID/article/22/4/15-1192-Techapp1.pdf>). After treatment, the patient's symptoms resolved and titers returned to low levels.

The long symptom-to-diagnosis interval (range 21–97 days) for patients in this report is alarming (Table). Treatment delays are associated with increased focal complications and relapse rates (3). Further, high case-fatality rates, allegedly due to low physician awareness, were reported in a largely immigrant cohort of brucellosis patients in Germany (4).

Several circumstances might have led to the failure to include brucellosis in the initial differential diagnosis for these patients, even in a disease-endemic region. First, we can assume that physicians are unfamiliar with the ceremonial slaughter central to the celebrations of Ethiopian Jews.

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## Technical Appendix



**Technical Appendix Figure.** Major structural features of plasmid pK351 indicated with bold type, compared with closely related *bla*<sub>NDM-1</sub>-positive plasmid pNDM-Ec1GN574 (KJ812998), pKOX\_NDM1 (NC\_021501), pRJF866 (KF732966), pNDM1\_EC14653 (KP868647.1), and pCR38-KP-NDM-1 (partial, KP826710.1). Light gray shades indicate shared regions with a high degree of homology. ORFs are portrayed by arrows and colored according to their putative functions. Dark-blue arrows indicate replication associated genes. Genes associated with plasmid conjugal transfer are indicated by green arrows, and genes involved in plasmid stability are indicated by brown arrows. Red and yellow arrows indicate antimicrobial resistance genes and mobile elements genes, respectively. Grey arrows indicate genes for hypothetical proteins as well as proteins of unknown function.