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Outbreak of NDM-1-Producing *Klebsiella pneumoniae* Causing Neonatal Infection in a Teaching Hospital in Mainland China

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The emergence and spread of bacteria carrying the bla_{NDM-1} gene has become a worldwide concern. Here, we report eight cases of *Klebsiella pneumoniae* with bla_{NDM-1} in the neonatal ward of a teaching hospital in mainland China. Multilocus sequence typing showed that seven isolates were clonally related and confirmed them as sequence type 17 (ST17). One isolate belonged to ST433. These findings suggest continuous spread of bla_{NDM-1} in mainland China and emphasize the need for intensive surveillance and precautions.

The emergence of carbapenem-resistant Gram-negative bacteria is a global threat. Antibiotics of the carbapenem family have been a mainstay for the treatment of antibiotic-resistant bacterial infections (1). Carbapenems can be hydrolyzed by bacterial enzymes, e.g., the New Delhi metallo-beta-lactamase NDM-1. NDM-1 makes bacteria resistant to a broad range of beta-lactam antibiotics, including those of the carbapenem family (2). NDM-1 was first detected in *Escherichia coli* and *Klebsiella pneumoniae* isolated from a Swedish patient of Indian origin in 2008 (3). Since then, infections associated with NDM-1-positive strains have been reported worldwide, including in India, the United Kingdom, the United States, Canada, Australia, France, Holland, China, Pakistan, Italy, Japan, and Spain (4). The majority of these reported cases were strains isolated from adult patients (5). Here, we report an outbreak of NDM-1-producing *K. pneumoniae* in the neonatal ward of a tertiary teaching hospital in mainland China.

In August 2012, a premature neonate was admitted to our hospital due to poor response after vaginal delivery. The patient developed neonatal sepsis and necrotizing enterocolitis. A carbapenem-resistant *K. pneumoniae* strain was isolated from the blood culture. There was no documented clinical history of the neonate's parents having a link to an area where NDM-1 is endemic. However, his mother suffered from acute appendicitis in the sixth month of pregnancy and was hospitalized in another hospital in Hunan. The neonate received a 5-day course of meropenem and a 3-day course of ciprofloxacin; however, no adequate clinical response was noted. His parents requested discharge from the hospital against medical advice, and the neonate subsequently died (case 1). In September 2012, another neonate had recurrent fever with cytomegalovirus infection and was also positive for a carbapenem-resistant *K. pneumoniae* isolate in blood culture. Ceftazidime was given for 10 days, after which the patient recovered (case 2). Successively, four cases were identified. In February 2013, there was also a preterm neonate who had decreased response after birth and displayed severe dyspnea. The patient was initially treated with mezlocillin-sulbactam. An isolate of *K. pneumoniae* was detected from the sputum. A combination of meropenem and cefoperazone-sulbactam was given for 10 days, and then the patient recovered and was discharged (case 7). By March 2013, eight cases had occurred in the neonatal ward in total. The carbapenem-resistant *K. pneumoniae* isolates were detected from blood and sputum samples individually. The clinical profiles of all patients are shown in Table 1.

All isolates were identified as *K. pneumoniae* by using the BD Phoenix automated microbiology system. Routine determination of antimicrobial susceptibilities was performed using the disk diffusion agar method according to the CLSI standards (6). It showed that all the isolates were resistant to beta-lactams, including carbapenems, but were susceptible to quinolones and aminoglycosides (Table 2). Production of carbapenemases was detected by the modified Hodge test (6), and carbapenemase production by all isolates was confirmed. Detection of bla_{NDM-1} was performed by PCR with designed primers NDM-1-F (5′-GGAAAACTTGATG GA-3′) and NDM-1-R (5′-TAAAACGCCTCTGTC-3′). The PCR products were sequenced and showed 100% identity with bla_{NDM-1} of *K. pneumoniae* that is deposited in GenBank with the accession number FN396876.1 (3). To clarify the mechanisms of carbapenem resistance, PCR screening and sequencing for other beta-lactamase genes (bla_{SHV}, bla_{IMP}, bla_{VIM}, bla_{KPC-2}, bla_{TEM-1}, $bla_{CTX-M-14}$, $bla_{CTX-M-15}$, bla_{CMY-4}, bla_{CMY-8}, bla_{OXA-1}, bla_{OXA-2}, bla_{OXA-9}, bla_{OXA-48}, and $bla_{OXA-181}$), fluoroquinolone resistance genes (*qnrA*, *qnrB1*, and *qnrS*), and aminoglycoside resistance genes [*aac(3′)*, *aac(6′)*, *aph(3′)*, and *armA*] were performed (7, 8). The results revealed the presence of bla_{TEM-1} in addition to bla_{NDM-1} in all isolates. Seven isolates coharbored bla_{SHV-1}, bla_{CMY-4}, $bla_{CTX-M-15}$, and *qnrS*. In isolate 7, only bla_{OXA-2} was detected in addition to bla_{NDM-1}.

Random amplified polymorphic DNA (RAPD) analysis (9)

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TABLE 1 Clinical characteristics of the neonates in the study

Case	Sex[a]	Pregnancy duration (wk)	Dates of hospital stay	Date isolate identified	Type(s) of infection	Antimicrobial therapy[b]	Clinical outcome
1	M	31	30 Aug–7 Sep 2012	1 Sep 2012	Neonatal sepsis, necrotizing enterocolitis	MEM+CIP	Death
2	F	39	9 Sep–11 Oct 2012	16 Sep 2012	Neonatal sepsis, neonatal pneumonia	CAZ	Improvement
3	M	30	23 Oct–28 Nov 2012	29 Oct 2012	Neonatal respiratory distress syndrome, neonatal pneumonia	MEM	Improvement
4	M	33	26 Oct–11 Nov 2012	7 Nov 2012	Neonatal sepsis; neonatal pneumonia	MEM	Improvement
5	M	28	29 Oct–6 Nov 2012	5 Nov 2012	Neonatal respiratory distress syndrome, neonatal pneumonia	PIP-TZB+CAZ	Poor prognosis
6	M	31	14 Nov 2012–12 Jan 2013	22 Dec 2012	Neonatal sepsis, neonatal pneumonia	MEM	Improvement
7	F	28	1 Feb–22 Mar 2013	18 Feb 2013	Neonatal sepsis, neonatal pneumonia	MEM+CFP-TZB	Improvement
8	F	33	1 Mar–14 Mar 2013	15 Mar 2013	Neonatal pneumonia	CAZ	Improvement

[a] M, male; F, female.
[b] MEM, meropenem; CIP, ciprofloxacin; CAZ, ceftazidime; PIP-TZB, piperacillin-sulbactam sodium; CFP-TZB, cefoperazone-sulbactam sodium.

showed that seven of the *K. pneumoniae* strains belonged to the same clone. The clonal relationship was further analyzed using multiple locus sequence typing (MLST) according to protocols provided on the MLST websites (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). Seven of the *K. pneumoniae* isolates were defined as sequence type 17 (ST17), while one belonged to ST433. Neither ST belonged to the most common *K. pneumoniae* STs (ST14 and ST11) that are reported to harbor NDM-1 (10). Only one case of NDM-1-positive ST17 has been confirmed, in Guatemala (11).

The transferability of $bla_{\text{NDM-1}}$ associated with plasmids was confirmed by broth mating conjugation assays, using *E. coli* K12J53 as the recipient strain. Transconjugants were selected on MacConkey agar plates containing sodium azide (100 μg/ml) and meropenem (0.25 μg/ml). Transconjugants revealed resistance to all beta-lactams and carbapenems. PCR and sequencing for transconjugants further confirmed the successful transfer of $bla_{\text{NDM-1}}$ in all the isolates and cotransfer of $bla_{\text{CTX-M-15}}$ in four isolates. This transmissibility with plasmids implies an alarming potential for rapid spread to diverse bacteria.

This report describes a neonatal outbreak of NDM-1-producing *K. pneumoniae* in mainland China. Since seven isolates analyzed in this outbreak were of the same clonal type, the first neonate was likely the source of this outbreak. His mother had a history of hospitalization during pregnancy and had high risk factors for fetal intrauterine infection. Although this organism was not detected from the mother, we could assume the NDM-1-producing *K. pneumoniae* might potentially have been colonized in small numbers in the mother's body and infected the fetus through the placental circulation or through the birth canal. Then, the isolate was transmitted between patients and caused nosocomial infection in the ward. In China, various $bla_{\text{NDM-1}}$-carrying strains of the *Enterobacteriaceae* have been sporadically identified. The first $bla_{\text{NDM-1}}$-positive *K. pneumoniae* isolate in China was identified in Hunan Province in 2012 (12). Now, this study reports more isolates in the same area. This indicates that Hunan province may be a reservoir of $bla_{\text{NDM-1}}$ that may be unrelated to other areas of NDM endemicity, e.g., India. Among the cases in this study, six patients responded to treatment with ceftazidime and meropenem, which seems inconsistent with the resistance characteristics of the isolates. We considered that five isolates from sputum might be normal flora colonizing the respiratory tract rather than the real pathogen of infection.

After March 2013, more cases occurred, not only in the neona-

TABLE 2 Antimicrobial susceptibilities of the eight NDM-producing *K. pneumoniae* isolates

Case	Sample type	ST	Resistance mechanisms	MIC(s) (mg/liter) of[a]: AMC	SAM	PIP	CAZ	CTX	CRO	FEP	IPM	MEM	LVX	CIP	AMK	GEN
1	Blood	17	NDM-1, TEM-1, CTX-M-15, CMY-4, SHV-1, *qnrS*	>16/8	>16/8	>64	>16	>32	>32	>16	≥8	>8	≤2	≤1	≤16	≤1
2	Blood	17	NDM-1, TEM-1, CTX-M-15, CMY-4, SHV-1, *qnrS*	>16/8	>16/8	>64	>16	>32	>32	>16	≥8	>8	≤2	≤1	≤16	≤1
3	Sputum	17	NDM-1, TEM-1, CTX-M-15, CMY-4, SHV-1, *qnrS*	>16/8	>16/8	>64	>16	>32	>32	>16	≥8	>8	≤2	≤1	≤16	≤1
4	Sputum	17	NDM-1, TEM-1, CTX-M-15, CMY-4, SHV-1, *qnrS*	>16/8	>16/8	>64	>16	>32	>32	>16	≥8	>8	≤2	≤1	≤16	≤1
5	Blood	17	NDM-1, TEM-1, CTX-M-15, CMY-4, SHV-1, *qnrS*	>16/8	>16/8	>64	>16	>32	>32	>16	≥8	>8	≤2	≤1	≤16	≤1
6	Sputum	17	NDM-1, TEM-1, CTX-M-15, CMY-4, SHV-1, *qnrS*	>16/8	>16/8	>64	>16	>32	>32	>16	≥8	>8	≤2	≤1	≤16	≤1
7	Sputum	433	NDM-1, TEM-1, OXA-2	>16/8	>16/8	≤16	>16	>32	>32	>16	4	8	≤2	≤1	≤16	≤1
8	Sputum	17	NDM-1, TEM-1, CTX-M-15, CMY-4, SHV-1, *qnrS*	>16/8	>16/8	>64	>16	>32	>32	>16	≥8	>8	≤2	≤1	≤16	≤1

[a] AMC, amoxicillin-clavulanic acid; SAM, ampicillin-sulbactam; PIP, piperacillin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxon; FEP, cefepime; IPM, imipenem; MEM, meropenem; LE, levofloxacin; CI, ciprofloxacin; AM, amikacin; GE, gentamicin.

tal ward but spreading to other departments, including the intensive care unit, neurology ward, and pediatrics ward. Additionally, the bla_{NDM-1} gene was identified in *Escherichia coli*, *Enterobacter cloacae*, and *Citrobacter* isolates. It is uncertain whether the latter NDM-1-producing isolates were related to the neonatal ward or were imported from the outside. There were reports on NDM-producing bacteria originating from food animals (13), which indicated a reservoir in the community and connection to animals in China. The progenitor of the NDM-1 remains undefined. In its worrisome situation, the hospital implemented transmission-based precautions and enhanced environmental cleaning in order to prevent expanded spread. A new study has found that the human gut microbiota is a reservoir of antibiotic resistance genes, but little is known about their diversity and richness within the gut (14). Further studies are needed to confirm whether NDM-1 is spread from bacteria in the human gut due to excessive use of antibiotics. These findings highlight that epidemiological surveillance of multidrug-resistant microorganisms is indispensable for implementing appropriate interventions.

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REFERENCES

1. **Sidjabat H, Nimmo GR, Walsh TR, Binotto E, Htin A, Hayashi Y, Li J, Nation RL, George N, Paterson DL.** 2011. Carbapenem resistance in *Klebsiella pneumoniae* due to the New Delhi metallo-beta-lactamase. Clin Infect Dis **52:**481–484. http://dx.doi.org/10.1093/cid/ciq178.
2. **Kumarasamy KK, Toleman MA, Walsh TR, Baqaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M.** 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis **10:**597–602. http://dx.doi.org/10.1016/S1473-3099(10)70143-2.
3. **Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR.** 2009. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother **53:**5046–5054. http://dx.doi.org/10.1128/AAC.00774-09.
4. **Bushnell G, Mitrani-Gold F, Mundy LM.** 2013. Emergence of New Delhi metallo-β-lactamase type 1-producing Enterobacteriaceae and non-Enterobacteriaceae: global case detection and bacterial surveillance. Int J Infect Dis **17:**e325–e333. http://dx.doi.org/10.1016/j.ijid.2012.11.025.
5. **Roy S, Viswanathan R, Singh AK, Das P, Basu S.** 2011. Sepsis in neonates due to imipenem-resistant *Klebsiella pneumoniae* producing NDM-1 in India. J Antimicrob Chemother **66:**1411–1413. http://dx.doi.org/10.1093/jac/dkr068.
6. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing; 21st informational supplement. CLSI document M1-00-S21. CLSI, Wayne, PA.
7. **Yu Y, Ji S, Chen Y, Zhou W, Wei Z, Li L, Ma Y.** 2007. Resistance of strains producing extended-spectrum beta-lactamases and genotype distribution in China. J Infect **54:**53–57 http://dx.doi.org/10.1016/j.jinf.2006.01.014.
8. **Pérez-Pérez FJ, Hanson ND.** 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol **40:**2153–2162. http://dx.doi.org/10.1128/JCM.40.6.2153-2162.2002.
9. **Abou-Dobara MI, Devab MA, Elsawy EM, Mohamed HH.** 2010. Antibiotic susceptibility and genotype patterns of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from urinary tract infected patients. Pol J Microbiol **59:**207–212.
10. **Giske CG, Fröding I, Hasan CM, Turlej-Rogacka A, Toleman M, Livermore D, Woodford N, Walsh TR.** 2012. Diverse sequence types of *Klebsiella pneumoniae* contribute to the dissemination of bla_{NDM-1} in India, Sweden, and the United Kingdom. Antimicrob Agents Chemother **56:**2735–2738. http://dx.doi.org/10.1128/AAC.06142-11.
11. **Pasteran F, Albornoz E, Faccone D, Gomez S, Valenzuela C.** 2012. Emergence of NDM-1-producing *Klebsiella pneumoniae* in Guatemala. J Antimicrob Chemother **67:**1795–1797. http://dx.doi.org/10.1093/jac/dks101.
12. **Zou MX, Wu JM, Li J, Dou QY, Zhou RR, Huang Y, Liu WE.** 2012. NDM-1-producing *Klebsiella pneumoniae* in mainland China. Zhongguo Dang Dai Er Ke Za Zhi **14:**616–621. (In Chinese.)
13. **Wang Y, Wu C, Zhang Q, Qi J, Liu H, He T.** 2012. Identification of New Delhi metallo-β-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. PLoS One **7:**e37152. http://dx.doi.org/10.1371/journal.pone.0037152.
14. **Hu Y, Yang X, Qin J, Cheng G, Wu N.** 2013.Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. Nat Commun **4:**2151. http://dx.doi.org/10.1038/ncomms3151.