

# Extensively Drug-Resistant New Delhi Metallo- $\beta$ -Lactamase–Encoding Bacteria in the Environment, Dhaka, Bangladesh, 2012

Mark A. Toleman, Joachim J. Bugert,  
Syed A. Nizam

Carriage of the New Delhi metallo- $\beta$ -lactamase variant 1 (NDM-1) enables drug resistance to move between communities and hospitals. In Bangladesh, we found the *bla*<sub>NDM-1</sub> gene in 62% of environmental waters and in fermentative and nonfermentative gram-negative bacteria. *Escherichia coli* sequence type (ST) 101 was most commonly found, reflecting a common global relationship between ST101 and NDM-1.

Carbapenemases, bacterial enzymes that typically inactivate most of the  $\beta$ -lactam class of antimicrobial drugs, have emerged rapidly over the past decade (1). These resistance mechanisms are often accompanied by other resistance alleles, and together they can confer extensive drug resistance, leaving minimal treatment options (2). The New Delhi metallo- $\beta$ -lactamase variant 1 (NDM-1), a chimera formed by the fusion of 2 resistance genes, is unique among the carbapenemases (3). Since its description in 2009, NDM-1 has spread rapidly to many countries worldwide and appears to be endemic in South Asia (1,4,5). A study of the environment in New Delhi, India, showed that  $\approx$ 30% of surface waters and sewage was contaminated with NDM-1; the enzyme was also detected in drinking water (6). In addition, high rates of NDM-1 gut carriage have been found in the community and in hospitals in Pakistan (7). High rates of gut carriage can lead to contamination of drinking water and food through inadequate sewage treatment. Furthermore, gut carriage of NDM-1–encoding *Escherichia coli* can lead to common community-acquired infections (e.g., urinary tract infections), which often require hospitalization (8) and enable resistance mechanisms to move between community and hospital sectors. Indirect studies in 2009 and 2010 showed that NDM-1 was not present in the Bangladesh environment (9,10). To determine whether NDM-1 is now present in Bangladesh, we surveyed the environmental waters of Dhaka.

## The Study

During October 19–27, 2012, we collected environmental water/sewage samples from 7 regions (58 sites) in Dhaka,

Author affiliation: Cardiff University, Heath Park Campus, Cardiff, Wales, UK

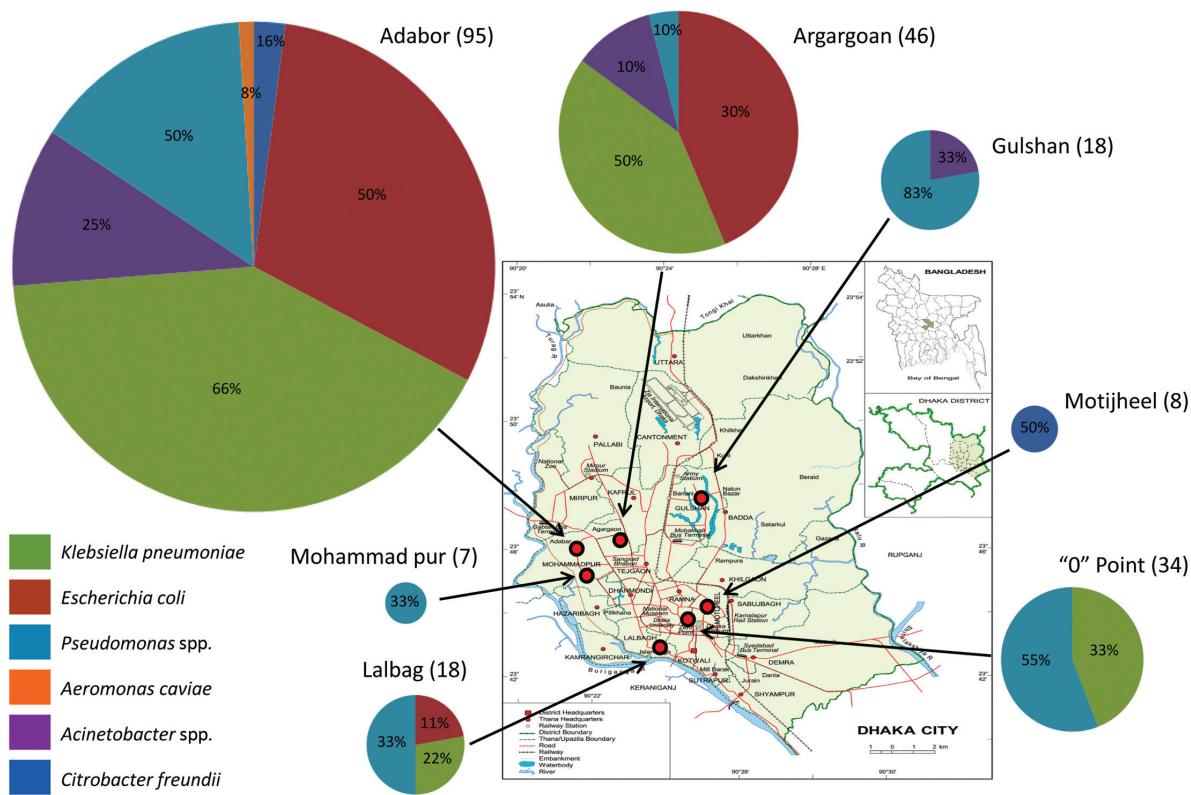
DOI: <http://dx.doi.org/10.3201/eid2106.141578>

Bangladesh (Figure 1). Control samples were from the United Kingdom. Each sample was investigated for bacterial growth on UTI brilliance agar plates (Thermo Fischer Scientific, Basingstoke, UK) containing vancomycin (30 mg/L) plus meropenem (0.5 mg/L). The species of individual colonies of different colors and morphologies were determined by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Bacteria were genetically characterized by *bla*<sub>NDM-1</sub>-specific PCR. Genetic location of the *bla*<sub>NDM-1</sub> gene was determined by probing S1 nuclease pulsed-field gels. A subset of isolates of each species was further investigated for MICs of relevant antimicrobial drugs. All *E. coli* isolates were genotyped to determine multilocus sequence typing group; examples of each group were characterized for additional relevant resistance mechanisms. Details are provided in the online Technical Appendix (<http://wwwnc.cdc.gov/EID/article/21/6/14-1578-Techapp1.pdf>).

The carbapenemase and extended-spectrum  $\beta$ -lactamase genes *bla*<sub>NDM-1</sub> and *bla*<sub>CTX-M-15</sub> were detected by PCR in 36 (62%) and 41 (71%), respectively, of the 58 water samples. Both genes were found at all 7 sample region sites in Dhaka. Gene *bla*<sub>CTX-M-15</sub>, but not *bla*<sub>NDM-1</sub>, was detected in sewage samples from the United Kingdom; neither was detected in UK water samples from the River Thames.

We identified 226 gram-negative NDM-1–producing isolates to the species level (Figure 1; online Technical Appendix Table 1); 15 isolates harboring *bla*<sub>NDM-1</sub> could not be identified and were not investigated further. The most widely disseminated bacteria in samples from Dhaka were pseudomonads (6/7 regions) and *Klebsiella pneumoniae* (4/7 regions). Nine different species of *Pseudomonas* spp. and 5 *Acinetobacter* spp., mostly belonging to nonpathogenic strains, were among the nonfermentative bacteria (online Technical Appendix Table 1). Carbapenem resistance in the *Pseudomonas* spp. isolates was unstable; all strains lost the *bla*<sub>NDM-1</sub> gene after 2 days' growth or when frozen for storage.

With the exception of 4 isolates, all bacterial isolates contained the original *bla*<sub>NDM-1</sub> allele; 3 *E. coli* sequence type (ST) 101 isolates carried the *bla*<sub>NDM-3</sub> variant, and 1 ST648 isolate carried the *bla*<sub>NDM-4</sub> variant (online Technical Appendix Table 2). S1 nuclease pulsed-field gel electrophoresis combined with *bla*<sub>NDM-1</sub> probes detected *bla*<sub>NDM-1</sub> on plasmids of limited size diversity in *E. coli* (ST101, 160 kb; ST405, 100 kb; ST648, 150 kb); however, other species included *bla*<sub>NDM-1</sub>–positive plasmids in



**Figure 1.** Diversity of New-Delhi metallo- $\beta$ -lactamase variant 1–encoding species and the number found in 58 locations in 7 regions (red circles on map) of Dhaka, Bangladesh, October 2012. Individual sampling sites were within 2 km of each sampling region, and the number of sites varied from 6 to 12 per region. Pie charts indicate the proportions of different  $bla_{NDM-1}$ –positive bacteria isolated in each region; colors indicate specific species. The diameter of each pie chart is directly proportional to the number of  $bla_{NDM-1}$ –positive isolates collected in each region; actual numbers are shown in parentheses after the region name. Numbers within pie charts indicate the percentage of sites in each region in which the individual positive  $bla_{NDM-1}$ –positive species were found.  $bla_{NDM-1}$  was detected in samples from all 7 regions and from 36 (62%) of the 58 sampling sites.

a wide diversity of sizes (30 kb–450 kb); some of these species had multiple positive plasmids, and  $bla_{NDM-1}$  was also found on the chromosome (online Technical Appendix Table 1 and Figure 1).

The *E. coli* isolates were further analyzed by PCR to identify additional resistance mechanisms often associated with  $bla_{NDM-1}$  (Table).  $bla_{CTX-M-15}$  and 16s ribosomal methylase genes (*armA* or *rmtB*) were associated with most *E. coli* strains, which explains the extensively drug-resistant phenotype of the *E. coli* isolates (online Technical Appendix Table 3). Plasmids of plasmid incompatibility groups *incFII* (ST101, ST405, ST648) and *incX* (ST405, ST648) were also closely associated with *E. coli* strains (Table). *E. coli* harboring  $bla_{NDM-1}$  were isolated from 10 sampling sites (Figure 1; online Technical Appendix Table 1). The *E. coli* isolates belonged to 3 different multilocus sequence typing groups: ST101 (phylogroup B1, 20/53 samples); ST405 (phylogroup D, 5/53 samples);

and ST648 (phylogroup D, 28/53 samples) (online Technical Appendix Table 2). ST101, which was found in samples from 6 (10.3%) of the 58 sites, was the most prevalent NDM-1–encoding *E. coli* genotype. ST648 represented an intermediate prevalence (5/58 [8.6%] sites), and ST405 was the least prevalent (1/58 [1.7%] sites) (online Technical Appendix Tables 1, 2).

## Conclusions

Our findings indicate that NDM-1 is widespread in the Dhaka environment. We detected 241 NDM-1–encoding bacterial isolates; they were found in all 7 sampled regions and at 36 (62%) of the 58 sampling sites. This high level of environmental  $bla_{NDM-1}$  contamination is of concern, especially because drinking water in Bangladesh usually carries high levels of sewage-derived bacteria (11). It is therefore likely that  $bla_{NDM-1}$  carriage rates will rise rapidly. Future environmental studies could provide

**Table.** Resistance genes and plasmid profiles for a subset of *Escherichia coli* strains in a study of extensively drug-resistant New Delhi metallo- $\beta$ -lactamase–encoding bacteria in the environment, Dhaka, Bangladesh, October 2012\*

<i>E. coli</i> strain, ST	Resistance genes								
	<i>bla</i> <sub>CTX-M-15</sub>	<i>bla</i> <sub>NDM</sub>	16S methylase	<i>bla</i> <sub>ampC</sub>	<i>incX</i>	<i>incFII</i>	<i>incL/M</i>	<i>incA/C</i>	<i>incN2</i>
18, ST101	+	NDM-3	<i>rmtB</i>	–	–	+	–	–	–
24, ST101	+	NDM-3	<i>rmtB</i>	–	–	+	–	–	–
25, ST101	+	+	<i>rmtB</i>	–	–	+	–	–	–
28, ST101	+	NDM-3	<i>rmtB</i>	–	–	+	–	–	–
221, ST101	+	NDM-1	<i>rmtB</i>	–	–	+	–	–	–
34, ST648	+	+	<i>armA</i>	<i>cmv, dha</i>	+	+	–	–	–
192, ST648	+	NDM-4	<i>armA</i>	<i>cmv, dha</i>	+	+	–	–	–
346, ST648	+	+	<i>armA</i>	<i>cmv, dha</i>	+	+	–	–	–
43, ST405	+	NDM-1	<i>armA</i>	<i>cmv, dha</i>	+	+	–	–	–
54, ST405	+	NDM-1	<i>armA</i>	<i>cmv, dha</i>	+	+	–	–	–

\**armA* and *rmtB*, aminoglycoside methylase genes; CTX-M-15, *cmv*, and *dha*,  $\beta$ -lactamases; *inc*, plasmid incompatibility group; NDM, New-Delhi metallo- $\beta$ -lactamase; ST, sequence type; –, negative; +, positive.

indicators of epidemics of emerging resistant bacteria before they are realized in hospitals.

Despite the widespread presence of NDM-1 in Dhaka, it appears that this carbapenemase has recently emerged in the Bangladesh environment. Studies in northern Bangladesh did not find NDM-1 in wild ducks and poultry in 2009 (9) or in crow and gull feces in 2010 (10). Similarly, NDM-1 was not detected in drinking water in Dhaka during 2008–2009 (11) even though all samples had high levels of fecal and *bla*<sub>CTX-M-15</sub> contamination. Furthermore, a study of 1,879 clinical *E. coli* and *Shigella* spp. isolates collected during 2009–2010 in Bangladesh did not detect *bla*<sub>NDM-1</sub> (12). The first known clinical isolates date from 2008 (12),

and the first evidence of human gut carriage of *bla*<sub>NDM-1</sub> was found in samples collected in Dhaka (13) a month before our study.

Because *E. coli* is the leading cause of human urinary tract infections, bloodstream infections, and neonatal meningitis, the ability of NDM-1 to give this bacterium clinical resistance to carbapenems is of concern (14). *E. coli* is also universally carried in the human gut. Therefore, we focused on this species because it is likely to be the greatest threat to human health. *E. coli* encoding NDM-1 were found in 3 of the 7 sampled regions, and genotyping showed they belonged to only 3 STs: ST648, ST101, and ST405. These same 3 *E. coli* genotypes are



**Figure 2.** Sites where New-Delhi metallo- $\beta$ -lactamase variant 1 (NDM-1)–encoding *Escherichia coli* sequence type (ST) 101 isolates have been detected worldwide. Stars indicate countries where NDM-encoding *E. coli* ST101 has been detected: Australia, Bangladesh, Belgium, Bulgaria, China, Canada, Denmark, France, Germany, India, Korea, New Zealand, Pakistan, the United Kingdom, and the United States.

responsible for 80% of clinical NDM-1–encoding *E. coli* isolates in the United Kingdom (15). Furthermore, ST101 is the most common *E. coli* genotype in the Bangladesh environment (10.3% prevalence) and in clinical isolates from the United Kingdom (50%). Results of a literature search for NDM-1–encoding *E. coli* belonging to ST101 showed that this genotype has been detected in 15 nations (Figure 2). Thus, *E. coli* ST101 appears to be a successful global genotype that is often associated with NDM-1. This association with a single global genotype is analogous to the association between *E. coli* ST131 and the cephalosporinase CTX-M-15. Because of the critical nature of extensively drug-resistant bacteria, we are investigating the underlying factors responsible for the success of these particular antimicrobial drug-resistant strains.

This work was funded by grants from the National Institute for Social Care and Health Research (grant no. HF-11-24) and from the Medical Research Council (grant no. G1100135).

Dr. Toleman is a senior lecturer at Cardiff University. His recent work includes the discovery of the *ISCR* (insertion sequence common region) elements, NDM-1, and the formation of NDM-1 by an unusual genetic fusion event.

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Address for correspondence: Mark A. Toleman, Medical Microbiology, Department of Infection and Immunity, Cardiff University, Rm 179, Main Bldg, Heath Park Campus, Cardiff, Wales, UK: email: [Tolemanma@Cardiff.ac.uk](mailto:Tolemanma@Cardiff.ac.uk)



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# Extensively Drug-Resistant New Delhi Metallo- $\beta$ -Lactamase–Encoding Bacteria in the Environment, Bangladesh, October 2012

## Technical Appendix

### Supplemental Methods

#### Sample Collection and Transport

The samples included surface waters, water from a lake, gray water from residences and sewage samples all from Dhaka city, Bangladesh. Sample sites included the Motijheel, Agargaon, Gulshan, Adabor, Lalbag, “0” point and Mohammad Pur areas of Dhaka city and included 8-10 samples within a 2 kilometer radius of each location. Each sample consisted of about 100  $\mu$ l of liquid absorbed onto a sterile charcoal swab and was collected by dipping the sterile swab in the water source. Samples were transported in UN 3373 approved sealed containers by personal courier to Cardiff, UK. Control water samples were collected from sewage processing plants in South Wales (Nash, Ponthir, Weycock, Coslech, Cogmoors, Creigau, New Drope, Cowbridge, East Bon and Rhydylafar) and the river Thames. All sewage samples from the UK were influent samples before any sewage processing had taken place.

#### Initial Gene Detection

The NDM-1 gene (*bla*<sub>NDM-1</sub>) and the CTX-M-15 gene (*bla*<sub>CTX-M-15</sub>) were initially detected by PCR directly from the swab using 1  $\mu$ l of sample water squeezed from the swab as template and standard primers and conditions.

#### Bacterial Selection, Identification and MIC Determination

Each swab was streaked on UTI brilliance agar plates containing vancomycin (30 mg/L) plus meropenem (0.5 mg/L) and grown overnight at 37°C. Following incubation, bacterial growth was collected by sweeping a sterile loop several times across the plate ensuring that colonies of many different colors and morphologies were collected. The bacterial culture was

transferred to 0.5 ml of sterile water in an Eppendorf tube and resuspended. One  $\mu$ l of this bacterial suspension was then used for PCR reactions to detect the NDM-1 gene (*bla*<sub>NDM-1</sub>) and the CTX-M-15 gene (*bla*<sub>CTX-M-15</sub>) and the rest stored at 4°C. Samples that were positive for *bla*<sub>NDM-1</sub> in the initial PCR were then re-plated at several dilutions to ensure well-spaced colonies. From these plates at least twenty further well-spaced colonies were re-grown and individually investigated by PCR and sequencing for the presence of *bla*<sub>NDM-1</sub>. These represented a range of colonies of different color and morphology to ensure detection of as many different bacterial NDM-1 hosts at each site as possible.

Positive individual cultures were again streaked on brilliance agar plates to confirm purity and then speciated by Maldi-TOF using the Bruker MALDI Biotyper system, Microflex LT and Biotyper 3.0 software (Bruker Daltonics, Germany) and NDM presence re-confirmed by PCR. Isolates of each species were tested for MICs against several antibiotics (ETest, bioMérieux, Basingstoke, UK. Liofilchem, Roseto degli Abruzzi, Italy) on Mueller-Hinton plates (Becton Dickinson, Oxford, UK) and the results determined according to the manufacturer's instructions.

#### **NDM Allele**

Approximately 10 positive PCR products from each genus including duplicate examples from each individual species were sequenced to confirm the accuracy of the PCR reaction and also to determine the NDM variant present (these were amplified using primers NDMVF-TGGCTTTTGAAACTGTCGCACC and NDMVR-CTGTACATCGAAATCGCGCGA designed up and downstream of the gene to ensure the entire gene was sequenced).

#### **Gene Location**

The location of the *bla*<sub>NDM-1</sub> gene in each bacterial isolate was determined by pulsed field gel electrophoresis (PFGE) following S1 digestion of macro DNA in agarose plugs. The genomic location was detected using in-gel hybridization with a P<sup>32</sup>-labeled *bla*<sub>NDM-1</sub> probe at 65°C as described previously.

#### **Additional Resistance Alleles**

*E. coli* *bla*<sub>NDM-1</sub> positive isolates (10) collected from a range of sites that were positive for these species were further investigated for additional resistance alleles that are often found

associated with *bla*<sub>NDM-1</sub> including the 16S ribosomal methylase genes *armA*, *rmtB*, *rmtC*, *rmtF*, *ampC* genes and *bla*<sub>CTX-M</sub> genes by multiplex PCR using published primers and conditions.

### **Common NDM Plasmid Types**

Plasmid types that are commonly associated with *bla*<sub>NDM-1</sub> were investigated by PCR in the above *E. coli* isolates using the custom designed primer pairs itemized below. These were designed to amplify plasmid backbone genes from NDM harboring plasmids available in the genetic data bases and amplified using relevant controls.

*incN2* F/R-ACTCACGTTTCGCTGGATTT and GCCACCCTTAACCTGTTCGA;  
JQ349085

*incFII* F/R-TGCAGAGTTCGCTGCCGGTG and TACGCCCGGCATCTCCCACA;  
HG003695

*incL/M* F/R GGTCAGCACCGTTGACCGGG and CGTCTTTCGGCAGCGTCCGT;  
JX988621

*incX* F/R ATGGGCGCATCTTTTTGCGAAGGA and  
TTTCCTGTCGCTCAGGACTTCA; JX104760

*incAC* F/R GAGAACCAAAGACAAAGACCTGGA and  
TTCTGGAGTTCGTACAGAGTGAAC. JF503991

### ***E. coli* Phylogeny**

The 53 *bla*<sub>NDM-1</sub> positive *E. coli* isolates collected in this study were further investigated for phylogenetic grouping using the recently updated (2013) Clermont multiplex PCR method and also by the high resolution 2 loci clonal EXPEC typing method, using the described primers and conditions and applicable sequencing. Several examples of each different group determined by these methods were also subjected to full multi-locus sequence typing using the MLST *E. coli* typing system, primers and conditions described at the university of Warwick <http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>.

## Supplemental Results

### Initial Species Identification

Initial species identification was by colony color on brilliance agar plates. Swabs from each site were plated out on individual plates. Clear colony types >100 were found on all plates, indicative of carbapenem resistant non-fermentative bacteria being found at all sites. Red colonies indicative of *E. coli* were found on 11 plates varying in number from 1 or 2 colonies to 50-80 per plate. Dark blue colonies suggestive of *K. pneumoniae* were found on 19 plates and varied in number from 1 or 2 to more than 100 per plate.

### Comparison of *E. coli* Phylogeny Methods

The 2 locus clonal EXPEC typing method correctly identified all isolates belonging to ST101 and ST405 whereas the Clermont method was inaccurate for many of the strains analyzed placing ST648 and ST405 (phylogroup D) as B2 as well as half of the ST101 (phylogroup B1) as phylogroup E (Technical Appendix Table 2). Interestingly, all ST648 phylogroup D *E. coli* strains were *fimH* negative and *fumC* allele 4 (Technical Appendix Table 2). Only a single phylogroup B1 strain was present with this 2 locus designation in the database and so this system incorrectly identified this ST648 as phylogroup B1 instead of D.

### Resistance Profile of *bla*<sub>NDM-1</sub> Bacterial Species

Overall the bacterial isolates displayed antibiotic resistance profiles that are very similar to clinical isolates that carry *bla*<sub>NDM-1</sub> (Technical Appendix Table 3). The *E. coli* and *K. pneumoniae* isolates were resistant to all  $\beta$ -lactams with the single exception of one *K. pneumoniae* strain that was sensitive to Aztreonam. They were also resistant to all clinical aminoglycosides and only sensitive to fosfomycin, tigecycline and colistin reflecting the additional presence of 16s RNA methylases in all strains classifying the majority of these isolates as XDR. In general, the MICs to meropenem were higher than those to imipenem, validating the inclusion of meropenem in the initial screening. The various *Acinetobacter* spp. and *Citrobacters* were generally less resistant, several being sensitive to aminoglycosides and occasionally ciprofloxacin, the *Acinetobacters* were additionally generally sensitive to rifampicin.

**Technical Appendix Table 1.** Isolate list/NDM plasmid profile\*

Area/site, isolate no.	Species	NDM plasmid size, kb,
<b>MOT/1.1</b>		
1	<i>Citrobacter freundii</i>	50, 250
177	<i>C. freundii</i>	50, 250
<b>MOT/1.2</b>		
5	<i>C. freundii</i>	50, 250
<b>MOT/1.3</b>		
13	<i>C. freundii</i>	50, 250
14	<i>C. freundii</i>	50, 250
16	<i>C. freundii</i>	50, 250
179	<i>C. freundii</i>	50, 250
180	<i>C. freundii</i>	50, 250
<b>ARG/2.1</b>		
17	<i>Escherichia coli</i>	150
18	<i>E. coli</i>	150
19	<i>E. coli</i>	150
20	<i>E. coli</i>	150
23	<i>E. coli</i>	150
24	<i>E. coli</i>	150
25	<i>E. coli</i>	150
83	<i>Acinetobacter tandoii</i>	240
84	<i>A. tandoii</i>	ND
86	<i>Klebsiella pneumoniae</i>	50,120,180,250
87	<i>K. pneumoniae</i>	100,180,250
89	<i>K. pneumoniae</i>	100
93	<i>Acinetobacter genomospecies</i>	240
94	<i>A. genomospecies</i>	ND
95	<i>K. pneumoniae</i>	100
97	<i>K. pneumoniae</i>	100
98	<i>Acinetobacter towneri</i>	ND
<b>ARG/2.2</b>		
27	<i>E. coli</i>	150
28	<i>E. coli</i>	150
29	<i>E. coli</i>	150
30	<i>E. coli</i>	150
31	<i>E. coli</i>	150
32	<i>E. coli</i>	150
33	<i>E. coli</i>	150
34	<i>E. coli</i>	150
<b>ARG/2.6</b>		
35	<i>K. pneumoniae</i>	100
36	<i>K. pneumoniae</i>	100
<b>ARG/2.7</b>		
43	<i>E. coli</i>	100
44	<i>E. coli</i>	100
45	<i>E. coli</i>	100
46	<i>E. coli</i>	100
47	<i>K. pneumoniae</i>	100
49	<i>K. pneumoniae</i>	100
50	<i>K. pneumoniae</i>	100
51	<i>K. pneumoniae</i>	50, 250
54	<i>E. coli</i>	100
59	<i>K. pneumoniae</i>	145,160
182	<i>K. pneumoniae</i>	145,160
183	<i>Pseudomonas putida</i>	ND
184	<i>K. pneumoniae</i>	50, 250
185	<i>Pseudomonas alcaligenes</i>	ND
<b>ARG/2.8</b>		
186	<i>K. pneumoniae</i>	145,160
69	<i>K. pneumoniae</i>	145,160
70	<i>K. pneumoniae</i>	145,160
<b>ARG/2.9</b>		
73	<i>K. pneumoniae</i>	120
80	<i>K. pneumoniae</i>	120
<b>GUL/3.1</b>		
99	<i>A. towneri</i>	50
100	<i>A. towneri</i>	ND
<b>GUL/3.2</b>		

Area/site, isolate no.	Species	NDM plasmid size, kb,
103	<i>Pseudomonas</i> spp.	ND
105	<i>P. putida</i>	ND
107	<i>A. tandoii</i>	50
108	<i>A. tandoii</i>	ND
GUL/3.3		
109	<i>Pseudomonas</i> spp.	ND
112	<i>P. putida</i>	ND
116	<i>P. putida</i>	ND
118	<i>P. putida</i>	ND
120	<i>Pseudomonas otitidis</i>	ND
GUL/3.4		
121	<i>P. putida</i>	ND
125	<i>P. otitidis</i>	ND
128	<i>P. putida</i>	ND
GUL/3.5		
138	<i>P. otitidis</i>	ND
GUL/3.6		
144	<i>P. putida</i>	ND
146	<i>P. otitidis</i>	ND
147	<i>Pseudomonas monteillii</i>	ND
ADA/4.3		
151	<i>K. pneumoniae</i>	120
153	<i>K. pneumoniae</i>	120
155	<i>A. townneri</i>	ND
156	<i>K. pneumoniae</i>	100, 350
158	<i>K. pneumoniae</i>	100, 350
159	<i>P. aeruginosa</i>	ND
160	<i>A. townneri</i>	ND
ADA/4.4		
161	<i>Acinetobacter baumannii</i>	250, 450
162	<i>Pseudomonas mendocina</i>	ND
163	<i>A. genomospecies</i>	280
165	<i>A. genomospecies</i>	280
166	<i>P. mendocina</i>	ND
167	<i>K. pneumoniae</i>	ND
170	<i>A. genomospecies</i>	ND
174	<i>A. genomospecies</i>	ND
175	<i>A. genomospecies</i>	ND
176	<i>A. genomospecies</i>	ND
ADA/4.5		
188	<i>E. coli</i>	150
189	<i>E. coli</i>	150
190	<i>K. pneumoniae</i>	45, 150, 250
191	<i>E. coli</i>	130
192	<i>E. coli</i>	130
193	<i>E. coli</i>	130
194	<i>E. coli</i>	130
195	<i>E. coli</i>	130
197	<i>K. pneumoniae</i>	45, 150, 250
199	<i>K. pneumoniae</i>	45, 150, 250
200	<i>E. coli</i>	270
201	<i>K. pneumoniae</i>	100, 350
202	<i>Pseudomonas monteillii</i>	ND
204	<i>P. putida</i>	ND
205	<i>K. pneumoniae</i>	100, 350
ADA/4.6		
207	<i>K. pneumoniae</i>	50, 100, 250
208	<i>E. coli</i>	100
209	<i>K. pneumoniae</i>	100, 300
210	<i>K. pneumoniae</i>	50, 120, 140
213	<i>E. coli</i>	140
214	<i>E. coli</i>	140
211	<i>E. coli</i>	130
212	<i>K. pneumoniae</i>	50, 120, 140
216	<i>K. pneumoniae</i>	50, 120, 140
217	<i>K. pneumoniae</i>	120, 200
218	<i>K. pneumoniae</i>	120, 200

Area/site, isolate no.	Species	NDM plasmid size, kb,
219	<i>K. pneumoniae</i>	80, 130, 150
220	<i>P. putida</i>	ND
ADA/4.9		
221	<i>E. coli</i>	130
222	<i>E. coli</i>	130
225	<i>P. putida</i>	ND
226	<i>P. putida</i>	ND
227	<i>P. putida</i>	ND
228	<i>C. freundii</i>	50, 250
229	<i>K. pneumoniae</i>	80, 130, 150
230	<i>K. pneumoniae</i>	150, 170, 300
231	<i>K. pneumoniae</i>	150, 170, 300
233	<i>K. pneumoniae</i>	50, 150, 200
236	<i>P. mendocina</i>	ND
ADA/4.10		
237	<i>Pseudomonas fulva</i>	ND
238	<i>Pseudomonas aeruginosa</i>	ND
239	<i>E. coli</i>	130
241	<i>K. pneumoniae</i>	50, 150, 200
242	<i>P. aeruginosa</i>	ND
243	<i>C. freundii</i>	50, 250
245	<i>Aeromonas caviae</i>	ND
249	<i>P. aeruginosa</i>	ND
ADA/4.11		
250	<i>E. coli</i>	130
251	<i>E. coli</i>	130
252	<i>E. coli</i>	130
253	<i>E. coli</i>	130
254	<i>E. coli</i>	130
255	<i>E. coli</i>	130
256	<i>E. coli</i>	130
257	<i>E. coli</i>	130
258	<i>K. pneumoniae</i>	110
259	<i>K. pneumoniae</i>	110
260	<i>K. pneumoniae</i>	150
261	<i>K. pneumoniae</i>	150
262	<i>A. baumannii</i>	ND
264	<i>K. pneumoniae</i>	150
265	<i>K. pneumoniae</i>	150
266	<i>K. pneumoniae</i>	150
267	<i>K. pneumoniae</i>	120
ADA/4.12		
268	<i>E. coli</i>	130
269	<i>E. coli</i>	130
271	<i>E. coli</i>	140
273	<i>E. coli</i>	140
274	<i>E. coli</i>	130
275	<i>E. coli</i>	130
276	<i>K. pneumoniae</i>	120
277	<i>K. pneumoniae</i>	120
278	<i>K. pneumoniae</i>	120
279	<i>K. pneumoniae</i>	120
280	<i>K. pneumoniae</i>	120
281	<i>K. pneumoniae</i>	120
282	<i>K. pneumoniae</i>	ND
283	<i>K. pneumoniae</i>	ND

Area/site, isolate no.	Species	NDM plasmid size, kb,
LAL/5.1		
342	<i>K. pneumoniae</i>	ND
343	<i>K. pneumoniae</i>	ND
344	<i>K. pneumoniae</i>	ND
345	<i>K. pneumoniae</i>	ND
346	<i>E. coli</i>	130
347	<i>E. coli</i>	130
348	<i>E. coli</i>	120
349	<i>E. coli</i>	140
LAL/5.2		
352	<i>P. putida</i>	ND
355	<i>P. mendocina</i>	ND
358	<i>K. pneumoniae</i>	ND
360	<i>P. mendocina</i>	ND
361	<i>P. oleovorans</i>	ND
362	<i>P. oleovorans</i>	ND
LAL/5.7		
363	<i>P. oleovorans</i>	ND
364	<i>Pseudomonas pseudoalcaligenes</i>	ND
LAL/5.8		
367	<i>P. fulva</i>	ND
368	<i>P. fulva</i>	ND
369	ND	ND
370	ND	ND
0-P/6.2		
287	<i>P. monteilii</i>	ND
288	<i>P. putida</i>	ND
289	<i>P. putida</i>	ND
291	<i>P. putida</i>	ND
292	<i>P. putida</i>	ND
0-P/6.3		
293	<i>K. pneumoniae</i>	ND
294	<i>K. pneumoniae</i>	ND
295	<i>P. putida</i>	ND
296	<i>K. pneumoniae</i>	ND
297	<i>K. pneumoniae</i>	ND
298	<i>K. pneumoniae</i>	ND
299	<i>P. otitidis</i>	ND
300	<i>P. otitidis</i>	ND
301	<i>P. otitidis</i>	ND
303	<i>P. putida</i>	ND
304	<i>P. putida</i>	ND
305	<i>P. putida</i>	ND
306	ND	ND
0-P/6.4		
307	<i>K. pneumoniae</i>	ND
308	<i>K. pneumoniae</i>	ND
309	ND	ND
310	<i>P. mendocina</i>	ND
311	ND	ND
312	ND	ND
313	<i>P. otitidis</i>	ND

Area/site, isolate no.	Species	NDM plasmid size, kb,
314	ND	ND
315	<i>P. putida</i>	ND
316	<i>P. putida</i>	ND
0-P/6.8		
323	<i>K. pneumoniae</i>	ND
324	<i>K. pneumoniae</i>	ND
325	<i>K. pneumoniae</i>	ND
326	<i>K. pneumoniae</i>	ND
327	<i>K. pneumoniae</i>	ND
328	ND	ND
330	<i>K. pneumoniae</i>	ND
331	ND	ND
332	ND	ND
333	ND	ND
335	<i>Pseudomonas oleovorans</i>	ND
0-P/6.9		
336	<i>P. otitidis</i>	ND
337	<i>P. otitidis</i>	ND
338	ND	ND
339	ND	ND
MOH/7.5		
340	ND	ND
341	ND	ND
371	<i>P. mendocina</i>	ND
372	<i>P. pseudoalcaligenes</i>	ND
MOH/7.6		
374	<i>P. oleovorans</i>	ND
375	<i>P. mendocina</i>	ND
376	<i>P. oleovorans</i>	ND
377	<i>P. pseudoalcaligenes</i>	ND
378	<i>P. oleovorans</i>	ND

\*Arg, Argargoan; Gul, gulshan; Lal, Lalbag; Moh, Mohammad pur; Mot, Motijheel; ND, not determined; NDM, New Delhi metallo- $\beta$ -lactamase; 0-p, 0 point (Dhaka city regions).

**Technical Appendix Table 2.** Phylogenetic analysis of NDM-positive *Escherichia coli* from the environment, Bangladesh\*

Strain no.	NDM	Site	Area in Bangladesh	Clermont strain typing analysis				2 -loci typing analysis			MLST, true designation
				<i>chuA</i> PCR	<i>yjaA</i> PCR	<i>tspE</i> PCR	Apparent designation	<i>fimH</i> allele	<i>fumC</i> allele	Designation by 2 loci	
17	+	2.1	Argargoan		+	+	B2	258	41	B1	B1-ST101
18	NDM-3	2.1	Argargoan	+			E	258	41	B1	B1-ST101
19	+	2.1	Argargoan	+			E	258	41	B1	B1-ST101
20	+	2.1	Argargoan	+			E	258	41	B1	B1-ST101
23	+	2.1	Argargoan	+			E	258	41	B1	B1-ST101
24	NDM-3	2.1	Argargoan	+		+	B2	258	41	B1	B1-ST101
25	+	2.1	Argargoan	+			E	258	41	B1	B1-ST101
27	+	2.2	Argargoan	+			E	258	41	B1	B1-ST101
28	NDM-3	2.2	Argargoan	+		+	E	258	41	B1	B1-ST101
29	+	2.2	Argargoan	+			E	258	41	B1	B1-ST101
30	+	2.2	Argargoan	+		+	B2	258	41	B1	B1-ST101
31	+	2.2	Argargoan	+		+	B2	258	41	B1	B1-ST101
32	+	2.2	Argargoan	+			E	258	41	B1	B1-ST101
33	+	2.2	Argargoan	+			E	258	41	B1	B1-ST101
34	+	2.2	Argargoan	+		+	B2	258	41	B1	B1-ST101
43	NDM-1	2.7	Argargoan	+	+	+	B2	37	27	D	D-ST405
44	+	2.7	Argargoan	+	+	+	B2	37	27	D	D-ST405
45	+	2.7	Argargoan	+	+	+	B2	37	27	D	D-ST405
46	+	2.7	Argargoan	+	+	+	B2	37	27	D	D-ST405
54	NDM-1	2.7	Argargoan	+	+	+	B2	37	27	D	D-ST405
188	+	4.5	Adabor	+		+	B2	Null	4	B1	D-ST648
189	+	4.5	Adabor	+		+	B2	Null	4	B1	D-ST648
191	+	4.5	Adabor	+		+	B2	Null	4	B1	D-ST648
192	NDM-4	4.5	Adabor	+		+	B2	Null	4	B1	D-ST648
193	+	4.5	Adabor	+		+	B2	Null	4	B1	D-ST648
194	+	4.5	Adabor	+		+	B2	Null	4	B1	D-ST648
195	+	4.5	Adabor	+	+	+	B2	Null	4	B1	D-ST648

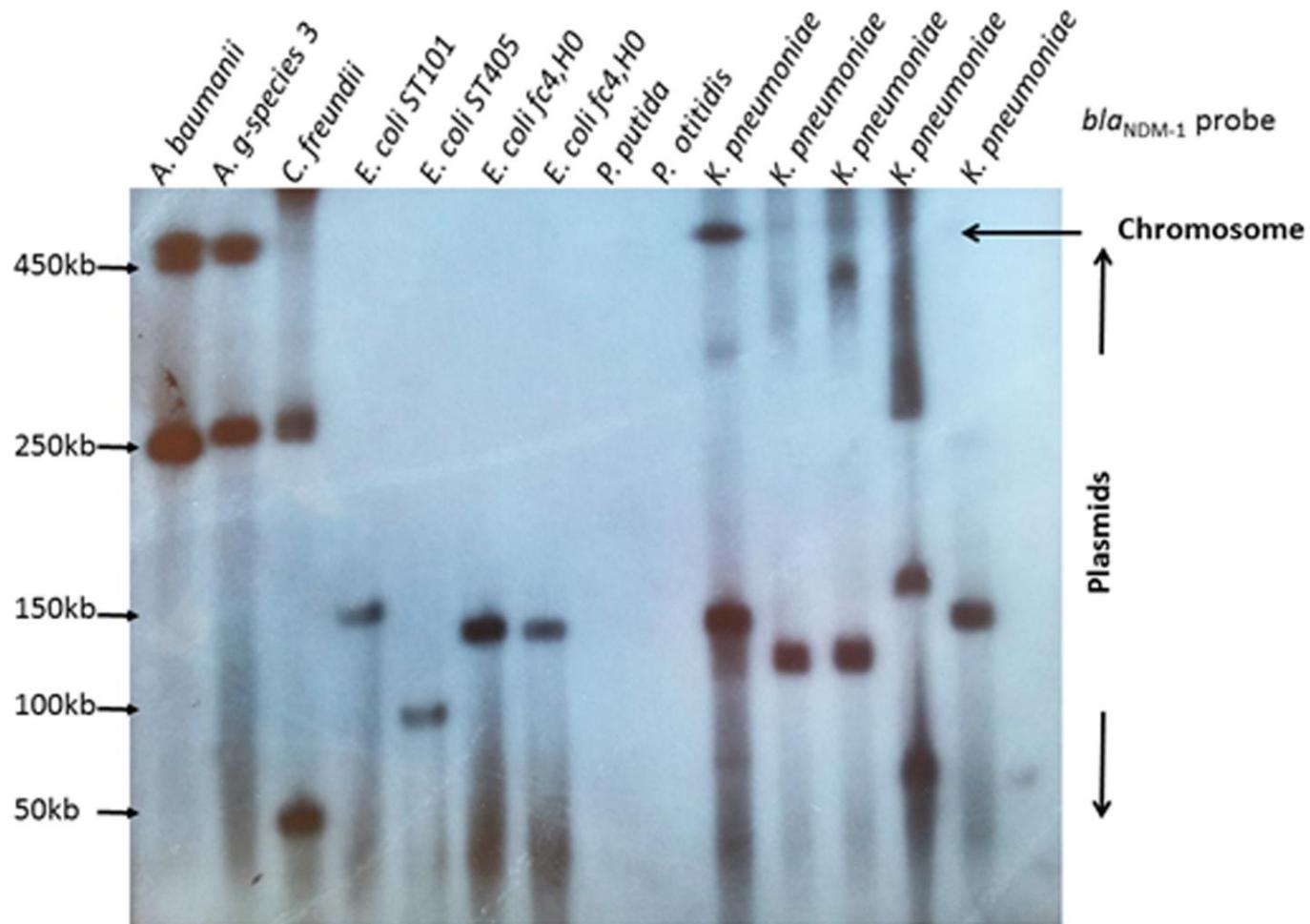
200	+	4.5	Adabor		+	+	B2	258	41	B1	B1-ST101
208	+	4.6	Adabor	+		+	B2	Null	4	B1	D-ST648
211	+	4.6	Adabor	+		+	B2	Null	4	B1	D-ST648
213	+	4.6	Adabor	+	+	+	B2	Null	4	B1	D-ST648
214	+	4.6	Adabor	+		+	B2	Null	4	B1	D-ST648
221	NDM-1	4.9	Adabor		+	+	B2	258	41	B1	B1-ST101
222	+	4.9	Adabor		+		E	258	41	B1	B1-ST101
239	+	4.10	Adabor		+	+	B2	258	41	B1	B1-ST101
250	+	4.11	Adabor	+	+	+	B2	Null	4	B1	D-ST648
251	+	4.11	Adabor	+	+	+	B2	Null	4	B1	D-ST648
252	+	4.11	Adabor	+	+	+	B2	Null	4	B1	D-ST648
253	+	4.11	Adabor	+	+	+	B2	Null	4	B1	D-ST648
254	NDM-1	4.11	Adabor	+	+	+	B2	Null	4	B1	D-ST648
255	+	4.11	Adabor	+	+	+	B2	Null	4	B1	D-ST648
256	+	4.11	Adabor	+	+	+	B2	Null	4	B1	D-ST648
257	+	4.11	Adabor	+	+	+	B2	Null	4	B1	D-ST648
268	+	4.12	Adabor	+	+	+	B2	Null	4	B1	D-ST648
269	+	4.12	Adabor	+	+	+	B2	Null	4	B1	D-ST648
271	NDM-1	4.12	Adabor			+	<i>E. albertii</i>	258	41	B1	B1-ST101
273	+	4.12	Adabor	+	+	+	B2	Null	4	B1	D-ST648
274	+	4.12	Adabor				?	Null	4	B1	D-ST648
275	+	4.12	Adabor	+	+	+	B2	Null	4	B1	D-ST648
346	+	5.1	Lalbag	+	+	+	B2	Null	4	B1	D-ST648
347	NDM-1	5.1	Lalbag	+	+	+	B2	Null	4	B1	D-ST648
348	+	5.1	Lalbag	+	+	+	B2	Null	4	B1	D-ST648
349	+	5.1	Lalbag	+	+	+	B2	Null	4	B1	D-ST648

\*NDM, New Delhi-metallo-β-lactamase; MLST, multilocus sequence typing.

**Technical Appendix Table 3.** MICs of various antibiotics to a subset of individual NDM encoding environmental bacteria\*

Genus sp., ST, strain no.	Antimicrobial drug																			
	IMP	MEM	ERT	PIP	AMX/CLA	PIP/TAZ	AMP	CAZ/CLA	CTX/CLA	FEP/CLA	ATM	AMK	TOB	GEN	CIP	TGC	FOF	NIT	CST	RIF
<i>Escherichia coli</i>																				
ST101																				
18	4	12	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.19	2	>512	1	ND
24	12	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.25	1.5	>512	0.25	ND
25	4	16	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.5	24	192	1	ND
28	>32	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.25	1.5	>512	0.75	ND
221	6	6	12	>32	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.38	2	>512	1	ND
ST648																				
40	6	12	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.19	2	48	1	ND
192	12	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.38	1	192	1	ND
346	4	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.25	1	>256	1	ND
ST405																				
43	8	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.38	3	48	1	ND
54	6	12	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.19	2	48	1	ND
<i>Citrobacter freundii</i>																				
1	4	6	6	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	12	0.5	>32	1	2	>512	0.75	32
5	2	3	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	12	0.38	>32	0.75	3	16	1	32
172	4	16	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	12	0.38	>32	1	2	24	0.75	16
228	2	4	8	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	12	1.5	3	1	48	16	0.5	24
243	6	6	6	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	12	0.75	3	1	2	16	1	24
<i>Klebsiella pneumoniae</i>																				
35	8	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.5	16	>512	0.38	ND
49	>32	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.75	12	384	0.5	ND
70	6	6	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.5	12	>512	1	ND
86	8	6	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.5	12	>512	0.75	ND
151	>32	12	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	0.25	>256	ND	>32	64	16	>512	2	ND
182	8	24	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.5	12	>512	1	ND
201	3	8	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	6	0.5	6	96	0.75	ND
297	>32	16	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	12	1	4	384	0.5	ND
323	6	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.75	16	>512	0.38	ND
325	12	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	>256	ND	>32	0.75	16	>512	0.75	ND
<i>Acinetobacter towneri</i>																				
83	32	16	>32	64	>256	32	>256	>32/>4	>16/>1	>16/>4	>256	>256	3	48	0.25	0.19	12	12	0.5	1.5
93	>32	>32	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	256	192	>32	0.5	32	32	0.75	16
<i>Actinobacillus genomosp.</i>																				
98	>32	>32	>32	96	>256	48	>256	>32/>4	>16/>1	>16/>4	>256	>256	0.38	0.19	0.5	0.094	8	8	0.75	1.5
155	>32	>32	>32	96	>256	24	>256	>32/>4	>16/>1	>16/>4	>256	>256	1.5	24	32	0.125	8	8	0.5	1.5
<i>Acinetobacter tandoii</i>																				
161	>32	>32	>32	>256	>256	64	>256	>32/>4	>16/>1	>16/>4	>256	>256	12	192	>32	0.19	32	32	0.75	4
<i>A. baumannii</i>																				
174	>32	>32	>32	96	>256	32	>256	>32/>4	>16/>1	>16/>4	>256	>256	6	128	0.19	0.125	32	32	0.75	2
262	32	16	>32	>256	>256	>256	>256	>32/>4	>16/>1	>16/>4	>256	>256	256	256	>32	0.75	64	64	1	6

\*AMK, amikacin; AMP, ampicillin; AMX, amoxicillin; ATM, aztreonam; CAZ, Ceftazidime; CIP, ciprofloxacin; CLA, clavulanic acid; CST, colistin; CTX, cefotaxime; ERT, ertapenem; FEP, cefipime; FOF, fosfomicin; GEN, gentamicin; IMP, imipenem; MEM, meropenem; ND, not determined; NDM, New Delhi-metallo-β-lactamase; NIT, nitrofurantoin; PIP, piperacillin; RIF, rifampin; ST, sequence type; TAZ, tazobactam; TGC, tigecycline; TOB, tobramycin



**Technical Appendix Figure.** Genomic location of *bla*<sub>NDM-1</sub> in environmental bacteria from Dhaka, Bangladesh. Pulsed field gel electrophoresis of macro DNA of a subset of *bla*<sub>NDM-1</sub>-harboring strains collected from environmental waters in Dhaka. The gel was probed with a radio-labeled *bla*<sub>NDM-1</sub> probe and detected by using photographic film. Samples were run against a concatenated  $\lambda$  molecular size standard.