Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study

Timothy R Walsh, Janis Weeks, David M Livermore, Mark A Toleman

Summary

Background Not all patients infected with NDM-1-positive bacteria have a history of hospital admission in India, and extended-spectrum β-lactamases are known to be circulating in the Indian community. We therefore measured the prevalence of the NDM-1 gene in drinking water and seepage samples in New Delhi.

Methods Swabs absorbing about 100 μL of seepage water (ie, water pools in streets or rivulets) and 15 mL samples of public tap water were collected from sites within a 12 km radius of central New Delhi, with each site photographed and documented. Samples were transported to the UK and tested for the presence of the NDM-1 gene, blaNDM-1, by PCR and DNA probing. As a control group, 100 μL sewage effluent samples were taken from the Cardiff Wastewater Treatment Works, Tremorfa, Wales. Bacteria from all samples were recovered and examined for blaNDM-1 by PCR and sequencing. We identified NDM-1-positive isolates, undertook susceptibility testing, and, where appropriate, typed the isolates. We undertook Inc typing on blaNDM-1-positive plasmids. Transconjugants were created to assess plasmid transfer frequency and its relation to temperature.

Findings From Sept 26 to Oct 10, 2010, 171 seepage samples and 50 tap water samples from New Delhi and 70 sewage effluent samples from Cardiff Wastewater Treatment Works were collected. We detected blaNDM-1 in two of 50 drinking-water samples and 51 of 171 seepage samples from New Delhi; the gene was not found in any sample from Cardiff. Bacteria with blaNDM-1, were grown from 12 of 171 seepage samples and two of 50 water samples, and included 11 species in which NDM-1 has not previously been reported, including Shigella boydii and Vibrio cholerae. Carriage by enterobacteria, aeromonads, and V cholerae was stable, generally transmissible, and associated with resistance patterns typical for NDM-1; carriage by non-fermenters was unstable in many cases and not associated with typical resistance. 20 strains of bacteria were found in the samples, 12 of which carried blaNDM-1 on plasmids, which ranged in size from 140 to 400 kb. Isolates of Aeromonas caviae and V cholerae carried blaNDM-1 on chromosomes. Conjugal transfer was more common at 30°C than at 25°C or 37°C.

Interpretation The presence of NDM-1 β-lactamase-producing bacteria in environmental samples in New Delhi has important implications for people living in the city who are reliant on public water and sanitation facilities. International surveillance of resistance, incorporating environmental sampling as well as examination of clinical isolates, needs to be established as a priority.

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Introduction The spread of mobile carbapenemases among bacterial pathogens is of great concern, not only because these enzymes confer resistance to carbapenems and other β-lactam antibiotics, but also because such pathogens typically are resistant to multiple other antibiotic classes, leaving very few treatment options available.1 This concern is certainly warranted for Enterobacteriaceae that produce NDM-1 β-lactamase.2 Plasmids carrying the gene for this carbapenemase, blaNDM-1, can have up to 14 other antibiotic resistance determinants (M A Toleman, unpublished data) and can transfer this resistance to other bacteria, resulting in multidrug-resistant or extreme drug-resistant phenotypes.3 Resistance of this scale could have serious public health implications because so much of modern medicine is dependent on the ability to treat infection.2,4

So far, the NDM-1 enzyme has been found clinically in Enterobacteriaceae and Acinetobacter baumannii;5,6 and both our previous study5 and findings by Deshpande and colleagues5 showed that NDM-1 is widely disseminated in the Indian subcontinent. The first NDM-1-positive isolate described was from a Swedish resident of Indian origin who contracted a Klebsiella pneumoniae infection while in India in late 2007. The patient also had an NDM-1-positive Escherichia coli strain in his gut flora. A recent surveillance study7 showed that isolates containing NDM-1 were circulating in New Delhi as early as 2006, 2 years before the first European case was identified. Since 2008, there has been repeated import of NDM-1-positive bacteria from the Indian subcontinent to Europe, the USA, Canada, Asia, and Australasia, which was often mediated via transfers of patients, as well as some direct transmission in Europe and some unaccounted clusters linked to the Balkans.8


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Many of the patients from Europe, North America, Asia, and Australasia had been admitted to hospital in India, Pakistan, or Bangladesh, but a few had a history of travel to the subcontinent without hospital admission. For example, some individuals had gut colonisation with producer strains found coincidentally while being assessed for traveller’s diarrhoea. Also, the known problem of poor sanitation in some parts of India and the fact that many travellers to the Indian subcontinent experience gut colonisation with other multiresistant bacteria led us to investigate the extent to which *bla*<sub>NDM-1</sub> is circulating in community waste seepage and tap water in urban New Delhi and to assess the transmissibility of *bla*<sub>NDM-1</sub>-encoding elements.

**Methods**

**Sample collection**

An employee of Channel 4 News who holds a UK biology degree oversaw the sample collection and was given strict verbal and written instructions on collection of samples. The corresponding author spoke to the team several times to ensure that proper procedures were followed. Swabs of seepage water and tap water samples from individual sites, picked by team members, within a 12 km radius of central New Delhi were collected. Swabs were taken from waste seepage sites (ie, water pools in streets or rivulets) and absorbed about 100 μL of liquid. Swabs were transported in charcoal agar to preserve bacteria and DNA. 15 mL samples of tap water were taken from public water supplies that local residents use for drinking, washing, and food preparation. The sources and times of all samples were recorded on large-scale maps and photographs of each site were taken. Samples were transported to the UK in airtight UN-approved sealed containers via a personal courier. Seepage water and tap water samples are not listed in the DGFT Notification number 27/2007 issued by the Government of India, Ministry of Commerce and Industry, Department of Commerce, and therefore no permit is required for export. As a control group, we took 100 μL sewage effluent samples from the Cardiff Wastewater Treatment Works, Tremorfa, Wales.

**Procedures**

Swabs were squeezed out in 0·5 mL volumes of sterile molecular grade water (Sigma Aldrich, Poole, UK) in Eppendorf tubes and centrifuged at 3000 g for 30 s to remove residual charcoal agar. *bla*<sub>NDM-1</sub> was detected by PCR amplification with 1 μL of the swab suspension as a DNA template. We also used in-gel DNA hybridisation with a *bla*<sub>NDM-1</sub> probe, as previously described, with the modification that PCR annealing was done at 68°C and the reaction continued for 40 cycles. Resuspended swab samples (in molecular grade water) were also spotted directly onto a hybridisation membrane and probed with *bla*<sub>NDM-1</sub>. We assayed water samples both as obtained and after any bacteria had been concentrated 15-fold by centrifugation at 12000 g for 15 min and resuspended in 1 mL of sterile molecular grade water. PCR analysis and hybridisation was then done as for the swab samples. We used *K pneumoniae* IR25-P and K15-D6-37 as NDM-1-positive controls.

To isolate and characterise the phenotypes of NDM-1-positive bacteria, swabs and 50 μL of concentrated tap water were repeatedly spread onto Mueller-Hinton agar (Becton Dickinson, Oxford, UK) containing 100 mg/L vancomycin plus 20 mg/L cefotaxime or 100 mg/L vancomycin plus 0·5 mg/L meropenem until single colonies were obtained. Colonies that grew during overnight incubation (37°C) were selected on the basis of morphology and were transferred with sterile toothpicks onto Mueller-Hinton media containing 0·5 mg/L meropenem. These colonies were screened by use of colony DNA hybridisation and PCR analysis as previously described. All PCR products were confirmed by sequencing.

Positive samples were subcultured onto MacConkey agar (Oxoid Thermfoisher, Basingstoke, UK) containing 0·5 mg/L meropenem to ensure culture purity and were identified with the Phoenix automated identification system (Becton Dickinson) or with API 20E strips (bioMérieux, Basingstoke, UK). For samples with putative NDM-1 positive *Vibrio cholerae* and *Shigella* spp, we used strain-specific antisera and species-specific PCR typing. We calculated minimum inhibitory concentrations by micro-broth dilution (Phoenix, Becton Dickinson) or Etest (bioMérieux). NDM-1-positive isolates were sloped and frozen at −80°C. We used *K pneumoniae* IR25-P and K15-D6-37 as positive controls and *E coli* J53 as a negative control.

If several NDM-1-positive isolates of the same species were isolated from one sample, genomic DNA was prepared in agarose blocks and digested with the restriction enzyme *XhoI* (Roche Diagnostics, Mannheim, Germany). We separated DNA fragments by pulsed-field gel electrophoresis on a CHEF-DR III apparatus (Bio-Rad, Hercules, CA, USA) for 20 h at 6 V/cm at 8°C, with initial and final pulse times of 0·5 s and 45 s, respectively.

Transfer of *bla*<sub>NDM-1</sub> plasmids from the environmental isolates was done with the following recipients, all handled under appropriate safety conditions: the standard *E coli* J53 azide resistant strain; a clinical strain of *Shigella sonnei* (provided by the Special Antimicrobial Chemotherapy Unit [SACU], NHS Wales); and a clinical strain of *Salmonella enterica* serotype enteritidis (*S enteritidis*; also provided by SACU). We made suspensions of overnight cultures, with the recipient at about one-tenth of the cell density of the donor. About 10<sup>9</sup> colony-forming units of the donor and 10<sup>7</sup> colony-forming units of the recipient were then added to 1 mL of nutrient broth (Becton Dickinson) and left overnight at 25°C, 30°C, or 37°C before being diluted in physiological saline (10<sup>3</sup> and 10<sup>9</sup>) and plated onto selective media. Conjugation was also attempted on
nutrient agar by adding a defined inoculum ($10^8$) of the donor directly onto the recipient and incubating overnight at 25°C, 30°C, or 37°C. After 18 h of incubation, the cells were harvested from the agar surface, suspended in 10 mL of water, and diluted in physiological saline ($10^{-3}$, $10^{-5}$, and $10^{-7}$). In all cases, the donor and recipient suspensions were also diluted in physiological saline ($10^{-5}$) and plated onto MacConkey agar (Oxoid, Basingstoke, UK) to confirm purity and to assess the colony count.

We used the following media to recover NDM-1-positive transconjugants: MacConkey agar containing 100 mg/L sodium azide and 0.5 mg/L meropenem for _E coli_ J53 transconjugants; MacConkey agar containing 0.5 mg/L meropenem (in this case only lactose fermenters were used as _bla_{NDM-1}, donors) for _S sonnei_ transconjugants; and XLD medium (Oxoid) containing 0.5 mg/L meropenem for _S enteritidis_ transconjugants. We confirmed putative transconjugants by biochemical profiling and by detection of _bla_{NDM-1} with PCR and DNA hybridisation, as described previously. Plasmids were isolated and typed on the basis of their origin or origins of replication by in-gel hybridisation, as previously described.

**Figure 1:** Map of NDM-1-positive samples from New Delhi centre and surrounding areas

**Figure 2:** PCR screening and hybridisation of selected seepage samples
(A) PCR screening from swabs of seepage samples. Negative and positive controls were run with each batch (data not shown). All corresponding gels were probed with _bla_{NDM-1}. (B) Lane 5 does not show a PCR product but the increased sensitivity of Southern hybridisation detects low copy numbers of _bla_{NDM-1}. PCR products were confirmed by sequencing. M=DNA size marker.
We assessed plasmid stability by serial passage of NDM-1-positive isolates on antibiotic-free and 0·5 mg/L meropenem-containing Mueller-Hinton media (Becton Dickinson). Carriage of \emph{bla}_{NDM-1} was assessed as previously described.\(^4\) Plasmids were deemed unstable if they were lost after two consecutive passages. Genomic DNA in agarose blocks was digested with the endonuclease S1 (Invitrogen, Abingdon, UK), and DNA fragments were separated by pulsed-field gel electrophoresis, as described earlier. We then did in-gel hybridisation with a \emph{bla}_{NDM-1} \(^{32}\)P-labelled probe  (Strategene, Amsterdam, Netherlands) with a random-primer method, as previously described.\(^3\) Conﬁrmation of A/C plasmids carrying \emph{bla}_{NDM-1} was done by hybridisation with \emph{IncA/C} probes, as previously described.\(^4\) We used \emph{K pneumoniae} IR25-P and K15-D6-37 as positive controls and \emph{E coli} J53 as a negative control.\(^1\)

Role of the funding source
The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author, at all times, had full access to all the data in the study and had final responsibility for the decision to submit for publication.
Results

From Sept 26 to Oct 10, 2010, 171 swabs of seepage water and 50 tap water samples from New Delhi and 70 sewage effluent samples from Cardiff Wastewater Treatment Works were collected. Analyses of samples was done from Oct 12, 2010, to Jan 10, 2011. Two of 50 water samples and 51 of 171 seepage samples from New Delhi were NDM-1 positive (figure 1); however, eight seepage samples did not give a positive result with PCR alone (figure 2). All 70 sewage effluent samples from Cardiff were negative for NDM-1.

The two positive water samples were from designated public water supplies; one was taken west of the Yamuna River in the district of Ramesh Nagar and the other just south of the Red Fort. The NDM-1-positive seepage samples were collected from sites on both sides of the Yamuna River, including locations close to the main commercial and financial district around Connaught Place. Positive samples were also collected close to the Sir Ganga Ram Hospital and other public areas, including Gol Market.

All seepage samples grew bacteria (including Enterobacteriaceae) that were resistant to cefotaxime, and 166 of 171 grew bacteria on media containing 0·5 mg/L meropenem. All the concentrated water samples grew bacteria on media containing cefotaxime and 14 of 50 grew on media containing meropenem. blaNDM-1-positive bacteria was recovered from 12 of 171 seepage samples and two of 50 water samples. Some samples contained multiple blaNDM-1-positive species, although we cannot exclude the possibility that some organisms gained or lost the gene during the handling procedures.

20 NDM-1-positive strains were present in the samples (table 1), including the opportunistic Enterobacteriaceae Citrobacter freundii, E coli, and K pneumoniae, which commonly carry blaNDM-1 in hospitals in India and elsewhere,4,5 as well as the pathogenic species Shigella boydii and V cholerae, and Aeromonas caviae (a probable cause of gastroenteritis). Non-fermentative Gram-negative bacteria not previously reported to carry blaNDM-1 were also present comprising Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas pseudoalcaligenes, Pseudomonas oryzihabitans, Sutonella indologenes, Stenotrophomonas maltophilia, Achromobacter spp, and Kingella denitrificans.

Detailed plasmid analysis, by methods previously described,6,8 was done on isolates that were positive for blaNDM-1. Passaging over 14 days in media with and without meropenem 0·5 mg/L showed that 12 of these 20 isolates, including all the Enterobacteriaceae, carried stable blaNDM-1 plasmids; however, many of the non-fermenters—isolates B-3-2, 1-19, 21-9, 72-28, W32-17, W38-14, W38-16, and W38-17—lost their plasmids within 48 h (two passages) with or without meropenem (0·5 mg/L).

Two isolates, A caviae (107-5) and V cholerae (116-14), carried blaNDM-1 on their chromosomes, although the V cholerae isolate also carried blaNDM-1 on a 400 kb plasmid (table 1). Plasmid profiling of the other isolates showed that they carried blaNDM-1 on plasmids ranging in size from 140 to 400 kb (table 1, figure 3). All the plasmids from Enterobacteriaceae isolates were the same size (140 kb). Where typeable, these plasmids, and a 170 kb plasmid from V cholerae 116-17, were IncA/C types, as identified by Southern blotting first to detect blaNDM-1, followed by use of a specific rep/inc probe with a method modified from that of Carattoli and colleagues.36 Four of the 12 isolates that had stable plasmids possessed A/C replicon regions, whereas the remainder had non-typeable replicon regions.

### Table 2: Effects of different temperatures on transfer of NDM-1 encoding plasmids from environmental bacteria strains to Escherichia coli, Salmonella enterica serotype enteritidis, and Shigella sonnei

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency of transfer</th>
</tr>
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<tbody>
<tr>
<td>E coli J53</td>
<td></td>
</tr>
<tr>
<td>3·1  Escherichia coli</td>
<td>4·3×10⁻⁷</td>
</tr>
<tr>
<td>25·4 Klebsiella pneumoniae</td>
<td>8·1×10⁻⁷</td>
</tr>
<tr>
<td>33·5 Escherichia coli</td>
<td>1·1×10⁻⁷</td>
</tr>
<tr>
<td>65·4 Escherichia coli</td>
<td>2·1×10⁻⁷</td>
</tr>
<tr>
<td>65·5 Shigella boydii</td>
<td>&lt;1×10⁻⁹</td>
</tr>
<tr>
<td>79·6 Pseudomonas pseudoalcaligenes</td>
<td>&lt;1×10⁻⁹</td>
</tr>
<tr>
<td>107·5 Aeromonas caviae</td>
<td>3·3×10⁻⁷</td>
</tr>
<tr>
<td>107·7 Pseudomonas putida</td>
<td>3·5×10⁻⁷</td>
</tr>
<tr>
<td>116·4 Stenotrophomonas maltophilia</td>
<td>1·5×10⁻⁷</td>
</tr>
<tr>
<td>116·14 Vibrio cholerae</td>
<td>5·5×10⁻⁸</td>
</tr>
<tr>
<td>116·17 V cholerae</td>
<td>2·1×10⁻⁷</td>
</tr>
<tr>
<td>117·4 Citrobacter freundii</td>
<td>6·7×10⁻⁸</td>
</tr>
</tbody>
</table>

Table 2: Effects of different temperatures on transfer of NDM-1 encoding plasmids from environmental bacteria strains to Escherichia coli, Salmonella enterica serotype enteritidis, and Shigella sonnei.
All of the Enterobacteriaceae isolates positive for \( \text{bla}_{\text{NDM-1}} \) had multiresistant phenotypes, including raised carbapenem minimum inhibitory concentrations (table 1), thus resembling NDM-1-positive bacteria from previous studies.\(^{8,10,11} \) Typical NDM-1-type resistance was also seen in both \( V \) cholerae isolates and, aside from a low ceftazidime minimum inhibitory concentration, in \( P \) putida 107-7. Assessment was harder for the \( A \) caviae and the \( S \) maltophilia isolates, because these species and genera often have chromosomal carbapenemases distinct from NDM-1; nevertheless, these isolates also had broad resistance compatible with carriage and expression of \( \text{bla}_{\text{NDM-1}} \). Despite being \( \text{bla}_{\text{NDM-1}} \)-positive, nine non-fermenters were less resistant than the Enterobacteriaceae (table 1) but were substantially more resistant than typical members of these species. Antibiogram profiling and molecular typing by pulsed-field gel electrophoresis showed that the two \( V \) cholerae isolates from site 116 were distinct.

Table 1 shows that \( \text{bla}_{\text{NDM-1}} \) plasmids vary in type and size, and occur in disparate bacteria. We therefore attempted to transfer \( \text{bla}_{\text{NDM-1}} \) from those bacteria that had stable plasmids into recipient species (\( E \) coli, \( S \) enteritidis, and \( S \) sonnei), and assessed the transfer frequency at temperatures relevant to the Indian environment. Isolates were classed as being unable to transfer \( \text{bla}_{\text{NDM-1}} \) into the recipient species if the frequency of transfer was 10\(^-9 \) or lower. All the isolates with stable \( \text{bla}_{\text{NDM-1}} \)-encoding plasmids were able to transfer these to \( E \) coli J53 at 30°C and all except \( S \) boydii 65-5 did so at 25°C, whereas only \( E \) coli 3-1 achieved conjugative transfer at 37°C (table 2). Transfer to \( S \) enteritidis was less efficient, with seven of 12 capable of transfer at 30°C, only one (\( K \) pneumoniae 25-4) capable of transferring its plasmid at 25°C, and no transfers at 37°C. The transfer of \( \text{bla}_{\text{NDM-1}} \) plasmids into \( S \) sonnei was even less efficient, with only 117-4 (\( C \) freundii) and 25-4 (\( K \) pneumoniae) able to transfer at 30°C, and no transfers at 25°C or 37°C. Where transfer was achieved, frequencies at 30°C were one to 10,000 times higher than at 25°C and were one to 100,000 times higher than at 37°C; transfer into the \( S \) enteritidis and \( S \) sonnei recipients was ten to 1000 times less efficient than into \( E \) coli J53. A caviae 107-5 transferred \( \text{bla}_{\text{NDM-1}} \) into \( E \) coli J53 despite the fact that its \( \text{bla}_{\text{NDM-1}} \) was chromosomally encoded.

**Discussion**

NDM-1 β-lactamase is widely disseminated in New Delhi and has spread into key enteric pathogens. These data present an account of the environmental distribution of bacteria carrying the NDM-1 gene in New Delhi (panel). The two water sources that were \( \text{bla}_{\text{NDM-1}} \)-positive are used for drinking and food preparation as well as personal washing and cleaning of clothes. Whether these data can be extrapolated to other Indian cities is unknown, but clearly there is an urgent need for broad epidemiological and environmental studies to be done, not just in India, but also in Pakistan and Bangladesh, which are source countries for other exported cases of infection and colonisation with \( \text{bla}_{\text{NDM-1}} \)-positive bacteria.\(^{10,11} \)

Some of the seepage samples failed to give a positive result by PCR alone, seemingly reflecting the increased sensitivity of DNA hybridisation. Also, although direct probing of PCR products showed good discrimination between negative and positive samples, some PCR products from seepage swabs appeared smeared, suggesting that DNA degradation by endonucleases occurred during handling. Such degradation probably occurred when the swabs were washed in 0.5 mL of molecular grade water before PCR, and suggests a high concentration of bacterial endonucleases in the samples. This inference is further supported by the finding that, when washed swabs were stored in a fridge overnight and the experiment repeated, 46 of 51 of the initially positive samples then appeared negative.

The sample volumes collected were very small and some \( \text{bla}_{\text{NDM-1}} \) plasmids were unstable. Consequently, the dissemination of \( \text{bla}_{\text{NDM-1}} \) is probably more extensive than indicated by the data presented here.

This study also emphasises the extent to which \( \text{bla}_{\text{NDM-1}} \) can disseminate among species, including not only enterobacteria but also aeromonads, vibrio, and various non-fermenters. These data reflect the gene's association with promiscuous plasmids—most of them IncA/C or non-typeable elements. This result is in keeping with previous data from clinical isolates, where \( \text{bla}_{\text{NDM-1}} \) was found in a wide range of species recovered from infected or colonised patients.\(^2\) Despite possessing an intrinsic metallo-β-lactamase gene, \( \text{bla}_{\text{NDM-1}} \) \( S \) maltophilia 116-4 also carried a stable NDM-1-positive plasmid, not suggesting that carbapenem resistance alone is not the sole selection criterion for maintaining \( \text{bla}_{\text{NDM-1}} \)-encoding plasmids.

Some of the isolates—including all the Enterobacteriaceae, aeromonads, and \( V \) cholerae that were positive for \( \text{bla}_{\text{NDM-1}} \)—had the extreme drug-resistance profiles typical of clinical isolates with the NDM-1 enzyme.\(^{14,15} \) Along with stable, transferable expression of \( \text{bla}_{\text{NDM-1}} \). Others, consisting of many of the non-fermenters, were little or no more resistant than typical members of their species, despite being \( \text{bla}_{\text{NDM-1}} \)-positive, as confirmed by sequencing. This finding could be because high-level resistance requires additional factors not present on these plasmids or because, owing to its promoter sequences, \( \text{bla}_{\text{NDM-1}} \) is not well expressed in some non-fermenters.

Two samples contained \( \text{bla}_{\text{NDM-1}} \)-positive strains of the classic pathogens \( V \) cholerae and \( S \) boydii. Although the primary treatment of cholera is based on rehydration,\(^2\) antibiotics are used to reduce excretion of the bacteria, and any accumulation of resistance in the species must be viewed with concern. Antibiotics reduce the duration and severity of shigellosis,\(^2\) but the genus shows increasing resistance to the typical treatments used—namely, ampicillin, co-trimoxazole, nalidixic acid, and ciprofloxacin.\(^{7,21} \) Clinical isolates of \( S \) boydii from India are mostly susceptible to these antibiotics;\(^{26} \) by contrast,
NDM-1-positive Enterobacteriaceae carried similarly sized (140 kb) plasmids, corresponding to the size reported for \( \text{bla}_{\text{NDM-1}} \) plasmids by others. These transferred to recipient \( E\ coli \), \( S\ enteroxidis \), and \( S\ sonnei \) at higher frequencies at 30°C than at 25°C or, particularly, at 37°C; however, whether this finding is a result of species or strains difference is unclear, especially in view of the close relation between \( E\ coli \) and \( Shigella\) spp. This finding has serious implications for environmental transfer given that the average daily peak temperature in New Delhi reaches 30°C and a temperature of 30°C lies within the daily high–low range for the 7 months from April to October inclusive (figure 4). This period includes the monsoon season, when floods and drain overflows are most likely, which potentially disseminates resistant bacteria. In view of the lower transfer frequencies at 37°C than at 30°C, transfer in the environment might be more important than in the gut, even for those species of Enterobacteriaceae represented in the typical human flora. This aspect also highlights a deficiency with many published plasmid studies, which attempt in-vitro conjugation experiments only at 37°C. However, thermostensitivity plays a part in the conjugal apparatus of plasmids of the IncHI families, and a key gene in the transfer locus is transcribed in a temperature-dependent manner, with substantially reduced levels of expression at 37°C compared with 30°C.

The results of this study suggest widespread environmental contamination and are in keeping with the facts that not all of the UK or European patients with \( \text{bla}_{\text{NDM-1}} \)-positive bacteria who had visited India were admitted to hospital there, and that many patients from Chennai who had \( \text{bla}_{\text{NDM-1}} \)-positive clinical isolates had no recent history of hospital admission. Rather than being a purely nosocomial problem, bacteria with this resistance seem to be circulating in the community; thus they may be imported into hospitals with admitted patients and probably enriched within the gut flora when these patients, for whatever reason, receive antibiotic treatment or prophylaxis. The risk posed by the community circulation of multiresistant bacteria is emphasised by the recent study by the ReAct group, which showed that seven of eight tourists returning to Sweden from India had colonisation by gut bacteria that produced extended-spectrum \( \beta\) lactamases that they did not carry before departure, and is further supported by the high concentration of extended-spectrum \( \beta\) lactamases in community-onset as well as hospital-onset intra-abdominal infections in India. Similar colonisation studies on travellers and residents are urgently needed for NDM-1 carbapenemase.

Oral–faecal transmission of bacteria is a problem worldwide, but its potential risk varies with the standards of sanitation. In India, this transmission presents a serious problem, as highlighted by a recent UN report that showed that 650 million citizens do not have access to a flush toilet and even more probably do not have access to clean water. The sewerage system in New Delhi is unable to cater for the city’s population and only 60% of the population is served by the sewerage system, as emphasised by studies sponsored by Water Aid India. This study shows that international surveillance of resistance needs to be established. Such surveillance must incorporate environmental sampling as well as examination of clinical isolates and cover Pakistan and Bangladesh, because these are also source countries for exported cases. These are pressing needs if the ability to treat severe infections in vulnerable patients is to be maintained. The next step in this analysis should be to sample carriage in south Asia, both in residents and travellers. We would be delighted to advise and help in any capacity to take such studies forward in partnership with Indian, Pakistani, and Bangladeshi scientists, clinicians, and government officials.
Articles

Contributors
Authors from Cardiff University (TRW, JW, and MAT) undertook the laboratory work and wrote the manuscript. Channel 4 News collected the swabs and water samples and documented their location. DML interpreted the data and contributed to the editing of the manuscript.

Conflicts of interest
DML has received conference support from numerous pharmaceutical companies, and also holds shares in AstraZeneca, Merck, Pfizer, Dechra, and GlaxoSmithKline, and, as Enduring Attorney, manages further holdings in GlaxoSmithKline and Eco Animal Health. TRW has received payment for writing from Pfizer and travel, accommodation, or meeting expenses from bioMérieux. All other authors declare that they have no conflicts of interest.

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