DISSEMINATION OF NDM-1(SUPERBUG) IN HOLY GANGES AND ITS IMPLICATIONS FOR HUMAN HEALTH

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ABSTRACT-

New Delhi metallo-β-lactamase-1 (NDM-1) producing Escherichia coli constitute a serious threat to global health since it is found to be highly resistant to most of the currently available antibiotics including carbapenems. Gram-negative bacterial strains with NDM-1 (New Delhi Metallo-beta-lactamase-1) gene, also called the superbug, detected in Ganges water. The New Delhi metallo-β-lactamase (NDM-1) increases bacterial resistance to a broad range of antibiotics, and bacteria that produce it can cause mutation in genes through different processes in wastewater treatment plants (WWTPs). NDM-1 genes prevailed through several treatment units including disinfection by chlorination in WWTPs in Ganga River near Haridwar and Rishikesh (Uttarakhand). The study brings out various reasons of breeding superbugs in Ganges and measures to combat them. The overall prevalence of NDM-1 varied between 8.1% in isolates of E coli and 38.02% in the isolates of K pneumoniae — two types of bacteria found commonly in post-operative patients and immune-compromised patients. NDM-1 genes were present at much higher concentrations in dewatered waste sludge that is applied to soils. This concern was validated by a conjugation experiment with Ganga River sediment isolated from a WWTP.

KEYWORDS – metallo, pneumoniae, carbapenems.

Introduction-

It is a known established fact that exposing bacteria to the right kind of antibiotics will kill most of them, but leave a few mutants that happen to resist the drug better than the rest. These mutants go on to multiplying, and eventually the whole strain evolves as a strain resistant to antibiotics. A major concern over WWTPs is largely beyond resistant bacteria, as they deal with drugs and chemicals either dumped into the system, or excreted from humans in their waste. In recent years we’ve seen a number of reports on detectable levels of drugs in rivers and streams that passed relatively intact through treatment facilities. As Waste Water Treatment Plants (WWTPs) depend upon microbial activity to break down sewage, use of large quantities of antibiotics in the sewage could inhibit microbial activity, resulting in the failure of WWTPs and the discharge of under-treated wastewater into the environment. The gene that encodes for NDM-1 is called blaNDM-1 and has been identified on bacterial chromosomes and plasmids. Plasmids are small segments of genetic material that are easily transferred among bacteria. In this way, the ability to produce NDM-1 can pass from one bacterial strain to another and even from one bacterial genus to another.

Cases of NDM-1 infection are usually caused by gram negative bacteria from the Enterobacteriaceae family. This family includes common bacteria like Escherichia coli (E. coli) and Klebsiella. These bacteria reside in the bowel and may spread from person to person if hands or items are contaminated with fecal material. To date, strains of Klebsiella, Escherichia, and Acinetobacter genera of bacteria are known to possess the gene for NDM-1. Unfortunately, there is rapid scattering of blaNDM-1 genes, data are still lacking for epidemical research. As the climate changes there is seasonal mass migration of pilgrims from large cities in India to religious areas, such as sacred sites along the Upper Ganges River. Cities such as Rishikesh (pop. 78,805;
2011 census) and Haridwar (pop. 220,767) in the foothills of the Himalayas receive up to 500,000 additional visitors in May and June, providing a large influx of nonresidents who may carry NDM-1 positive strains in their guts. The waste treatment is limited in such areas of Rishikesh and Haridwar, where the treatment plant capacity is for about population of 80,000. Therefore large quantities of improperly treated wastes are released into the Upper Ganges regions of Haridwar and Rishikesh, especially during the pilgrimage season. The potential for increased blaNDM-1 exposure is disastrous because pilgrims generally suppose that the water quality in the Upper Ganges is good, not considering how their visiting in impact local water quality. Therefore, by comparing fecal coliform and blaNDM-1 abundances in waters and residues before and during the pilgrimage season, one can determine possible exposure risks to guests and residents during pilgrimage visits, which can be used to guide new waste management methods. This is critical from a health point of view, because drinking water and bathing are key elements of pilgrimages to the region. Here we used seasonal tourist and resident population data in conjunction with water quality, quantitative molecular microbiology on Anti Resistances Gene (ARG) abundances (including blaNDM-1), and fecal microbial indicators to assess human impacts to ARG before and during the pilgrimage season. Specifically, we compared blaNDM-1, other ARG, and the total and fecal coliform levels in sediment and water samples between seasons at selected sites on the Upper Ganges River near Rishikesh-Haridwar.

We surveyed waste water treatment plants of Haridwar and Rishikesh. There’s no antibiotic that can kill these bacteria. We often think about sewage treatment plants as a way to protect us, to get rid of all of these disease-causing constituents in waste water. But the microbes are continuously increasing. They’re eating sewage, so they proliferate.

The Ganges River is 2525 km long, and its watershed covers more than one million km$^2$, spanning India, Nepal, Bangladesh, and China. The Upper Ganges River, which is the focus of this study, originates from glaciers at Gaumukh and enters the northern Indian plain at Rishikesh, a small town with many religious shrines and ashrams near the river. Below Rishikesh, the river flows 30 km south to the city of Haridwar, the first larger city on the river’s path across the Upper Ganges basin. Within this region, seven shoreline sites were sampled, including three nearer to Rishikesh and four nearer to Haridwar.

**Results**
Water column and river sediment samples were collected in February and June 2012 at seven locations on the Upper Ganges River near Rishikesh-Haridwar. Rishikesh and Haridwar are located in the Himalayan foothills where residential human population densities are low and there is minimal agricultural activity. The Upper Ganges is primarily fed by glacial and snowmelt from the mountains; however, it also receives increased rainfall inputs during the monsoon season (July to September).

Given the pristine upland watershed, water quality conditions in the Upper Ganges River are generally good, which was confirmed by our monitoring data (Table 1). High ambient dissolved oxygen (DO; 8.9–9.6 mg/L) and low total dissolved solids levels (TDS; 68–107 mg/L) suggest the river is well oxygenated and has minimal extraneous organic and inorganic matter. TDS levels were slightly higher in June versus February (107 vs 81.3 mg/L at Haridwar sites; Table 1), which was probably due to greater human waste inputs during the pilgrimage season. As background, pilgrims usually visit the region for a few weeks to a month, staying in hotels, dharamshala (spiritual dwellings), guest houses, temples, and private accommodations, which overall taxes local waste management facilities.

<table>
<thead>
<tr>
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<th>DO (mg/L)</th>
<th>TDS (mg/L)</th>
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<tr>
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<td>Feb Jun</td>
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<tr>
<td>Rishikesh</td>
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<td>9.4 (0.20)</td>
<td>68.3 (4.3)</td>
</tr>
<tr>
<td>Haridwar</td>
<td>9.7 (0.30)</td>
<td>9.0 (0.30)</td>
<td>81.3 (3.0)</td>
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Table 1: Seasonal Water Quality Conditions in the Upper Ganges River near Rishikesh-Haridwar.[1]

ARG levels were significantly higher in both water column and sediment samples in June relative to February levels (Wilcoxon Two-sample nonparametric test (WT); p < 0.01), which is consistent with increased human waste releases when the seasonal visitors are present. However, the largest seasonal increase in ARG was seen in sediment-associated blaNDM-1 levels (see Figure c), which parallels bacterial 16S-rRNA gene levels at the same sites.

In the Upper Ganges, all measured ARG were significantly more enriched in June relative to February (WT; p < 0.05). The normalized ARG levels were only high in the Upper Ganges when visitors were present, especially blaNDM-1.

Figure (c): log(16S rRNA bacterial gene) and log(blaNDM-1) abundances in sediment and water column samples collected in February (blue) and June (red) near Rishikesh, Haridwar Ganges River. (A) 16S and (B) blaNDM-1 levels are provided per gram dry weight of sediment collected, whereas (C) 16S and (D) blaNDM-1 represent levels per mL of water column sample. Error bars present standard errors.[1]
Boxes represent 25–75% quartile ranges, and error bars indicate the standard errors.

Such differences might be explained by the larger human populations present in June, but when one normalizes ARG abundances to human population size in the region (including visitors), significantly greater blaNDM-1 and 16S rRNA gene abundances per capita were apparent in June in the Upper Ganges River (see Figure e; WT; p < 0.01). Interestingly, the other four ARG tested did not significantly vary seasonally on a per capita basis (WT; p > 0.05). This suggests that visitor wastes, which are more apparent in June, have higher intrinsic blaNDM-1 abundances than local residents, i.e., elevated blaNDM-1 levels in the river in June are primarily associated with waste inputs from the visitors.

Figure (d) Sediment gene abundances of 16S rRNA gene, blaNDM-1, blaOXA, tet(M), tet(W), and tet(Q) normalized to estimated human populations in the Rishikesh-Haridwar area in February (local residents only) and in June (visitors plus local residents). Human populations are assumed to be 320,000 and 820,000, respectively. Only 16S rRNA and blaNDM-1 per capita levels significantly differ between seasons, indicating visitor wastes contain more blaNDM-1 than local residents. Boxes present the 25–75% quartile range, and error bars show standard errors.

Antibiotic resistance is ancient, but AR levels are increasing worldwide due to increased use of antibiotics and many other factors [3][4]. However, there has been considerable debate about the dominant mechanisms by which AR is transmitted around the world. Recent evidence has shown that dissemination of AR can be rapid. For example, blaNDM-1 gene and its protein were first seen in patients in India about six years ago and are now detected in patients around the world, including many new variants. Original discovery of strains carrying blaNDM-1 was in hospital settings, but it has since been detected in surface waters, implying this plasmid-borne gene is migrating outside of hospital environments, including waters, soils, and sediments.

Figure (e): Sediment gene abundances of 16S rRNA gene, blaNDM-1, blaOXA, tet(M), tet(W), and tet(Q).[1] We compare blaNDM-1 and other ARG levels in human waste-impacted waters from a pristine region of India (Rishikesh-Haridwar). The data suggest that permanent residents of Rishikesh-Haridwar carry lower levels of blaNDM-1 genes in their gut relative to seasonal visitors to the region (see Figure e). The average visitor appears to carry at least >20 times more blaNDM-1 genes than Rishikesh-Haridwar residents in 2012, and increased waterborne blaNDM-1 exposure occurs during the pilgrimage season, both to local and pilgrimage residents. The pilgrimage areas may act as key areas for the broader transmission of blaNDM-1 and other ARG, especially considering bathing and water consumption occur in Ganges waters and exposed visitors return home after their visit to the region.

Other ARG data suggest otherwise Figure d and e. Sediment blaOXA, tet(W), and tet(M) levels in the Upper Ganges are roughly the same in February and June, which is consistent with the probable presence of such genes in the gut and wastes from both visitors and residents. Further, one might contend waste releases to the river may be impacted by nonhuman animal wastes, such as cattle, which also contain elevated blaOXA, tet(W), tet(M), and tet(Q). However, tet(Q) tends to be less associated with human wastewater, and there is significant decrease in per capita tet(Q) levels in June, implying tet(Q) in the river is not as strongly linked with seasonal human populations. Finally, FC and blaNDM-1 significantly correlate in the river water column including periods when per capita blaNDM-1 levels were elevated; therefore, higher blaNDM-1 levels in
June are likely associated with greater quantities of visitor fecal matter in the Upper Ganges.

These results have two major implications. First, blaNDM-1 genes do not appear to be equally present across the Indian population. However, human mass migrations almost certainly increase the probability of transmission of ARG across the broader community. We do not suggest pilgrimages are not important, nor do we suggest they should not continue, but results show pilgrimage areas, such as Rishikesh-Haridwar, are potential for AR transmission at large scales. This is particularly true because areas like Rishikesh-Haridwar often have inadequate waste treatment facilities, which are especially overloaded when visitor populations increase; i.e., human populations are greatest when waterborne blaNDM-1 levels are highest. Therefore, local officials are urged to consider improving waste treatment facilities in pilgrimage regions to better protect ritual bathing waters. Although full-scale waste treatment plants may not feasible, especially given the transient visiting population, providing greater access to pit privies and providing greater local waste management would reduce blaNDM-1 releases into surface waters.

A second major implication of this work is that we have established an ideal site for studying the role of surface waters and sediments on broader AR transmission. We do not yet have comprehensive epidemiological information on blaNDM-1 resistant clinical cases in Rishikesh-Haridwar versus national averages, but this is clearly an important next step. We predict that multiresistant blaNDM-1 positive clinical pathogens are currently less probable in the Rishikesh-Haridwar region (relative to urban India), but such information might be gathered and used to develop strategies for reducing both the clinical and environmental transmission of mobile genes, like blaNDM-1, to strains of clinical importance. There is no direct connection environmental reservoirs of blaNDM-1 gene to a change in health in the local population, abundances of such genes appear to be significantly different between Rishikesh-Haridwar residents and visitors from outside the region. We are not questioning pilgrimages because they provide huge social benefit, but waste handling and treatment facilities should improve and to decrease the spreading of ARG and blaNDM-1.

**Conclusion**

Treatment is guided by the antibiotic resistance pattern. Many NDM-1 strains are resistant to all antibiotics except for colistin and tigecycline. Colistin is an older antibiotic that has not been used much in recent decades, because it is somewhat more toxic than other antibiotics. A few NDM-1 strains have been sensitive to tigecycline (Tygacil), but this agent should be used cautiously in serious infections because it does not achieve high levels in the bloodstream. A few strains have also been sensitive to aztreonam, although the U.S. strains were not. Researchers have identified a new antibiotic compound that may inhibit NDM-1 containing bacterial topoisomerase function so the bacterial replication is inhibited or stopped. Unfortunately, the compound has not gone through any clinical trials and is not commercially available.

**Acknowledgement**

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