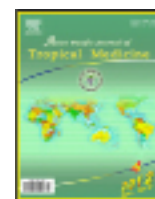




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: www.elsevier.com/locate/apjtm



Document heading doi:

NDM-1 in the Indian environment: hitherto the problem is not disquieting

Mohd Shahid^{1,*}, Fatima Khan¹, M Salman Shah², Indu Shukla¹, Fatima Shujatullah¹, Haris M Khan¹, Abida Malik¹, Iqbal M Khan²¹Section of Antimicrobial Agents & Drug Resistance Research and Molecular Biology, Department of Medical Microbiology, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India²Department of Community Medicine, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India

*bla*_{NDM-1} is a recently described novel plasmid-mediated resistance determinant that confers resistance to carbapenems. Bacteria harbouring NDM-1 have been reported from the clinical specimens^[1,2]. These are also reported and warned as impending gut colonists^[1,3], and recently described from the environment of the capital city of India^[4], indicating the potential as a reservoir for future infections. However, it is too early to proclaim that the NDM-1 has disseminated widely, and that the situation is worse in whole of the country, before some more studies are executed in this connection. Strung to the recent report of Walsh and colleagues^[4], as discussed earlier^[5], we performed an analogous study on the environmental samples (drinking water, drains, sewage) collected from Aligarh, a city nearby the Indian-capital. Keeping in view the reports on faecal carriage, we also studied faecal samples collected from 40 healthy volunteers. Moreover, we also analyzed the data, for any carbapenem resistance, of a parallel study performed on the stool samples taken from 150 children (age group ranging between 1–12 years) that were admitted in the wards of JN Medical College & Hospital as suspected cases of malaria during years 2008–2010. In addition we also retrospectively analyzed the departmental records, of 2008–2010, for the frequency of occurrence of carbapenem resistance in clinical isolates. Polymerase chain reaction (PCR), for the detection of *bla*_{CTX-M} (multiplex-PCR) and *bla*_{NDM-1} (monoplex-PCR), was performed in the third-generation cephalosporin (3GC) resistant isolates (because none of the isolates was found carbapenem-resistant) obtained from the environmental-samples and stool samples from the healthy volunteers.

With all aseptic precautions a total of 90 environmental samples (drinking water=77; drain=10; sewage=3), in an approximate volume of 15 ml, were collected by the community health team visiting the urban and rural

areas of the Aligarh city. Of the 90 samples, 20 yielded bacterial growth; 10.4% (8/77) from water samples, 90% (9/10) from drains and 100% (3/3) from the sewage (Table 1). Of these 20 culture positive specimens, a total of 43 bacterial isolates were obtained; majority being *Escherichia coli* (*n*=19) followed by *Pseudomonas* spp (*n*=9) (Table 1). It is noteworthy that none of the samples yielded important pathogenic bacteria such as *Shigella* spp. and *Vibrio cholerae* as reported in a recent study^[4]. It is also noteworthy that the water consumed by the privileged population (in urban areas) utilizing the commercial sterilizers such as “aquaguard”, and hand pumps (in rural areas) did not yield any growth of bacteria. Two samples from the bucket water (used for cooking) and 6 from the tap water yielded bacterial growth (Table 1). On antibiotics susceptibility testing, none of the bacterial isolate was found carbapenem-resistant; however 65.1% (28/43) isolates were found resistant to a third-generation cephalosporin (3GC) (Table 1). *bla*_{CTX-M} was detected in all of the 3GC-resistant isolates; however none of the bacterial isolate showed the presence of *bla*_{NDM-1} by PCR.

From the bacterial isolates obtained from 40 stool samples of healthy volunteers, 3GC-resistance was noticed in 82.5% (33/40) isolates and none showed resistance to carbapenem. On molecular analyses, *bla*_{CTX-M} was detected in 52.5% (21/40) isolates and none yielded *bla*_{NDM-1}. Similarly, of the 150 stool samples from children, 78.6% of the bacterial isolates showed resistance to 3GC and none demonstrated resistance to carbapenem. On analyzing the departmental records, during 2008–2010, a total of 6 408 culture yielded growth of Gram-negative bacteria; 3GC resistance was noticed in 83.5% isolates and the resistance to carbapenem was found in 0.79% isolates. Because of the retrospective analyses in these two latter cases testing for *bla*_{NDM-1} could not be performed.

Aligarh is a city situated 132 km from the New Delhi from where the first and further cases of NDM-1 were detected. Patients from Aligarh are usually referred to the hospitals in New Delhi for higher referrals. Moreover, a significant proportion of people work in New Delhi, while living in Aligarh, and hence daily move to and fro between the two cities utilizing the intercity train services. Therefore, in response to prior warnings regarding the spread of NDM-1-harboring bacteria, we performed this preliminary study and did not find *bla*_{NDM-1} in the Aligarh-environment and

*Corresponding author: Dr. Mohd. Shahid, M.B.B.S., M.D., PhD, FNZIMLS (New Zealand), Associate Professor & Consultant Microbiologist, Department of Microbiology, JN Medical College & Hospital, Aligarh Muslim University, Aligarh-202002, U.P., India.

Tel: +91-571-2720382, 009411802536

Fax: +91-571-2721776; +91-571-2720382

E-mail: shahidsahar@yahoo.co.in, drmohdshahid123@yahoo.com

Foundation project: Partially funded by Ministry of Science & Technology, Government of India, as Young Scientist Project (SR/FT/L-111/2006).

in stool samples of healthy volunteers. This reflects that the situation is not yet utterly miserable in other Indian cities. A recent study from Mumbai also reported the absence of *bla*_{NDM-1} from the stool samples of their patients[6]. The current preliminary report is in concordance with our previous study, performed on the vegetations (environment) of the Aligarh city, where at that time we did not find the resistance to carbapenems, and even not to 3GC in the environmental isolates[7]. However, future dissemination of NDM-1 in the environment (of Aligarh) cannot be denied, provided the recent appearance of CTX-M in the environmental isolates, if appropriate measures for their containment are not taken at this stage.

We are expanding our study looking the wider geographic area for the presence of *bla*_{NDM-1} in the environment of our city, as well as in the clinical isolates, and feel that such types of studies are required from other Indian cities; especially a collaborative multicenter study looking in this aspect is urgently required.

If our current data and that of Walsh & colleagues[4] are analyzed critically, it is evident that the resistance

mechanism(s) that is prevalent in the faecal flora (seepage/sewage) is probably detected in the drinking water and that could be due to leakage and mixing of faecal flora with the water supplies. However, to reach a final conclusion, further extensive studies are needed on the environmental and stool samples on a larger scale. We applaud the study of Walsh and colleagues[4] that warned us at an earlier stage and helped us to stand vigilant regarding the potential for dissemination of NDM-1 in the environment.

At this instance, we feel that the situation is not yet grave as far as NDM-1 is concerned and we may be able to cope up with the problem, if act promptly and sensibly. As a first line preventive measure, we suggest usage/replacement of corrosion-free metal pipes for the supply of public water. Also the joints in the pipelines should be in a manner to prevent water leakage. The concerned authorities should seriously think and take appropriate measures in this regard. Working in this line, and by proper execution of the antibiotics prescribing and antibiotics resistance monitoring policies, we are sure that we may easily combat this emerging threatening problem of drug resistance.

Table 1

Water, drain and sewage cultures and bacteria obtained from urban and rural areas of Aligarh city, India.

Samples sources	Total-studied	No-growth	Bacterial-growth	Organisms isolated [§]				
				<i>Escherichia coli</i>	<i>Klebsiella</i>	<i>Citrobacter</i>	<i>Pseudomonas</i>	<i>Acinetobacter</i>
Water Filtered(aquaguard)	19	19	–	–	–	–	–	–
	Hand pump	6	6	–	–	–	–	–
	Bucket water	4	2	2	2	1	2	1
Tap water	48	42	6	*3GCR=1	3GCR=0	3GCR=2	3GCR=0	–
				#3GCS=1	3GCS=1	3GCS=0	3GCS=1	–
				3GCR=2	3GCR=1	3GCR=0	3GCR=1	–
Drain	10	1	9	3GCS=4	3GCS=1	3GCS=1	3GCS=1	3GCS=0
				3GCR=9	3GCR=2	3GCR=2	3GCR=2	3GCR=1
				3GCS=0	3GCS=1	3GCS=0	3GCS=2	3GCS=0
Sewage	3	0	3	3GCR=1	3GCR=1	3GCR=2	3GCR=1	3GCR=1
				3GCS=1	3GCS=0	3GCS=1	3GCS=1	3GCS=1
				–	1	3	2	–
Total	90	70	20	19	4	7	9	4

§ Multiple organisms were isolated from individual cultures.

*3GCR= third-generation cephalosporin-resistant.

#3GCS= third-generation cephalosporin-sensitive.

Conflict of Interest

The authors reported no conflicts of interest.

Acknowledgements

M. Shahid is grateful to Department of Science & Technology, Ministry of Science & Technology, Government of India, for the award of Young Scientist Project (SR/FT/L-111/2006).

References

- [1] Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. 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* 2009; **53**: 5046–5054.
- [2] Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F,

- Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010; **10**: 597–602.
- [3] Nordmann P, Poirel L, Toleman MA, Walsh TR. Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? *J Antimicrob Chemother* 2011; **66**: 689–692.
- [4] Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria including *Vibrio cholerae* and *Shigella* spp., in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* 2011; **11**: 355–362. DOI: 10.1016/S1473-3099(11)70059-7.
- [5] Shahid M. Environmental dissemination of NDM-1: time to act sensibly. *Lancet Infect Dis* 2011; **11**: 334–335. DOI: 10.1016/S1473-3099(11)70074-3.
- [6] Deshpande P, Shetty A, Kapadia F, Hedge A, Soman R, Rodrigues C. New Delhi Metallo 1: Have Carbapenems Met Their Doom? *Clinical Infect Dis* 2010; **51**: 1222.
- [7] Shahid M, Malik A, Adil M, Jahan N, Malik R. Comparison of beta-lactamase genes in clinical and food bacterial isolates in India. *J Infect Dev Ctries* 2009; **3**: 593–598.