1 Short research article

2 Identification of *bla*_{GIM-1} in *Acinetobacter variabilis* isolated from the hospital 3 environment in Tamil Nadu, India

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10 Abstract

11 **Background:** Emergence of carbapenem resistance mechanisms among Gram-negative
12 bacteria is a worrisome health problem. Here, we focused on to identify the presence of
13 carbapenem-resistant bacteria among the samples collected from hospital environments in
14 Tamil Nadu. **Methods:** A total of 30 hospital environmental samples were collected between
15 August 2017 and January 2018 from hospitals located in Chennai and Vellore such as lift
16 switches, stair rails, switchboards, nursing desks, used nursing gloves, door handles,
17 wheelchairs, touch screens, chairs and from pillars inside the hospitals. **Results and**
18 **discussion:** A total of 22 carbapenem-resistant Gram-negative bacteria were isolated that
19 included *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp., *Salmonella* sp., *Pseudomonas*
20 *aeruginosa* and *Acinetobacter* sp. Interestingly, *bla*_{GIM-1} was detected in *Acinetobacter*
21 *variabilis* strain isolated in samples collected from hospitals. Unlike other studies, the
22 identified GIM-1 was not plasmid encoded, and this is the first report for the presence of
23 GIM-1 (German imipenemase) in India. **Conclusion:** Extensive surveillance programs are
24 necessary to trace the uncontrolled spread of carbapenem-resistance genes in order to reduce
25 the rapid spread of resistance.

26 **Keywords:** *Acinetobacter* species, *bla*_{GIM-1}, carbapenem resistance, Gram-negative bacteria,
27 German imipenemase.

28 Introduction

29 Antibiotic resistance has become one of the major clinical and public health concerns [1].
30 The spread of antibiotic resistance in clinical settings (nosocomial infections) is a well-

31 recognized problem, and this becomes overlooked with great importance [1]. Multidrug-
 32 resistant bacteria (MDR) are spreading at an uncontrolled rate, especially in hospital areas
 33 [2]. In recent years, it is found that there is a prevalence of a large population of multi-drug
 34 resistant bacteria in and around the hospital environment [1]. These are bacteria which are
 35 exposed to a variety of antibiotics and simultaneously becoming resistant, which makes a
 36 major threat to the public health [3]. Carbapenem is a subgroup in beta-lactam antibiotics
 37 which is considered as one of the last hope for the threatening multi-drug resistant bacterial
 38 infections [4,5]. But developing carbapenem-resistant Gram-negative bacteria is one of the
 39 serious concerns. More dangerously, an MDR bacterium having one or more beta-lactamase
 40 genes can involve in horizontal transfer of resistance genes to other bacterial species or
 41 strains [6]. Some of the prevalent carbapenem-resistance genes in India are; New Delhi
 42 Metallo-beta-lactamase (NDM), Imipenemase (IMP), Verona Integron-Mediated metallo-
 43 beta-lactamase (VIM), Oxacillinase (OXA) [7]. In another study, the identification of DIM-1
 44 gene in *Pseudomonas aeruginosa* was reported for the first time in India by our group [8].
 45 Antibiotic resistance surveillance programs in hospitals are essential to monitor and control
 46 the spread of antibiotic-resistant infections (nosocomial). Community-associated or
 47 community onset of carbapenem-resistant bacterial infections is reported to have a prevalence
 48 of 0-30% [9]. The main focus of this study is to identify the presence of carbapenem-resistant
 49 bacteria from samples collected in the hospital environment.

50 **Methods**

51 In the present study, a total of 30 samples were collected from in and around hospitals in
 52 Chennai and Vellore, Tamil Nadu during the period between August 2017 and August 2018.
 53 The samples were taken from lift switches, stair rails, switchboards, nursing desks, used
 54 nursing gloves, door handles, wheelchairs, touch screens, chairs and pillars inside the
 55 hospital. Sterile swabs dipped in sterile tryptone broth were used for sample collection, and
 56 collected samples were immediately transported and processed at Antibiotic Resistance and
 57 Phage Therapy Laboratory, VIT, Vellore. Bacterial isolation was carried out using the spread
 58 plate technique using MacConkey agar M081 (Himedia, India) amended with 8 µg/mL of
 59 meropenem. Minimal inhibitory concentration (MIC) was performed using micro-broth
 60 dilution method as per the CLSI guidelines. Modified Hodge test (MHT) was performed to
 61 evaluate the carbapenemase production as explained elsewhere [7]. Bacterial identification
 62 was carried out with the VITEK identification system (bioMerieux) and by 16S rRNA
 63 analysis using universal primers 27F and 1492R. DNA was isolated using a boiling lysis

method as explained elsewhere [7]. Further, molecular screening of beta-lactamase genes such as *bla_{VIM}*, *bla_{IMP}*, *bla_{KPC}*, *bla_{NDM}*, *bla_{OXA-48}*, *bla_{OXA-1}*, *bla_{OXA-4}*, *bla_{OXA-30}*, *bla_{OXA-23}*, *bla_{OXA-24}*, *bla_{OXA-51}*, *bla_{OXA-58}*, *bla_{AIM}*, *bla_{GIM}*, *bla_{DIM}*, *bla_{SIM}*, *bla_{BIC}* and *bla_{GES}* were performed using multiplex PCR using the primers and PCR conditions as explained earlier [7, 8]. Plasmid DNA was isolated (only for the isolates that were identified to harbour resistance genes) using HiPurA Plasmid DNA Miniprep Purification kit (Himedia, India). All the PCR products were sequenced (Eurofins Genomics India Pvt. Ltd., Bangalore) and sequence results were analysed against available BLASTN sequences and deposited in Genbank.

Results

A total of 22 non-repetitive, carbapenem-resistant, Gram-negative bacteria were isolated from 30 samples collected from hospital environments. Of the 22 carbapenem-resistant isolates, 15 were identified as lactose fermenters, and 7 were non-fermenters. The isolates were identified as *E. coli* (n=7), *Klebsiella* species (n=4), *Enterobacter* species (n=4), *Salmonella* species (n=2), *Acinetobacter* species (n=2) and *Pseudomonas aeruginosa* (n=3). The MIC results for meropenem/ imipenem were ranged between 4 µg/mL and >128 µg/mL (Table 1). None of the isolates was found to be positive for carbapenemase production by MHT. Molecular characterization results showed the presence of a *bla_{GIM}* gene in *Acinetobacter variabilis*. Genes *bla_{VIM}*, *bla_{IMP}*, *bla_{KPC}*, *bla_{NDM}*, *bla_{OXA-48}*, *bla_{OXA-1}*, *bla_{OXA-4}*, *bla_{OXA-30}*, *bla_{OXA-23}*, *bla_{OXA-24}*, *bla_{OXA-51}*, *bla_{OXA-58}*, *bla_{AIM}*, *bla_{DIM}*, *bla_{SIM}*, *bla_{BIC}* and *bla_{GES}* were absent in all the isolates tested. Plasmid DNA was isolated from *Acinetobacter variabilis* and presence of ~20kb plasmid was noted. Further screening for the presence of *bla_{GIM}* gene using plasmid DNA confirmed that *bla_{GIM}* gene was bound to chromosomal DNA, not in plasmid DNA. Sequencing results confirmed that the amplified *bla_{GIM}* gene is *bla_{GIM-1}*, and to the best of our knowledge this is a first report for the presence of *bla_{GIM-1}* in India. [Accession number: MG764090]

Table 1: Classification of resistance among the Gram-negative bacteria isolated from hospital environmental samples.

S. no.	Bacterial strain	Imipenem MIC (µg/mL)	Meropenem MIC (µg/mL)	Resistance gene
1.	<i>E. coli</i> EC1	8	8	-
2.	<i>E. coli</i> EC2	32	16	-
3.	<i>E. coli</i> EC3	8	4	-

4.	<i>E. coli</i> EC4	16	32	-
5.	<i>E. coli</i> EC5	8	8	-
6.	<i>E. coli</i> EC6	8	8	-
7.	<i>E. coli</i> EC7	32	32	-
8.	<i>Klebsiella</i> sp. KP1	8	4	-
9.	<i>Klebsiella</i> sp. KP2	128	64	-
10.	<i>Klebsiella</i> sp. KP3	32	16	-
11.	<i>Klebsiella</i> sp. KP4	8	8	-
12.	<i>Enterobacter</i> sp. EL1	32	64	-
13.	<i>Enterobacter</i> sp. EL2	64	32	-
14.	<i>Enterobacter</i> sp. EL3	32	32	-
15.	<i>Enterobacter</i> sp. EL4	128	>128	-
16.	<i>Salmonella</i> sp. SL1	32	16	-
17.	<i>Salmonella</i> sp. SL2	32	32	-
18.	<i>Acinetobacter</i> sp. AB1	8	16	-
19.	<i>Acinetobacter variabilis</i> AV1	>128	32	GIM-1
20.	<i>Pseudomonas</i> sp. PA1	32	64	-
21.	<i>Pseudomonas</i> sp. PA2	128	>128	-
22.	<i>Pseudomonas</i> sp. PA3	>128	>128	-

91 **Discussion**

92 Carbapenem-resistance among Gram-negative bacteria is one of the increasing public health
93 concerns. Nosocomial infections are considered as one of the serious health care problems,
94 notably; the rapid spread of antibiotic-resistant bacterial infections is scary [10]. In India, the
95 prevalence of carbapenem-resistant bacteria is being reported and multi-drug resistant
96 bacterial infections are on the rise [7]. Importantly, this study reports the emergence of
97 carbapenem-resistant bacteria in the hospital environment and presence of GIM-1 gene in *A.*
98 *variabilis*. In the past, GIM (German Imipenemase)-type carbapenem-resistance gene was
99 reported in *P. aeruginosa* in 2002 and in common, the reports of GIM genes were from the
100 bacteria isolated in and around Germany. To the best of our knowledge, this is the first study
101 to report the emergence of GIM-1 from India or even from the Asian subcontinent. Earlier,
102 the presence of GIM-1 genes was reported to be encoded on plasmids, and also in mobile
103 genetic elements such as integrons [11]. This is one of the rarest studies to report the presence

of GIM-1 gene in *A. variabilis* isolated from environmental samples, and the resistance gene was bound to chromosomal DNA and not in a plasmid. It is very common to identify GIM-1-type carbapenem resistance mechanism in *Enterobacteriaceae* and *P. aeruginosa*, but the prevalence of GIM-type carbapenemases in *Acinetobacter* is very rare. The isolation of *A. variabilis* in India indicates that GIM-1 is no longer confined to one geographical region as previously thought and these are more widespread among other non-*Enterobacteriaceae*. This clearly indicates the potential transmission of GIM-1-type carbapenemase genes among other Gram-negative bacteria. The increasing prevalence of carbapenem-resistant Gram-negative bacteria (CR-GNB) in the hospital environment, causing nosocomial infections, is challenging particularly during the clinical recovery of patients. This study opens the door for the detection of new carbapenemase genes in India; therefore, extensive resistance surveillance is needed to learn more about the emergence of GIM-1-producing bacteria in India.

Ethical Statement:

The ethical approval for this study was obtained from Institutional Biosafety Committee “Ref. No. VIT/IBSC/07/October, 2018”.

Conflict of Interest:

The authors declare no conflict of interest.

Author’s contribution:

PM designed the study and PM, MR, AA, H MV executed the study. PM analysed the data and validated the data. PM and MR wrote the manuscript draft. NR confirmed the research data and NR finalized the manuscript.

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