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1 **Heavy metal resistance genes are associated with *bla*_{NDM-1} and *bla*_{CTX-M-15-}**
2 **Enterobacteriaceae**

3

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13

14 **Abstract**

15 The occurrence of heavy metal resistance genes in multi-resistant Enterobacteriaceae possessing
16 *bla*_{NDM-1} or *bla*_{CTX-M-15} genes were examined by PCR and S1-PFGE. When compared with
17 clinical susceptible isolates (10.0-30.0%), the *pcoA*, *merA*, *silC* and *arsA* genes occurred with
18 higher frequencies in *bla*_{NDM-1} (48.8-71.8%) and *bla*_{CTX-M-15} (19.4-52.8%) positive isolates, and
19 they are mostly located on plasmids. Given the high association of metal resistance genes with
20 multidrug resistant Enterobacteriaceae, the use of heavy metals in hospitals and the environment
21 needs increased vigilance.

22

23 **Keywords:** heavy metal resistance, *bla*_{NDM-1}, *bla*_{CTX-M-15}, plasmids, co-resistance

24

25 The increasing spread of multidrug resistant ‘superbugs’ within clinical environments has
26 prompted worldwide concern, because antibiotic resistance genes such as *bla*_{NDM-1} and *bla*_{CTX-M-15}
27 leads to limit treatment options to combat bacterial infections (1-4). It is noteworthy that, in
28 addition to emerging antibiotic resistance, heavy metals represent another major sources of
29 environmental contamination that may select for antibiotic resistance (5). Heavy metal
30 compounds for growth promotion and therapeutic treatment, like zinc and cooper, have been used
31 in pig and poultry production and unlike antibiotic food additives, can accumulate in soil, water,
32 aquacultural and marine antifouling treatments or industrial effluent (6). It has been proposed that
33 antibiotic-resistant bacteria are enriched at locations contaminated with metals, and genes
34 conferring co-selection to heavy metal and antibiotic are often found together in many clinical
35 isolates (7-11). Furthermore, genes conferring heavy metal tolerance may coexist on the same
36 genetic element (e.g. plasmid), which could further promote co-dissemination and resistance (10,
37 12). Here, we characterize the phenotype and genotype of heavy metals resistance in a collection
38 of 95 clinical Gram-negative isolates including *Klebsiella pneumoniae*, *Escherichia coli*,
39 *Enterobacter cloacae*, *Klebsiella oxytoca* and *Providencia stuarti* isolated from the UK and India.
40

41 A total of 95 non-duplicate isolates were tested in this study (Table 1): 39 *bla*_{NDM-1}-positive
42 isolates originated from human lower respiratory and urinary tract samples from the United
43 Kingdom and Indian cities of Chennai and Haryana, as previously described (13); 36 *bla*_{CTX-M-15}-
44 carrying isolates, from burn, bacteraemia and UTI patients from a variety of Indian hospitals
45 (Haryana, Mumbai, Calcutta, Kerala, Delhi and Vellore); and 20 control *E. coli* and *K.*
46 *pneumoniae* susceptible to all known antibiotic classes as control samples, provided by Specialist
47 Antimicrobial Chemotherapy Unit (SACU), Public Health Wales. Minimal inhibitory
48 concentrations (MICs) of four heavy metals ions; CuSO₄·5H₂O for copper (Cu²⁺), HgCl₂ for
49 mercury (Hg²⁺), AgNO₃ for silver (Ag⁺), and AsNaO₂ for arsenic (As³⁺) were measured by agar
50 dilution using Müller-Hinton agar (Becton Dickinson, USA). *E. coli* (ATCC 25922) was used as
51 a negative control. MIC levels to Cu²⁺ (≥10 mM), As³⁺ (≥2 mM), Hg²⁺ (≥32 μM) and Ag⁺ (≥128
52 μM) were regarded as resistance (14-16). High MIC values to Cu²⁺ (10 mM), As³⁺ (20 mM) and
53 Hg²⁺ (128 μM) were obtained in the majority of *bla*_{NDM-1}-positive isolates, with a high resistance
54 rate of 82.1% (32/39), 76.9% (30/39) and 61.5% (24/39), respectively. Similarity with *bla*_{CTX-M-15}-
55 positive strains, 91.7% (33/36), 63.9% (23/36) and 52.8% (19/36) isolates were resistant to
56 Cu²⁺, As³⁺ and Hg²⁺, respectively. High MIC values (128-256 μM) for Ag⁺ were observed for all
57 isolates. Antibiotic susceptible control strains also gave high rates of resistance to Cu²⁺ (90%,
58 18/20), but remained sensitive to Hg²⁺ (15.0%, 3/20) and As³⁺ (25.0%, 5/20).

59

60 The presence of four heavy metal resistance genes was confirmed by PCR: *merA* for Hg²⁺, *arsA*
61 for As³⁺, *pcoA* for Cu²⁺ and *silC* for Ag⁺. Primers were designed by primer 3 (Geneious Pro 5.5.6)
62 and NCBI primer designing tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) (Table 2) and
63 PCRs were performed with the following condition: initial denaturation at 95°C for 5 min;

64 followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing at 58-60°C for 45
65 seconds and extension at 72°C for 45 seconds; final extension at 72°C for 5 min. The purified
66 PCR products were randomly selected for following sequencing analysis (Eurofins Genomics,
67 Germany). The *silC*, *merA*, *pcoA* and *arsA* genes were dispersed throughout our *bla*_{NDM-1}-positive
68 isolates, with 28/39 (71.8%), 26/39 (66.7%), 25/39 (64.1%) and 19/39 (48.7%), respectively (Fig.
69 1). Similarly, in *bla*_{CTX-M-15} producing isolates, the most prevalent heavy metal resistance gene
70 was *merA* (19/36, 52.8%). The genes of *arsA*, *pcoA* and *silC* were only detected in 7 (19.4%), 15
71 (41.7%) and 15 (41.7%) isolates, respectively. In contrast, the relative low prevalence of *pcoA*,
72 *silC*, *arsA* and *merA* genes were identified in susceptible isolates with detection rates of 30.0%
73 (6/20), 25.0% (5/20), 20% (4/20) and 10% (2/20), respectively (Fig. 1). In addition, the statistical
74 comparisons with these metal resistance genes in three groups of isolates, were conducted using
75 Chi-square (and fisher's exact) test, where *p* value equal or less than 0.05 was considered as
76 significant. The prevalence of *silC* (71.8% vs 25.0%, *p*=0.0009), *merA* (66.7% vs 10.0%,
77 *p*<0.0001), *pcoA* (64.1% vs 30.0%, *p*=0.0158) and *arsA* (48.7% vs 20.0%, *p*=0.0482) genes
78 detected in *bla*_{NDM-1}-positive isolates, are all markedly higher than those in susceptible isolates.
79 Furthermore, the detection rates of *silC* (71.8% vs 41.7%, *p*=0.0108) and *arsA* (48.7% vs 19.4%,
80 *p*=0.0144) in *bla*_{NDM-1}-positive isolates are also significantly higher, comparing to that in *bla*_{CTX-}
81 _{M-15}-producing isolates (Fig. 1).

82 Previous studies have proposed the role of plasmids in conferring resistance to both antibiotics
83 and heavy metals (7, 17, 18). In this study, the location of the *pcoA*, *merA*, *silC* and *arsA* genes
84 were analysed by Pulsed-field gel electrophoresis (PFGE) with S1 nuclease (Invitrogen
85 Abingdon, UK) (S1-PFGE). In brief, isolates carrying heavy metal resistance genes were
86 randomly selected and genomic DNA in agarose blocks was digested with S1 nuclease and

87 probed. In-gel hybridisation was performed with *pcoA*, *merA*, *silC* and *arsA* genes probe labelled
88 with ³²P with a random primer method (Stratgene, Amsterdam, Netherlands). The results showed
89 that *pcoA*, *merA*, *silC* and *arsA* genes are located on a diverse range of plasmids backbones,
90 differing from 50- to 500 kb in size (Fig. 2 and Fig. S1). Heavy metal resistance genes were
91 carried upon more than one plasmid in many strains and chromosomal located genes were also
92 identified (Fig. 2 and Fig. S1), suggesting significant plasticity.

93

94 Conjugation experiments were performed as described previously (13), to investigate co-transfer
95 of heavy metal and antibiotic resistance genes. Conjugations were performed with *bla*_{NDM-1} and
96 *bla*_{CTX-M-15}-positive donors with the rifampin-resistant recipient *E. coli* UAB190. Selection of
97 *bla*_{CTX-M-15}-positive transconjugants was performed on Brilliance UTI Clarity agar (Oxoid Ltd.,
98 Basingstoke, United Kingdom) supplemented with rifampicin (100 mg/L) (Sigma-Aldrich, St.
99 Louis, MO, USA) and cefotaxime (2 mg/L). *bla*_{NDM-1}-positive transconjugants were selected
100 using rifampicin with meropenem (0.5 mg/L) (AstraZeneca, London, United Kingdom). PCR for
101 *bla*_{NDM-1} and *bla*_{CTX-M-15} genes were used for further confirmation of gene transfer (13, 19).
102 Plasmid incompatibility groups were characterized by PCR-based replicon typing as previously
103 described (20). A total of 18 and 14 transconjugants were obtained in *E. coli* UAB190 from 39
104 *bla*_{NDM-1} and 36 *bla*_{CTX-M-15} isolates, respectively. In 11 of 18 transconjugants, *bla*_{NDM-1} was
105 located upon IncA/C-type plasmids, 78.6% (11/14) of plasmids carrying *bla*_{CTX-M-15} belonged to
106 IncFII, reflective of global molecular epidemiology (2, 21). Plasmids carrying *bla*_{NDM-1} from six
107 transconjugants could not be typed. The heavy metal resistance genes *arsA*, *merA* and *pcoA* were
108 found on two *bla*_{NDM-1} and one *bla*_{CTX-M-15} positive plasmids, respectively (Table 1).

109

110 Our data indicates the abundant and mobility of heavy metals resistance genes (*pcoA*, *merA*, *silC*
111 and *arsA*) that can contribute to antibiotic resistant genes dissemination and maintenance.
112 Furthermore, many of these genes are found on transmissible plasmids. Therefore, our findings
113 suggest that the co-selection of heavy-metal resistance genes in *bla*_{NDM-1} and *bla*_{CTX-M-15} positive
114 isolates have significant implications for hospital and environmental (industrial waste)
115 contamination with heavy metals.

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117

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121

122 Conflict of interest: none declared

123

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193 200.
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202 Table 1. Phenotypic and genotypic resistances to heavy metals in 95 clinical strains in this study

Strains ID	bacterial organism	Phenotype (MIC)				Genotype
		Ag(uM)	Hg(uM)	Cu(mM)	As(mM)	
39 bla _{NDM-1} strains						
N1	<i>Klebsiella pneumoniae</i>	128	128	10	0.625	<i>merA, silC</i>
N2	<i>Klebsiella pneumoniae</i>	128	128	10	2.5	<i>arsA, merA</i>
N3	<i>Citrobacter freundii</i>	128	128	10	2.5	<i>arsA, merA</i>
N4	<i>Enterobacter cloacae</i>	128	16	10	20	<i>pcoA, silC</i>
N5	<i>Enterobacter spp.</i>	128	16	5	1.25	neg.
N6	<i>Escherichia coli</i>	128	128	10	20	<i>arsA, merA, pcoA, silC</i>
N7	<i>Klebsiella pneumoniae</i>	128	128	10	10	<i>arsA, merA, pcoA, silC</i>
N8	<i>Klebsiella pneumoniae</i>	128	128	10	20	<i>arsA, merA, pcoA, silC</i>
N9	<i>Klebsiella pneumoniae</i>	128	16	10	0.625	<i>pcoA, silC</i>
N10	<i>Klebsiella pneumoniae</i>	128	16	10	0.625	<i>silC</i>
N11	<i>Klebsiella pneumoniae</i>	128	16	10	0.625	<i>silC</i>
N12	<i>Klebsiella pneumoniae</i>	256	128	10	10	<i>arsA, merA, pcoA, silC</i>
N13	<i>Citrobacter freundii</i>	256	128	10	10	<i>arsA, merA, pcoA, silC</i>
N14	<i>Escherichia coli</i>	128	128	10	10	<i>arsA, merA, pcoA, silC</i>
N15	<i>Escherichia coli</i>	128	16	5	1.25	<i>pcoA, silC</i>
N16	<i>Klebsiella pneumoniae</i>	128	128	10	1.25	<i>arsA, merA, pcoA, silC</i>
N17	<i>Klebsiella pneumoniae</i>	128	128	10	20	<i>arsA, merA, pcoA, silC</i>
N18	<i>Klebsiella pneumoniae</i>	128	64	10	10	<i>arsA, merA, pcoA, silC</i>
N19	<i>Klebsiella pneumoniae</i>	128	128	10	20	<i>arsA, merA, pcoA, silC</i>
N20	<i>Escherichia coli</i>	128	16	5	2.5	neg.
N21	<i>Klebsiella pneumoniae</i>	128	128	10	2.5	<i>merA, pcoA, silC</i>
N22	<i>Klebsiella pneumoniae</i>	128	128	10	2.5	<i>merA, pcoA, silC</i>
N23	<i>Escherichia coli</i>	128	128	5	0.625	neg.
N26	<i>Enterobacter spp</i>	128	128	10	10	<i>arsA, merA, pcoA</i>
N27	<i>Klebsiella pneumoniae</i>	128	128	5	10	<i>arsA, merA, pcoA, silC</i>
N28	<i>Klebsiella oxytoca</i>	128	16	10	5	<i>arsA, merA, pcoA, silC</i>
N29	<i>Escherichia coli</i>	128	16	10	10	<i>arsA, silC</i>
N31	<i>Enterobacter cloacae</i>	128	16	10	20	<i>pcoA, arsA, silC</i>
N32	<i>Enterobacter cloacae</i>	128	16	10	0.625	<i>pcoA, silC, merA, arsA</i>
K15	<i>Klebsiella pneumoniae</i>	128	16	10	5	<i>merA, pcoA, silC</i>
K7	<i>Klebsiella pneumoniae</i>	128	128	10	2.5	<i>merA, pcoA, silC</i>
IR25	<i>Klebsiella pneumoniae</i>	128	128	10	5	<i>merA</i>
IR18k	<i>Klebsiella pneumoniae</i>	128	128	10	20	<i>merA</i>
IR28k	<i>Klebsiella pneumoniae</i>	128	128	10	20	<i>merA, pcoA, silC</i>
IR29	<i>Escherichia coli</i>	128	128	5	5	<i>merA, pcoA, silC</i>
IR26	<i>Escherichia coli</i>	128	128	5	5	neg.
IR22	<i>Escherichia coli</i>	128	16	5	5	neg.
IR61	<i>Klebsiella oxytoca</i>	128	16	10	20	neg.
IR5	<i>Escherichia coli</i>	128	128	10	20	<i>arsA, merA, pcoA, silC</i>

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Table 1 continued.

Strains ID	bacterial organism	Phenotype (MIC)				Genotype
		Ag(μ M)	Hg(μ M)	Cu(mM)	As(mM)	
36 bla _{C_{TX-M-15}} strains						
A5/3	<i>Klebsiella pneumoniae</i>	128	16	10	5	<i>arsA, pcoA, silC</i>
A5/7	<i>Klebsiella pneumoniae</i>	128	128	10	20	<i>arsA, merA, pcoA, silC</i>
A5/4	<i>Klebsiella pneumoniae</i>	128	128	5	5	<i>pcoA, silC</i>
C5/8	<i>Klebsiella pneumoniae</i>			10	0.625	<i>arsA, merA</i>
C5/7	<i>Klebsiella pneumoniae</i>	128	128	10	10	<i>arsA, merA, pcoA, silC</i>
C5/5	<i>Klebsiella pneumoniae</i>	128	16	10	5	<i>neg.</i>
D5/12	<i>Klebsiella pneumoniae</i>	128	128	10	0.15	<i>merA</i>
D5/4	<i>Klebsiella pneumoniae</i>	128	16	10	0.625	<i>pcoA, arsA</i>
E5/14	<i>Klebsiella pneumoniae</i>	128	16	10	5	<i>merA, pcoA, silC</i>
E5/17	<i>Klebsiella pneumoniae</i>	128	128	10	2.5	<i>arsA, merA, pcoA, silC</i>
G5/2	<i>Klebsiella pneumoniae</i>	128	16	10	5	<i>arsA, pcoA, silC</i>
G5/6	<i>Klebsiella pneumoniae</i>	128	128	10	0.3	<i>merA</i>
G5/11	<i>Klebsiella pneumoniae</i>	128	128	10	0.3	<i>merA, pcoA, silC</i>
I5/5	<i>Klebsiella pneumoniae</i>	128	128	10	20	<i>merA, pcoA, silC</i>
F5/6	<i>Klebsiella pneumoniae</i>	128	16	10	0.3	<i>neg.</i>
E5/19	<i>Klebsiella pneumoniae</i>	128	128	10	5	<i>merA, pcoA, silC</i>
A4/8	<i>Escherichia coli</i>	128	16	10	0.3	<i>neg.</i>
F4/3	<i>Escherichia coli</i>	128	16	10	5	<i>neg.</i>
B4/6	<i>Escherichia coli</i>	128	16	10	2.5	<i>neg.</i>
A4/11	<i>Escherichia coli</i>	128	16	10	5	<i>neg.</i>
C4/3	<i>Escherichia coli</i>	128	128	10	2.5	<i>merA</i>
E4/4	<i>Escherichia coli</i>	128	128	10	2.5	<i>neg.</i>
D4/12	<i>Escherichia coli</i>	128	16	10	2.5	<i>merA</i>
C4/12	<i>Escherichia coli</i>	128	64	10	2.5	<i>merA</i>
G4/12	<i>Escherichia coli</i>	128	16	10	2.5	<i>neg.</i>
I4/9	<i>Escherichia coli</i>	128	128	10	2.5	<i>merA</i>
I4/3	<i>Escherichia coli</i>	128	16	10	0.3	<i>neg.</i>
I4/13	<i>Escherichia coli</i>	128	16	5	2.5	<i>merA, pcoA, silC</i>
H4/5	<i>Escherichia coli</i>	128	16	10	0.3	<i>neg.</i>
H6/20	<i>Salmonella spp.</i>	128	128	10	0.15	<i>neg.</i>
G6/9	<i>Salmonella spp.</i>	128	16	10	0.625	<i>merA, pcoA, silC</i>
G6/13	<i>Salmonella spp.</i>	128	64	10	0.15	<i>merA, silC</i>
I2/5	<i>Enterobacter spp.</i>	128	128	10	20	<i>pcoA, silC</i>
I2/2	<i>Enterobacter spp.</i>	128	128	10	20	<i>pcoA, silC</i>
F2/6	<i>Enterobacter spp.</i>	128	128	0.625	0.15	<i>merA</i>
B1/10	<i>Providencia stuarti</i>	128	128	10	20	<i>merA</i>

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Table 1 continued.

Strains ID	bacterial organism	Phenotype (MIC)				Genotype
		Ag(uM)	Hg(uM)	Cu(mM)	As(mM)	
20 Susceptible strains						
Kp ff160	<i>Klebsiella pneumoniae</i>	128	128	10	10	<i>arsA, merA, pcoA, silC</i>
Kpff217	<i>Klebsiella pneumoniae</i>	128	16	10	0.3	<i>pcoA, silC</i>
KpFF11	<i>Klebsiella pneumoniae</i>	128	128	10	5	<i>arsA, merA, pcoA, silC</i>
KpFF197	<i>Klebsiella pneumoniae</i>	128	16	10	0.625	<i>silC</i>
KpFF177	<i>Klebsiella pneumoniae</i>	128	16	10	0.3	<i>pcoA</i>
KpFF296	<i>Klebsiella pneumoniae</i>	128	16	10	10	<i>arsA, pcoA, silC</i>
KpFF101	<i>Klebsiella pneumoniae</i>	256	16	10	10	<i>neg.</i>
KpFF264	<i>Klebsiella pneumoniae</i>	128	16	10	0.15	<i>neg.</i>
KpFF267	<i>Klebsiella pneumoniae</i>	128	16	10	0.15	<i>neg.</i>
KpFF153	<i>Klebsiella pneumoniae</i>	128	16	10	0.3	<i>pcoA</i>
Ec66	<i>Escherichia coli</i>	128	8	10	0.15	<i>neg.</i>
Ec9	<i>Escherichia coli</i>	128	16	10	0.15	<i>neg.</i>
Ec63	<i>Escherichia coli</i>	128	8	10	0.15	<i>neg.</i>
Ec59	<i>Escherichia coli</i>	128	8	5	0.15	<i>neg.</i>
Ec60	<i>Escherichia coli</i>	128	16	5	0.15	<i>neg.</i>
Ec166	<i>Escherichia coli</i>	128	8	10	0.15	<i>neg.</i>
Ec284	<i>Escherichia coli</i>	128	8	10	0.625	<i>neg.</i>
Ec61	<i>Escherichia coli</i>	128	128	10	5	<i>neg.</i>
Ec141	<i>Escherichia coli</i>	128	16	10	0.15	<i>neg.</i>
Ec98	<i>Escherichia coli</i>	128	16	10	0.15	<i>neg.</i>
Transconjugants and control strains						
25922	<i>Escherichia coli</i>	64	16	5	0.15	<i>neg.</i>
GFP	<i>Escherichia coli</i>	64	16	5	1.25	<i>neg.</i>
TCE5/19	<i>Escherichia coli</i>	64	16	5	2.5	<i>pcoA</i>
TCN12	<i>Escherichia coli</i>	128	64	5	10	<i>arsA, pcoA, merA</i>
TCN22	<i>Escherichia coli</i>	128	8	5	2.5	<i>pcoA</i>

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216 Table 2. Details of primers used for heavy metal resistance genes detection in this study

metal ions	primers	sequence (5'-3')	Tm	size(bp)	Genbank ID or GI number
Hg ²⁺ (mercury)	<i>merA_F1</i>	CTGCGCCGGGAAAGTCCGTT	58°C	1035	DQ126685
	<i>merA_R1</i>	GCCGATGAGCCGTCCGCTAC			
	<i>merA_F2</i>	GAGCTTCAACCCTTCGACCA	60°C	849	575669924
	<i>merA_R2</i>	AGCGAGACGATTCTTAAGCG			
As ³⁺ (arsenic)	<i>arsA_F1</i>	CAGTACCGACCCGGCCTCCA	58°C	861	CP000648
	<i>arsA_R1</i>	AGGCCGTGTTCCTGCGAGC			
	<i>arsA_F2</i>	GGCTGGAAAAACAGCGTGAG	58°C	1002	387605479
	<i>arsA_R2</i>	CCTGCAAATTAGCCGCTTCC			
Cu ²⁺ (copper)	<i>pcoA_F</i>	CGGCCAGGTTACGTCCGTC	58°C	1371	NC_009649
	<i>pcoA_R</i>	TGCCAGTTGCCGCATCCCTG			
Ag ⁺ (silver)	<i>silC_F1</i>	CGTAGCGCAAGCGTGTGCGGA	58°C	1090	NC_009649
	<i>silC_R1</i>	ATATCAGCGGCCCGCAGCAC			
	<i>silC_F2</i>	TTCAACGTCACGGATGCAGA	60°C	872	157412014
	<i>silC_R2</i>	AGCGTGTGCGAAACATCCTT			

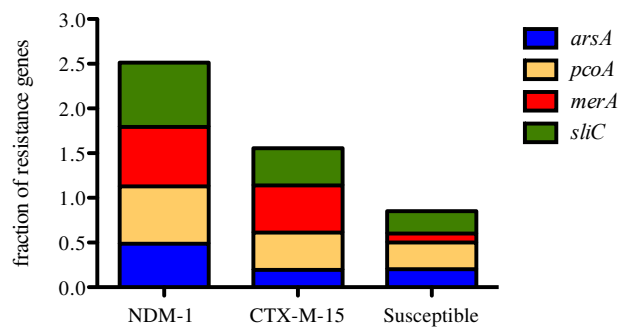
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220 Fig.1 occurrence of heavy metal resistance genes in 95 clinical isolates. *p* values were calculated
221 using Chi-square (and fisher's exact) test. *, ** and *** indicate $0.01 < p \text{ value} \leq 0.05$; $0.001 < p$
222 $\text{value} \leq 0.01$; *** indicates $p \text{ value} \leq 0.001$, respectively. 'ns' indicates not significant difference.
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225 Fig. 2. PFGE analysis of *bla*_{NDM-1}-positive strains digested with S1 nuclease, and hybridization with *pcoA*
226 gene probe (a), *silC* gene probe (b), respectively.
227 Isolates order of lanes 1-14 in A: N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, N11, N12, N13 and N14.
228 Isolates order of lanes 1-14 in B: N16; N17; N18; N19; N20; N21; N22; N23; N3; 26; N27; N28; N29;
229 N31.
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three groups of clinical isolates

Chi-square (Fisher's exact)test	Comparison of detection rates (<i>p</i> value)		
	<i>bla</i> _{NDM-1} vs susceptible	<i>bla</i> _{CTX-M-15} vs susceptible	<i>bla</i> _{NDM-1} vs <i>bla</i> _{CTX-M-15}
<i>arsA</i>	48.7% vs 20% (<i>p</i> =0.0482*)	19.4% vs 20% (<i>p</i> =1.0 ns)	48.7% vs 19.4% (<i>p</i> =0.0144*)
<i>pcoA</i>	64.1% vs 30% (<i>p</i> =0.0158*)	41.7% vs 30% (<i>p</i> =0.5653_ns)	64.1% vs 41.7% (<i>p</i> =0.0657_ns)
<i>merA</i>	66.7% vs 10% (<i>p</i> <0.0001***)	52.8% vs 10% (<i>p</i> =0.0016**)	66.7% vs 52.8% (<i>p</i> =0.2463(ns))
<i>sliC</i>	71.8% vs 25% (<i>p</i> =0.0009***)	41.7% vs 25% (<i>p</i> =0.2555_ns)	71.8% vs 41.7% (<i>p</i> =0.0108*)

