



Metallo-B-Lactamase and Carbapenemases Producing Bacteria Isolated from Animals and Their Environment at Epidemiology Laboratory of Indian Veterinary Research Institute

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Abstract

Over last six years (April 2011 to August 2017), of the 2968 bacterial isolates from different clinically sick animals and their environment tested for their antimicrobial sensitivity (by disc diffusion assay and E-test as per CLSI), 480 were resistant to carbapenem drugs (meropenem or imipenem or ertapenem). On further characterization of the 480 carbapenem-resistant bacteria, 124 were phenotypically characterized as Metallo-B-lactamase (MBL) producers using double disc diffusion assay and E-Test (for imipenem and imipenem+EDTA) and 51 were genotypically confirmed carrying one or more known Carbapenemase genes (Figure 1). Of the 51 genotypically MBL positive 43 were confirmed to carry New Delhi Metallo-B-lactamase (NDM), one *Acinetobacter lowffii* carried *Klebsiella pneumoniae* carbapenemase (KPC), two *Shewanella* sp. strains were positive for Verona integromediate carbapenemase (VIM), four strains (2 of *Escherichia coli*, one each of *Aeromonas bestiarum* and *Raoultella terrigena*) carried OXA beta-lactamases (OXA) and one *E. coli* strain had both NDM and

OXA on the plasmid. Seventy-three strains were phenotypically MBL type but no MBL gene was detected using primers for reported genes (Figure 2). Rest of the 356 carbapenem-resistant but negative for any of the known carbapenemase gene either genotypically or phenotypically belonged to more than 109 species of bacteria (Figure 3, 4, 5). Sources of carbapenem-resistant bacteria are shown in figure 6 and 7.

Table 1. List of bacteria (51) Genotypically positive for Carbapenemase gene(s) and Isolated at Epidemiology Laboratory of Indian Veterinary Research Institute from Different Sources (Clinical and Post-mortem Samples and Foods of Animals) from April 2011 to August 2017

Genes for Carbapenem resistance detected	Bacteria carrying genes (Source, number of isolates)
NDM+OXA	<i>Escherichia coli</i> (Pig 1)
KPC	<i>Acinetobacter lowffii</i> (Cattle 1)
OXA	<i>Escherichia coli</i> (Pigs 2)
OXA	<i>Raoultella terrigena</i> (Cattle 1)
OXA	<i>Aeromonas bestiarum</i> (Cattle 1)
VIM	<i>Shewanella</i> spp. (Pigs 2)
NDM	<i>Aeromonas jandaari</i> (Leopard 1)
NDM	<i>Aeromonas popoffii</i> (Cattle 2)
NDM	<i>Aeromonas trola</i> (Cattle 1)
NDM	<i>Budvicia aquatica</i> (Dog 1)
NDM	<i>Citrobacter freundii</i> (Hospital sewage 1)
NDM	<i>Edwardsiella ictaluri</i> (Leopard 1)
NDM	<i>Enterobacter agglomerans</i> (Human 1, Food 1)
NDM	<i>Escherichia coli</i> (Cattle 5, Dogs 9, Leopards 6, Pigs 9, Deer 1, Tiger 1)
NDM	<i>Salmonella enterica</i> ssp. <i>enterica</i> (Chicks 1)
NDM	<i>Staphylococcus felis</i> (Cattle 1)
NDM	<i>Staphylococcus xylosum</i> (Dog 1)

Fig. 1: Table 1. List of bacteria (51) Genotypically positive for Carbapenemase gene(s) and Isolated at Epidemiology Laboratory of Indian Veterinary Research Institute from Different Sources (Clinical and Post-mortem Samples and Foods of Animals) from April 2011 to August 2017

Table 2. List of Bacteria (73) Phenotypically MBL Positive (by double disc diffusion method and E-test) but Negative with PCR for any of the known Carbapenemase genes.

<i>Achromobacter</i> spp.	1	Cattle
<i>Acinetobacter calcoaceticus</i>	1	Cattle
<i>Aeromonas hydrophila</i>	1	Pig
<i>Alcaligenes faecalis</i>	1	Pig
<i>Citrobacter freundii</i>	1	Swage
<i>Enterobacter agglomerans</i>	2	Swamp b uffaloes
<i>Enterobacter sp.</i>	1	Soil
<i>Escherichia coli</i>	23	Cattle 7, Dogs 7, House floor 1, Horse 1, Humans 3, Pigs 3, Tiger 1 Cattle 3, Horse 3, Hospital sewage 1, Mithun (<i>Bos frontalis</i>) 1, Swamp buffaloes 3, Drinking water 3
<i>Klebsiella pneumoniae</i> spp. <i>pneumoniae</i>	14	
<i>Proteus mirabilis</i>	9	Dogs 2, Lions 2, Pigs 5
<i>Proteus vulgaris</i>	1	Tiger 1
<i>Pseudomonas aeruginosa</i>	9	Cattle 7, Black buck 1, Dog 1
<i>Pseudomonas</i> sp.	2	Dogs 2
<i>Raoultella terrigena</i>	3	Human 1, Lions 2
<i>Roseomonas</i> sp.	1	Dog 1
<i>Salmonella enterica</i> ssp. <i>enterica</i>	1	Pig 1
<i>Staphylococcus chromogenes</i>	1	Tiger 1
<i>Staphylococcus warneri</i>	1	Buffalo 1

Fig. 2: Table 2. List of Bacteria (73) Phenotypically MBL Positive (by double disc diffusion method and E-test) but Negative with PCR for any of the known Carbapenemase genes.

Table 3. List of Carbapenem (Meropenem or Imipenem or Ertapenem) Resistant Bacteria (356 of more than 109 species) isolated at Epidemiology Laboratory of Indian Veterinary Research Institute from April 2011 to August 2017 from Different Animal sources and their Environment were Phenotypically and Genotypically Negative for Metallo-β-lactamases and other Carbapenemases.

Bacteria	Nos.	Source of isolation
<i>Achromobacter</i> sp.	2	Cattle 2
<i>Acinetobacter calcoaceticus</i>	5	Cattle 1, House floor 1, Pigs 3
<i>Acinetobacter baumannii</i>	3	Cattle 1, Pig 1, Swamp buffalo 1
<i>Acinetobacter lovffii</i>	4	Cattle 2, House floor 2
<i>Acinetobacter schindleri</i>	4	Cattle 4
<i>Acinetobacter</i> sp.	5	Cattle 2, Human 1, Pigs 2
<i>Actinobacillus seminis</i>	2	Buffalo 2
<i>Actinobacillus</i> sp.	1	Human 1
<i>Actinomyces propionius</i>	2	Pigs 2
<i>Actinomyces pyogenes</i>	1	Horse 1
<i>Aerococcus</i> sp.	1	Human 1
<i>Aeromonas bestiarum</i>	6	Buffalo 1, Pigs 3, Tiger 1
<i>Aeromonas caviae</i>	2	Cattle 1, Pig 1
<i>Aeromonas euranophila</i>	2	Cattle 2
<i>Aeromonas hydrophila</i>	5	Human 2, Pig 1, Swamp buffaloes 2
<i>Aeromonas media</i>	7	Buffaloes 2, Pigs 3, Swam buffaloes 2
<i>Aeromonas popoffii</i>	3	Cattle 3
<i>Aeromonas tobiia</i>	3	Pigs 3
<i>Aeromonas troa</i>	1	Cattle 1
<i>Alcaligenes denitrificans</i>	5	Dog 1, Pigs 4
<i>Alcaligenes faecalis</i>	4	Cattle 2, Pig 1, Poultry birds 1
<i>Bacillus bishwiformis</i>	1	Horse
<i>Bacillus pumilus</i>	2	FMD Vaccine vials 2
<i>Bacillus sphaericus</i>	1	Dog 1
<i>Bacillus</i> spp.	4	Dog 1, Elephant 1, Hog deer 1, Horse 1
<i>Bordetella bronchiseptica</i>	2	Pig 1, Tiger 1
<i>Bruceella abortus</i>	2	Cattle 2
<i>Citrobacter freundii</i>	1	Fish 1
<i>Edwardsiella ictaluri</i>	1	Leopard
<i>Edwardsiella tarda</i>	1	Dog 1
<i>Enterobacter agglomerans</i>	22	Cattle 5, dogs 4, Farm air 4, Horse 2, Human 1, Pigs 3, Swamp buffaloes 2, Tiger 1
<i>Enterobacter amnigenus</i>	2	Buffalo 1, Cattle 1
<i>Enterococcus asaccharolyticus</i>	2	Bison 1, Porcupine 1
<i>Enterococcus durans</i>	1	Buffalo 1
<i>Enterococcus faecalis</i>	5	FMD vaccine vials 3, Human 1, Pig 1
<i>Enterococcus faecium</i>	9	Pigs 9
<i>Enterococcus malodoratus</i>	3	Sambhar deer 2, Spotted deer 1
<i>Enterococcus pseudoavium</i>	1	Dog 1
<i>Enterococcus raffinosus</i>	6	Elephants 2, Lions 2, Pigs 2
<i>Enterococcus solitarius</i>	2	Pigs 2

Fig. 3: Table 3a. List of Carbapenem (Meropenem or Imipenem or Ertapenem) Resistant Bacteria (356 of more than 109 species) isolated at Epidemiology Laboratory of Indian Veterinary Research Institute from April 2011 to August 2017 from Different Animal sources and their Environment were Phenotypically and Genotypically Negative for Metallo-β-lactamases and other Carbapenemases.

<i>Enterococcus</i> sp.	1	Pig 1
<i>Erwinia anglyvora</i>	1	Swamp buffalo 1
<i>Erwinia ananas</i>	1	Mithun 1
<i>Erwinia cactidis</i>	1	Elephant 1
<i>Erwinia carotovora</i>	1	Pig 1
<i>Erwinia chrysanthemi</i>	2	Elephant 1, Fish 1
<i>Erwinia cytophaga</i>	1	Buffalo
<i>Erwinia rhapsodicti</i>	1	Black buck 1
<i>Erwinia ureovorora</i>	1	Barling deer 1
<i>Escherichia coli</i>	48	Buffaloes 2, Cattle 20, Dogs 10, House floor 3, Horse 1, Humans 3, Pigs 5, Swamp buffalo 1, Swamp deer 1, Tiger 2 Cattle 1, Human 1
<i>Escherichia fergusonii</i>	2	
<i>Haemophilus</i> sp.	1	Pig 1
<i>Klebsiella pneumoniae</i> spp. <i>pneumoniae</i>	4	Cattle 1, Dogs 2, Elephant 1
<i>Micrococcus varians</i>	2	Buffaloes 2
<i>Micrococcus</i> sp.	3	Dog 1, Tigrisses 2
<i>Moraxella atlantae</i>	2	Cattle 2
<i>Moraxella canis</i>	2	Dogs 2
<i>Moraxella carvalhoi</i>	1	Dog 1
<i>Morganella morganii</i>	1	Dog 1
<i>Pasteurella canis</i>	1	Dog 1
<i>Pragia fontium</i>	1	Cattle 1
<i>Proteus mirabilis</i>	11	Cattle 6, Dog 3, Duckling 1, Goat 1
<i>Proteus penneri</i>	4	Cattle 2, Dog 1, Pig 1
<i>Proteus vulgaris</i>	2	Human 1, Mutton 1
<i>Providencia alkalicifera</i>	1	Goat 1
<i>Providencia alkalicifera</i>	1	Goat 1
<i>Providencia stuartii</i>	1	Horse 1
<i>Pseudomonas aeruginosa</i>	22	Cattle 6, Dogs 10, Horse 3, Pigs 3
<i>Pseudomonas alcaligenes</i>	2	Dog 1, Pig 1
<i>Pseudomonas fluorescens</i>	4	Cattle 1, Fish 3
<i>Pseudomonas paucibacillus</i>	1	Cattle 1
<i>Pseudomonas paucimobidis</i>	3	Horse 1, Pigs 2
<i>Pseudomonas pseudoalcaligenes</i>	2	Cattle 1, Swamp buffalo 1
<i>Pseudomonas stutzeri</i>	1	Horse 1
<i>Pseudomonas</i> sp.	3	Humans 2, Hospital air 1
<i>Raoultella terrigena</i>	4	Cattle 4
<i>Serratia plymuthica</i>	2	Fish 2
<i>Staphylococcus aureus</i>	1	Dog 1
<i>Staphylococcus aureus</i>	6	Cats 2, Cattle 1, Buffaloes 2, Aloo Tikka 1
<i>Staphylococcus aureus</i>	3	Dogs 2, Horse 1
<i>Staphylococcus capitis</i> spp. <i>urealyticus</i>	1	Rat 1
<i>Staphylococcus carnosus</i>	5	Humans 5
<i>Staphylococcus caseolyticus</i>	1	Cattle 1
<i>Staphylococcus cohnii</i>	3	Dog 1, Mutton 1, Kabab 1
<i>Staphylococcus epidermidis</i>	1	Human 1

Fig. 4: Table 3b. List of Carbapenem (Meropenem or Imipenem or Ertapenem) Resistant Bacteria (356 of more than 109 species) isolated at Epidemiology Laboratory of Indian Veterinary Research Institute from April 2011 to August 2017 from Different Animal sources and their Environment were Phenotypically and Genotypically Negative for Metallo-β-lactamases and other Carbapenemases.

<i>Staphylococcus haemolyticus</i>	8	Dogs 5, Goat 1, Human 1, Pig 1
<i>Staphylococcus hominis</i>	1	Human 1
<i>Staphylococcus hyicus</i>	3	Cattle 1, Dog 1, Horse 1
<i>Staphylococcus intermedius</i>	2	Dogs 2
<i>Staphylococcus schleiferi</i>	1	Horse 1
<i>Staphylococcus scrofae</i>	1	Dog 1
<i>Staphylococcus xylosum</i>	2	Milk 1, pig 1
<i>Staphylococcus sp.</i>	1	Human 1
<i>Streptococcus bovis</i>	3	Pigs 3
<i>Streptococcus canis</i>	1	Hyena 1
<i>Streptococcus dysgalactiae</i>	1	Cattle 1
<i>Streptococcus equi ssp. zooepidemicus</i>	3	Horses 3
<i>Streptococcus infantis</i>	1	Cattle 1
<i>Streptococcus milleri</i>	6	Cattle 1, Dogs 4, Human 1
<i>Streptococcus mitis</i>	1	elephant 1
<i>Streptococcus pyogenes</i>	2	Buffalo 1, Dog 1
<i>Streptococcus porcinus</i>	1	Dog 1
<i>Streptococcus pyogenes</i>	4	Goat 1, Hyena 1, Pigs 2
<i>Streptococcus sanguis</i>	3	Pigs 3
<i>Streptococcus suis</i>	1	Cattle 1
<i>Streptococcus uberis</i>	2	Blackbuck 1, Lion 1
<i>Streptococcus spp.</i>	7	Cattle 1, Horses 3, Pigs 3
<i>Vibrio anguillarum</i>	1	Swamp buffalo 1
<i>Xenorhabdus bovirhini</i>	1	Buffalo 1

Fig. 5: Table 3c. List of Carbapenem (Meropenem or Imipenem or Ertapenem) Resistant Bacteria (356 of more than 109 species) isolated at Epidemiology Laboratory of Indian Veterinary Research Institute from April 2011 to August 2017 from Different Animal sources and their Environment were Phenotypically and Genotypically Negative for Metallo--lactamases and other Carbapenemases.

Table 4. Carbapenem resistant bacteria from different animal sources and detection of different carbapenemases

Source of isolation	Carbapenem resistant	NDM	NDM+OXA	KPC	OXA	VIM
Environment	19	1	0	0	0	0
Deer	10	1	0	0	0	0
Bison	1	0	0	0	0	0
Buffalo	17	0	0	0	0	0
Cat	2	0	0	0	0	0
Cattle	114	9	0	1	2	0
Dog	85	11	0	0	0	0
Poultry birds including ducks	3	1	0	0	0	0
Elephant	7	0	0	0	0	0
Fish	7	0	0	0	0	0
FMD Vaccine	5	0	0	0	0	0
Food/ Feed	6	1	0	0	0	0
Goats	4	0	0	0	0	0
Horses	25	0	0	0	0	0
Humans	29	1	0	0	0	0
Hyena	2	0	0	0	0	0
Leopards	9	8	0	0	0	0
Lions	7	0	0	0	0	0
Mithun	2	0	0	0	0	0
Pigs	95	9	1	0	2	2
Porcupine	1	0	0	0	0	0
Rat	1	0	0	0	0	0
Swamp buffaloes	16	0	0	0	0	0
Tigers	12	1	0	0	0	0
Total	479	43	1	1	4	2

Fig. 6: Table 4. Carbapenem resistant bacteria from different animal sources and detection of different carbapenemases

Table 5. Carbapenem resistant bacteria associated with different affections in animals and detection of different carbapenemases

Affection/ Disorder	Carbapenem resistant isolates	Detection of different carbapenemases				
		NDM	NDM+OXA	KPC	OXA	VIM
Abortion	73	7	0	1	2	0
Abscess, Wounds and other topical infections	33	3	0	0	0	0
Apparently Healthy	29	13	1	0	2	0
Bronchitis	1	0	0	0	0	0
Death	51	2	0	0	0	0
Diarrhoea and enteritis	85	11	0	0	0	2
Infertility	2	0	0	0	0	0
Joint ill	1	0	0	0	0	0
Mastitis	7	0	0	0	0	0
Megacolon	3	1	0	0	0	0
Nasal discharge	14	0	0	0	0	0
Naval ill	3	0	0	0	0	0
Conjunctivitis	4	0	0	0	0	0
Orchitis	1	1	0	0	0	0
Otorrhoea	19	0	0	0	0	0
Prostatitis	1	0	0	0	0	0
Pyometra	3	1	0	0	0	0
Pyrexia of unknown origin	2	0	0	0	0	0
Respiratory tract infections	3	0	0	0	0	0
Urinary tract infections	33	2	0	0	0	0

Fig. 7: Table 5. Carbapenem resistant bacteria associated with different affections in animals and detection of different carbapenemases