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Epidemiology of carbapenem-resistant *Escherichia coli* and first report of *bla*VIM carbapenemases gene in calves from India

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Abstract

A cross-sectional study on six dairy farms was conducted to ascertain the occurrence of carbapenem-resistant *Escherichia coli* in calves. Two-hundred and seventy-nine isolates of *E. coli* were recovered from 90 faecal samples from apparently healthy (45) and diarrhoeal (45) calves. The isolates were screened for phenotypic susceptibility to carbapenems and production of metallo β-lactamase, as well as five carbapenemase resistance genes by PCR, and overexpression of efflux pumps. Eighty-one isolates (29.03%) were resistant to at least one of three carbapenem antibiotics [meropenem (23.30%), imipenem (2.15%) and ertapenem (1.43%)], and one isolate was positive for the *bla*VIM gene which was located on an Incl1 plasmid of a novel sequence type (ST 297) by multilocus sequence typing. The majority (83.95%) of isolates had an active efflux pump. Calves housed on concrete floors were approximately seven times more likely to acquire meropenem-resistant isolates than those housed on earthen floors (95% CI 1.27–41.54). In India, carbapenem drugs are not used in food animal treatment, hence carbapenem-resistant strains in calves possibly originate from the natural environment or human contact and is of public health importance. To our knowledge, this is the first report of *bla*VIM carbapenemases gene in calves from India.

Introduction

Carbapenems are one of the most important groups of antimicrobials and are considered as a last line of drugs for the treatment of severe infections. Consequently, the spread of carbapenem-resistant bacteria in food-producing and companion animals is a major public health concern [1]. Resistance to carbapenems is mediated by various factors such as the loss of outer membrane porins, production of carbapenemases and overexpressed efflux pumps [2]. The increasing frequency of Gram-negative bacteria producing extended spectrum β-lactamase enzymes has led to higher carbapenem usage which has resulted in the wider occurrence and spread of carbapenemase-producing *Enterobacteriaceae* [3–4]. These bacteria in sewage and waste water may contaminate the wider environment and spread resistance genes into many species [5]. Indeed, an epidemiological linkage between carbapenemase-producing *Escherichia coli* and *Klebsiella* spp., isolated from hospital-acquired and community infections, and a contaminated urban water supply has been previously made in India [6]. Moreover, the unregulated use of antimicrobials as prophylactics or therapeutics in food animals [7] has possibly impacted on the spread of resistant strains in India where no legally binding regulations are in force.

Here, we describe the results of a survey to ascertain and confirm by phenotypic and genotypic methods, the occurrence of carbapenem-resistant *E. coli* in healthy and diarrhoeal calves and explore associations of epidemiological factors with the isolation of such strains.

Materials and Methods

Farms and faecal sampling

Dairy farms located in Bareilly, India, were selected based on the owner's agreement to collect faecal samples from calves and provide details of husbandry practices (organised and unorganised) used; three each of the latter two groups of farms were chosen for sampling. In organised farms, animals were fed in stalls attended by dedicated workers, while in unorganised farms, animals were grazed in fields and daily activities were taken care of by family members. Both types of farms practiced weaning of calves after 90 days. Samples from apparently healthy (n = 45) and diarrhoeal calves (n = 45) of <3 months' age were collected at random between

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November 2013 and March 2014. Of the 90 samples, 30 and 60 samples were collected from cross-bred and native calves, respectively. Cross-bred calves were the progeny of Indian native and exotic breed cattle. Diarrhoea was defined as voiding of four or more loose stools by a calf in a 24 h period. Only calves that had not received antibacterials for a fortnight were included and diarrhoeal calves were matched with apparently healthy counterparts of the same age group. Faeces were sampled using sterile transport swabs.

Isolation of E. coli

Following enrichment in MacConkey broth at 37°C for 6–10 h, the faecal swabs were streaked on MacConkey agar and incubated at 37 °C for 12–18 h.

Antimicrobial susceptibility testing

Isolates were screened for susceptibility to the carbapenems, meropenem (10 µg), imipenem (10 µg) and ertapenem (10 µg) by disk diffusion testing (Beckton Dickinson, Sparks, Maryland, USA), and zone diameters were interpreted according to CLSI guidelines [8]. The MIC for meropenem was determined using Etests (HiMedia, Mumbai, India). Metallo β-lactamase (MBL) production was confirmed by the 'keyhole reaction' between a carbapenem and EDTA as previously described [9].

Molecular characterisation of carbapenem resistance

PCRs targeting *bla*NDM, *bla*IMP, *bla*OXA, *bla*KPC and *bla*VIM genes were performed as described [10, 11]. The reactions were optimised individually in 25 µl volumes with 10 picomole of each individual primer; amplicons were separated by electrophoresis with 0.5 X TBE buffer in 1.5% agarose gels and visualised under UV illumination (Syngene, Frederick, MD, USA). The *bla*VIM amplicon was purified and sequenced, and results were examined for homology using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence data were submitted to GenBank and assigned accession number KR296661.

Plasmid DNA from the *bla*VIM-positive *E. coli* isolate was extracted using the Qiagen Miniprep kit (Qiagen, Hilden, Germany) and subjected to multilocus sequence typing (MLST) using specific primers (https://pubmlst.org/plasmid/primers/) [12]. Amplicon sequences (Eurofins India Ltd, Bengaluru, India) were edited using BioEdit v7.0.5 and submitted to the Incl1 plasmid MLST site for allelic profile, sequence type and clonal complex, and assigned as a novel sequence type by the site curator (https://pubmlst.org/bigsdb?db=pubmlst_plasmid_seqdef).

Efflux pump screen

Isolates lacking a carbapenemase-mediated resistance mechanism were screened for overexpression of efflux pumps by the ethidium bromide agar cartwheel method described elsewhere [9, 13].

Statistical analysis

Data regarding various epidemiological factors related to calf management were collected in a suitable proforma and statistically compared with carbapenem-resistant isolates using SAS software (SAS India, Mumbai, India). Initially, χ^2/Fisher's exact tests were performed individually for each factor against the susceptibility pattern for each carbapenem. Those factors significant ($P < 0.05$) in univariate analysis were subjected to multinominal logistic regression analysis in a stepwise forward method to evaluate the association of risk factor with carbapenem susceptibility.

Results

Information about the herds was collected by the questionnaire at the time of sample collection, and data from organised farms are summarised in Table 1. The herd sizes ranged from 22 to 46 and 6 to 13 adult cattle in organised and unorganised dairies, respectively. Of the three organised farms, two housed animals on concrete floors, the other on a brick floor. Likewise, for the three unorganised farms, animals were housed on earthen (2) and brick (1) floors. All animals in the first group were fed with concentrate and roughage with drinking water supplied from a shallow well; animals in unorganised farms were fed principally with roughage. Female calves in both types of farm were fed with colostrum more frequently than male calves. None of the farms used antibiotics as a feed additive but cephalosporin and penicillin groups were commonly used for therapeutic purposes in adult animals on both types of farm. None of the farms used antibiotics for treating diarrhoea in calves.

Of 279 *E. coli* isolates recovered, 188 were from calves maintained on brick floors, 48 on earthen and 43 on concrete floors. Eighty-one (29.03%) were resistant to at least one of the three carbapenems tested in the order of meropenem (23.30%), imipenem (2.15%) and ertapenem (1.43%); 19 isolates were presumed to be MBL producers by a positive keyhole reaction.

Screens for carbapenemase genes revealed one isolate (32D) to be positive for the *bla*VIM gene, and this was resistant to meropenem (MIC = 6 µg/ml), but susceptible to the other carbapenems tested. This isolate was cultured from a 30 days old native breed male calf with diarrhoea which had been reared on a brick floor in an organised farm, and fed with colostrum and maintenance roughage alone. The *bla*VIM gene was located on an Incl1 plasmid with an MLST pattern of repl1-2; ardA-2; trbA-15; sogS-4; piL-17. This was assigned as sequence type (ST) 297. On efflux pump assay, 68/81 isolates (including isolate 32D) exhibited an active efflux pump under UV illumination. No carbapenemase resistance genes or efflux pump-mediated resistance were detected for the 13 remaining isolates.

Statistical analysis showed significantly higher imipenem resistance in *E. coli* from unorganised than organised dairies ($P < 0.01$), but there was no significant association between sex of calves and meropenem and imipenem resistance pattern ($P > 0.05$). However, ertapenem-resistant isolates were more frequently recovered from male animals ($P < 0.01$). No statistical difference was observed for meropenem ($P > 0.05$), imipenem ($P > 0.05$) and ertapenem ($P > 0.05$) resistance between *E. coli* isolates of cross-bred and native calves. Imipenem resistance was more associated with calves in the 31–60 and 61–90 days age groups ($P < 0.001$). No age group-specific resistance for meropenem ($P > 0.05$) or ertapenem ($P > 0.05$) was evident. Calves with diarrhoea harboured higher rates of meropenem ($P < 0.05$) and imipenem ($P < 0.001$) resistant isolates than healthy calves, but not for ertapenem ($P > 0.05$) resistance. Rearing on a concrete floor was associated with significantly higher meropenem ($P < 0.05$) resistance, and earthen floor calves with imipenem resistance of isolates ($P < 0.001$); moreover, imipenem resistance was more associated with calves not fed colostrum compared with their counterparts

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Table 1. Details of samples collected from organised farms

Factors		Number of faecal samples	Number of *E. coli* isolates
Floor type	Earthen	18	48
	Concrete	12	43
	Brick	60	188
Sector	Organised farm	45	159
	Unorganised	45	120
Health status	Diarrhoeal	45	163
	Apparently healthy	45	116
Sex	Male	47	130
	Female	43	149
Colostrum	Fed	84	267
	Non-fed	6	12
Greens	Fed	61	186
	Non-fed	29	93
Age group (days)	0–30	32	115
	31–60	29	87
	61–90	29	77
Deworming	Yes	29	93
	No	61	186

($P < 0.05$). Deworming status of the calves was significantly associated with the susceptibility pattern of meropenem ($P < 0.01$) and imipenem ($P < 0.001$) (Table 2).

Multinomial logistic regression of carbapenem susceptibility and epidemiological factors revealed that calves raised on concrete floors had approximately 7.2 times higher odds risk of acquiring meropenem-resistant isolates than those raised on earthen floors (OR = 7.25; 95% CI 1.27–41.54). Likewise, cross-bred calves had higher odds of acquiring meropenem intermediate resistant isolates than native calves (OR = 2.02; 95% CI 1.08–3.78) (Table 3).

Discussion

A key finding of this study was that almost 30% of *E. coli* isolates recovered from both healthy and diarrhoeal calves were not susceptible to at least one of three carbapenem agents, notably meropenem, which are not used in the dairy industry in India. The high rate of meropenem resistance might reflect the increased clinical use of both carbapenem and penem antimicrobials in the country [14]. Cited factors that may predispose to the occurrence of carbapenem resistance include overpopulation, poor sanitation and contaminated water, a tropical climate, and inappropriate antibiotic use, which together promote the spread of carbapenemase-producing bacteria among the human and animal population [15, 16].

The second noteworthy finding was the demonstration that one meropenem-resistant isolate (MIC 6 µg/ml) from a 30 days old native breed male calf with diarrhoea on an organised dairy unit harboured a plasmid-borne (Incl1) *bla*VIM gene. Such strains have been reported previously from a pig farm in Germany [17]. Recently in India, *E. coli* positive for *bla*NDM-1 and OXA-48 enzymes have been recovered from piglets [9, 18], as well as a mastitis milk sample [19]. More widely, β-lactamase-producing *E. coli* strains in pigs have been attributed to environmental contamination [20, 21], thus constituting a potential public health threat. To the best of our knowledge, this is the first report of the *bla*VIM gene in an *E. coli* strain from calf faeces in India. This strain along with 67 other isolates expressed an active efflux pump which is common among multidrug-resistant and carbapenem-resistant *E. coli* [9, 22].

Unorganised farms had a higher incidence of drug-resistant isolates than their organised counterparts which might be associated with inadequate management conditions such as hygiene and sanitation, etc., as these farms are generally run by family members in their leisure time. The finding that male calves harboured more resistant isolates may be linked with insufficient colostrum feeding and general poor care since male calves yield lower economic returns. Likewise, the higher frequency of resistant isolates from older calves could be attributable to diminishing maternal immunity and increased exposure to the environment [23].

The present study witnessed a higher incidence of antibiotic-resistant isolates in diarrhoeal calves of 3 months age, whereas Swedish dairies recorded a higher proportion of resistant isolates in neonatal diarrhoeal calves of 1–4 weeks age [20]. This finding may be associated with insufficient colostrum nutrition and reduced immune status leading to a predisposition to the development of infection and resistance. The calves maintained on concrete floors were seven times more likely to yield resistant isolates than earthen floors which might be a consequence of inefficient cleaning with mild antiseptic/detergent solutions and inadequate washing away of faecal material with clean water. In contrast, faecal material was scraped manually from earthen floors which were not cleaned with any antiseptics/detergent. It is noteworthy that an increased risk of diarrhoea in calves reared on concrete floors was also found in Norwegian dairy herds [24].

No significant differences in the resistance pattern of isolates from cross-bred and native calves were evident, although cross-bred calves are generally considered to be less immune to infections and not as adaptable to tropical climatic conditions, than native calves. Although not used in our veterinary clinical practice, the acquisition of carbapenem-resistant isolates might have occurred due to indiscriminate use of other antibiotic classes [25]. The farms in the study used penicillins, cephalosporins, tetracyclines and sulphonomides for treating adult animals but diarrhoeal calves were not treated with antibiotics. The recovery of a higher number of resistant isolates from the dairy calves might indicate the circulation of resistant pathogens in the farm environment. A specific study of the farm environment as a potential source of antimicrobial-resistant organisms for animals may therefore be worthwhile. Likewise, it has long been recognised that the increased use of carbapenems in human medicine could give rise to horizontal transfer of carbapenem resistance genes to zoonotic pathogens [2]. Indeed, it has been reported that the uncontrolled use of third-generation cephalosporins has resulted in a markedly higher likelihood of the isolation of carbapenem-resistant *Enterobacteriaceae* from human clinical settings [25]. It is therefore possible that acquisition of the *bla*VIM gene by strain 32D might have occurred through contact from humans or environmental sources, the latter being widely recognised as a primary source of antibiotic-resistant bacteria [26].

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Table 2. Univariate analysis of different epidemiological factors with carbapenem susceptibility pattern

		MRP				IMP				ETP			
Risk factor		R	I	S	*P* value	R	I	S	*P* value	R	I	S	*P* value
Floor	Brick	41 (21.81)	73 (38.83)	74 (39.36)	0.022*	0 (0.00)	14 (7.45)	174 (92.55)	0.000***	2 (1.06)	29 (15.43)	157 (83.51)	0.163
	Concrete	12 (27.91)	25 (58.14)	6 (13.95)		1 (2.33)	22 (51.1)	20 (46.51)		0 (0.00)	9 (20.93)	34 (79.01)	
	Earthen	12 (25.0)	21 (43.75)	15 (31.35)		5 (10.42)	10 (20.8)	33 (68.75)		2 (4.17)	12 (25)	34 (70.83)	
Sector	Organised	43 (27.04)	66 (41.51)	50 (31.45)	0.224	0 (0.00)	38 (23.90)	121 (76.10)	0.01**	1 (0.63)	30 (18.87)	128 (80.50)	0.414
	Unorganised	22 (18.33)	53 (44.17)	45 (37.50)		6 (5.00)	8 (6.67)	106 (88.33)		3 (2.50)	20 (16.67)	97 (80.83)	
Health status	Diarrhoeic	45 (27.61)	59 (36.20)	59 (36.20)	0.023*	4 (2.45)	16 (9.82)	143 (87.73)	0.001***	2 (1.23)	30 (18.40)	131 (80.37)	0.955
	Healthy	20 (17.24)	60 (51.72)	36 (3.03)		2 (1.72)	30 (25.8)	84 (72.41)		2 (1.72)	20 (17.24)	94 (81.03)	
Sex	Female	35 (23.49)	65 (43.62)	49 (32.89)	0.906	2 (1.34)	27 (18.1)	120 (80.54)	0.474	0 (0.00)	34 (22.82)	115 (77.18)	0.006**
	Male	30 (23.08)	54 (41.54)	46 (35.38)		4 (3.08)	19 (14.6)	107 (82.31)		4 (3.08)	16 (12.31)	110 (84.62)	
Breed	Cross-bred	19 (18.10)	57 (54.29)	29 (27.62)	0.08	2 (1.09)	14 (13.33)	89 (84.76)	0.78	2 (1.90)	23 (21.90)	80 (76.19)	0.56
	Native	21 (21.00)	39 (39.00)	40 (40.00)		3 (3.00)	11 (11.00)	86 (86.00)		2 (2.00)	16 (16.00)	82 (82.00)	
Colostrum	Fed	61 (22.85)	114 (42.70)	92 (34.46)	0.695	4 (1.50)	44 (16.4)	219 (82.02)	0.026*	4 (1.50)	44 (16.48)	219 (82.02)	0.023*
	Non-fed	4 (33.33)	5 (41.67)	3 (25.00)		2 (16.67)	2 (16.67)	8 (66.67)		0 (0.00)	6 (50.00)	6 (50.00)	
Greens	Fed	43 (23.12)	86 (46.24)	57 (30.65)	0.169	6 (3.23)	34 (18.2)	146 (78.49)	0.091	3 (1.61)	38 (20.43)	145 (77.96)	0.299
	Non-fed	22 (23.66)	33 (35.48)	38 (40.86)		0 (0.00)	12 (12.9)	81 (87.10)		1 (1.08)	12 (12.90)	80 (86.02)	
Age	0–30 days	24 (20.87)	44 (38.26)	47 (40.87)	0.171	0 (0.00)	9 (7.83)	106 (92.17)	0.001*	0 (0.00)	20 (17.39)	95 (82.61)	0.394
	31–60 days	18 (20.69)	40 (45.98)	29 (33.33)		3 (3.45)	20 (22.9)	64 (73.56)		3 (3.45)	15 (17.24)	69 (79.31)	
	61–90 days	23 (29.87)	35 (45.45)	19 (24.68)		3 (3.90)	17 (22.0)	57 (74.03)		1 (1.30)	15 (19.48)	61 (79.22)	
Deworming	Dewormed	21 (22.58)	52 (55.91)	20 (21.51)	0.002**	3 (3.23)	27 (29.03)	63 (67.74)	0.001***	1 (1.08)	22 (23.66)	70 (75.27)	0.188
	Not dewormed	44 (23.66)	67 (36.02)	75 (40.32)		3 (1.61)	19 (10.22)	164 (88.17)		3 (1.61)	28 (15.05)	155 (83.33)	

MRP, meropenem; IMP, imipenem; ERP, ertapenem.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

R, resistance; I, intermediate; S, susceptible. The numbers in the parenthesis indicates percent susceptibility.

Table 3. Multinominal logistic regression of epidemiological factors and carbapenem susceptibility, with susceptible isolates as reference category

Susceptibility pattern	Risk	Factor	B	*P* value	Exp (B)/odds ratio	95% CI
MRP resistant	Floor	Brick	0.070	0.89	1.07	0.37–3.09
		Concrete	1.981	0.03*	7.25	1.27–41.54
		Earthen	1 (Ref)			
MRP intermediate susceptible	Breed	Cross-bred	0.701	0.03*	2.02	1.08–3.78
		Native	1 (Ref)			
IMP intermediate susceptible	Sector	Organised	1.219	0.01**	3.38	1.33–8.61
		Unorganised	1 (Ref)			
ERP intermediate susceptible	Colostrum	Non-fed	1.870	0.01**	6.49	1.74–24.16
		Fed	1 (Ref)			

MRP, meropenem; IMP, imipenem; ERP, ertapenem.
*$P < 0.05$; **$P < 0.01$.

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References

1. **Nordmann P *et al.*** (2011) The emerging NDM carbapenemases. *Trends in Microbiology* **19**, 588–595.
2. **Bhardwaj M *et al.*** (2015) Emergence of carbapenemase producing pathogens in animals. *Pharmaceutica Analytica Acta* **6**, 1–6.
3. **Muller A *et al.*** (2018) Higher third-generation cephalosporin prescription proportion is associated with lower probability of reducing carbapenem use: a nationwide retrospective study. *Antimicrobial Resistance & Infection Control* **7**, 1–10.
4. **Kelly AM, Mathema B and Larson EL** (2017) Carbapenem-resistant *Enterobacteriaceae* in the community: a scoping review. *International Journal of Antimicrobial Agents* **50**, 127–134.
5. **Picao RC, Cardoso JP and Campana EH** (2013) The route of antimicrobial resistance from the hospital effluent to the environment: focus on the occurrence of KPC producing *Aeromonas spp.*, and *Enterobacteriaceae* in sewage. *Diagnostic Microbiology and Infectious Disease* **76**, 80–85.
6. **Castanheira M *et al.*** (2011) Early dissemination of NDM-1- and OXA-181-producing Enterobacteriaceae in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program. 2006–2007. *Antimicrobial Agents and Chemotherapy* **55**, 1274–1278.
7. **Page SW and Gautier P** (2012) Use of antimicrobial agents in livestock. *Revue Scientifique et Technique – OIE* **31**, 145–188.
8. **Clinical and Laboratory Standards Institute (CLSI)** (2014). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement 2014. M100-S24.* Wayne, PA, USA: CLSI, Vol 34, pp. 56–60.
9. **Pruthvishree BS *et al.*** (2017) Spatial molecular epidemiology of carbapenem-resistant and New Delhi metallo beta-lactamase (*bla*NDM)-producing *Escherichia coli* in the piglets of organized farms in India. *Journal of Applied Microbiology* **122**, 1537–1546.
10. **Poirel L *et al.*** (2011) Multiplex PCR for detection of acquired carbapenemase genes. *Diagnostic Microbiology and Infectious Disease* **70**, 119–123.
11. **Doyle D *et al.*** (2012) Laboratory detection of *Enterobacteriaceae* that produce carbapenemases. *Journal of Clinical Microbiology* **50**, 3877–3880.
12. **Johnson TJ *et al.*** (2007) Plasmid replicon typing of commensal and pathogenic *E. coli* isolates. *Applied and Environmental Microbiology* **15**, 1976–1983.
13. **Martins M *et al.*** (2013) A simple method for assessment of MDR bacteria for over-expressed efflux pumps. *The Open Microbiology Journal* **7**, 72–82.
14. **Gandra S *et al.*** (2016) Faropenem consumption is increasing in India. *Clinical Infectious Diseases* **62**, 1050–1052.
15. **Falagas ME, Karageorgopoulos DE and Nordmann P** (2011) Therapeutic options for infections with *Enterobacteriaceae* producing carbapenem-hydrolyzing enzymes. *Future Microbiology* **6**, 653–666.
16. **Rogers BA, Sidjabat HE and Silvey A** (2013) Treatment options for New Delhi metallo-beta-lactamase-harboring enterobacteriaceae. *Microbial Drug Resistance* **19**, 100–103.
17. **Fischer J *et al.*** (2012) *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm. *Journal of Antimicrobial Chemotherapy* **67**, 1793–1795.
18. **Nirupama KR *et al.*** (2018) Molecular characterization of *bla*OXA-48 carbapenemase, extended-spectrum β-lactamase-and Shiga toxin-producing *Escherichia coli* isolated from farm piglets in India. *Journal of Global Antimicrobial Resistance* **13**, 201–205.
19. **Ghatak S *et al.*** (2013) Detection of New Delhi metallo beta-lactamase and extended- spectrum beta-lactamase genes in *Escherichia coli* isolated from mastitic milk samples. *Transboundary and Emerging Disease* **60**, 385–389.
20. **de Verdier K *et al.*** (2012) Antimicrobial resistance and virulence factors in *E. coli* from Swedish dairy calves. *Acta Veterinaria Scandinavica* **54**, 1–10.
21. **Samanta I *et al.*** (2015) Approaches to characterize extended spectrum beta-lactamase/beta-lactamase producing *Escherichia coli* in healthy organized vis-a-vis backyard farmed pigs in India. *Infection, Genetics and Evolution* **36**, 224–230.
22. **Martins M, Viveiros M and Couto I** (2011) Identification of efflux pump-mediated multidrug-resistant bacteria by the ethidium bromide-agar cart-wheel method. *In Vivo* **25**, 171–178.
23. **Bendali F *et al.*** (1999) Risk factors associated with diarrhoea in newborn calves. *Veterinary Research* **30**, 509–522.
24. **Gullikse SM *et al.*** (2009) Enteropathogens and risk factors for diarrhea in Norwegian dairy calves. *Journal of Dairy Science* **92**, 5057–5066.
25. **Patel G *et al.*** (2008) Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. *Infection Control & Hospital Epidemiology* **29**, 1099–1106.
26. **Davies J and Davies D** (2010) Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews* **74**, 417–433.

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