

Prevalence of carbapenem resistance and its potential association with antimicrobial use in humans and animals in rural communities in Vietnam

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Background: Vietnam and Southeast Asia are hotspots for antimicrobial resistance; however, little is known on the prevalence of carriage of carbapenem resistance in non-hospitalized humans and in animals. Carbapenem-resistant Enterobacteriaceae (CRE), particularly *Escherichia coli* (CREC) and *Klebsiella pneumoniae* (CRKP) and also *Acinetobacter baumannii* (CRAB) are emerging threats worldwide.

Methods: We investigated healthy humans ($n=652$), chickens ($n=237$), ducks ($n=150$) and pigs ($n=143$) in 400 small-scale farms in the Mekong Delta of Vietnam. Samples (rectal swabs, faecal swabs) were investigated for carriage of CRE/CRAB and were further characterized phenotypically and genotypically.

Results: In the Mekong Delta of Vietnam, the prevalence of CRE isolates in human rectal swabs was 0.6%, including 4 CREC and 1 CRKP. One pig was infected with CREC (prevalence 0.7%). CRAB was isolated from chickens ($n=4$) (prevalence 2.1%) and one duck (prevalence 0.7%). CRKP was isolated from a human who was also colonized with CREC. The CRKP strain (ST16), from an 80 year-old person with pneumonia under antimicrobial treatment, genetically clustered with clinical strains isolated in a hospital outbreak in southern Vietnam. The prevalence of CRE was higher among humans that had used antimicrobials within 90 days of the sampling date than those had not (4.2% versus 0.2%) ($P=0.005$). All CRE/CRAB strains were MDR, although they were susceptible to colistin and neomycin. The carbapenemase genes identified in study strains were *bla_{NDM}* and *bla_{OXA}*.

Conclusions: The finding of a CRKP strain clustering with previous hospital outbreak raises concerns about potential transmission of carbapenem-resistant organisms from hospital to community settings or vice-versa.

Introduction

Carbapenems are β -lactam antimicrobials used for the treatment of infections caused by MDR Gram-negative bacteria.¹ Currently they are classified by WHO as high priority, critically important antimicrobials;² carbapenem-resistant Enterobacteriaceae (CRE) and *Acinetobacter baumannii* (CRAB) (alongside *Pseudomonas aeruginosa*) are regarded as ‘critical, high priority pathogens’ by WHO.³ Globally, the incidence of infections with both types of pathogen has been steadily increasing.^{4,5}

CRE infections are now being widely reported in Southeast Asian hospital settings.^{6,7} Data from Vietnamese hospitals have

documented that this leads to increased mortality and associated health care costs.^{8,9} Prevalence of infection with CRE among patients correlates with length of hospitalization (from 13% on admission to 89% at day 15).⁹ Laboratory data indicate increased prevalence of carbapenem resistance between 2012 and 2016 among *Escherichia coli* (CREC) (from 6% to 8%) and *Klebsiella pneumoniae* (CRKP) (from 17% to 24%).¹⁰ Recently, two nosocomial CRKP outbreaks caused by distinct lineages of sequence type (ST) 16 have been reported in Vietnam. CRKP strains from Vietnam are typically MDR, and are resistant to colistin.^{11,12} Studies in Ho Chi Minh City (southern Vietnam) (2010–12) indicated that resistance to carbapenems among *Acinetobacter* spp.

from ventilator-associated pneumonia patients was 84%–86%, and these had been steadily increasing since 2000.¹³ In another Vietnam-wide hospital study, the prevalence of carbapenem resistance among *A. baumannii* was seen to increase from 70% to 78% between 2012 and 2016.¹⁴

Carbapenemase production, often encoded by genes located on plasmids, is the most common carbapenem resistance mechanism among Enterobacteriaceae.⁵ Carbapenemases have been further classified into Class A (KPC); Class B (NDM, IMP, VIM); and Class D (OXA-type).¹⁵ Previous studies in Vietnam have reported a range of carbapenem resistance genes among CREC (*bla*_{KPC}, *bla*_{KPC-2}, *bla*_{NDM-1}, *bla*_{NDM-4}, *bla*_{NDM-5}, *bla*_{OXA}, *bla*_{OXA-48}), CRKP isolates (*bla*_{KPC-2}, *bla*_{NDM-1}, *bla*_{NDM-4}, *bla*_{OXA}, *bla*_{OXA-48} and *bla*_{VIM}),^{6,16} and CRAB isolates (*bla*_{OXA-51}, *bla*_{OXA-23}, *bla*_{OXA-58}, and *bla*_{NDM-1}).¹⁷

Few studies have investigated carriage of CRE among non-hospitalized human subjects. These were carried out in Spain (prevalence 0.4%);¹⁸ China (3.6% in children;¹⁹ 2.3% in the general population)²⁰ and Cambodia (1%).²¹ In the latter study, *bla*_{OXA-48} was identified both in *E. coli* and *K. pneumoniae*. In addition, a number of studies have demonstrated CRE in animal reservoirs (domestic, wild, companion) and food.²² CRE was not detected among 285 livestock samples (including ruminants, pigs and poultry) investigated in Cambodia.²¹ In contrast, a study in China identified CRE in 10.6% and 3.9% of pigs and chickens, respectively.²⁰ Another study from China detected CREC, CRKP and *Enterobacter cloacae* in, respectively, 21.8%, 7.4% and 3.9% of poultry samples along the production chain, with *bla*_{NDM} detected in 33.2% samples.²³

Studies on healthy populations in Netherlands and the USA have identified carriage of *A. baumannii* in 0.9% (faeces) and 10.4% (hands) individuals, respectively.^{24,25} Studies in Germany and Switzerland identified *A. baumannii* in 2.7%–45.7% of poultry samples investigated (choana and raw meat). However, none was identified as CRAB.^{26,27} Previous studies identified exposure to several classes of antimicrobials as a key explanatory factor for colonization with CRE.^{28–30}

In Vietnam, information on the prevalence of carriage of CREC/CRKP/CRAB in healthy livestock and in-contact human communities is limited. Vietnam and Southeast Asia are considered hot-spots for antimicrobial usage (AMU)/antimicrobial resistance (AMR).³¹ It is not known to what extent this may affect the colonization with CREC/CRKP/CRAB. This knowledge is essential for effective risk management of carbapenem resistance in the country. Using a One Health approach involving co-sampling of animals and human residents, we aimed to investigate: (1) the prevalence of carriage of carbapenem-resistant bacteria among livestock and in-contact humans in the Mekong Delta (Vietnam) and its potential relationship with antimicrobial use; and (2) the genetic determinants of carbapenem resistance in CREC, CRKP and CRAB in this area.

Materials and methods

Ethics

The project was conducted in accordance with the Declaration of Helsinki following institutional standards. The study was granted ethics approval by Oxford University Ethics Committee (OxTREC No. 503-20).

Sample and data collection

The study was carried out in Dong Thap province, considered to be representative of the Mekong Delta region of Vietnam in terms of human and animal demographics. The province has a census population of 1.6 million of whom 80.9% are classed as urban and a human population density of 494.1 per km² (versus 70.4% rural and a population density of 426.8 per km² in the region as a whole). Poultry and pig farm owners in Dong Thap province were randomly selected from the official farm census held by the veterinary authority (Dong Thap Sub-Department of Animal Health, Production and Aquaculture, SDAH-DT). We aimed to recruit 400 farms using a cluster sampling technique (i.e., based on random selection of 20 out of 141 communes in the province, and an average of 20 farms from each commune). Farms raising poultry (chickens or ducks) in flocks with >20 birds, and/or pigs (>2) were eligible. Farmers that consented to the study were enrolled, and their farms were visited during June and July 2020 by SDAH-DT (to collect animal data and samples) and Dong Thap Center for Disease Control (CDC-DT) staff (human data and samples). Data on AMU were collected using structured questionnaires aimed at the person with primary responsibility for animal husbandry. Farmers were also asked to provide all packages (bottles, sachets, etc.) of any antimicrobial-containing products used by humans or animals over the previous 90 and 7 days, respectively. Rectal swabs were collected from 1–3 consenting individuals living in each household. Pooled faecal samples were collected from each type of food-producing animal (chicken, duck and pig) present in the farm. This was achieved by swabbing with a cotton swab, three visibly fresh droppings from each of the target species. Swab samples were placed in 1 mL of sterile brain heart infusion broth (Oxoid, UK) plus 20% glycerol (Sigma, USA).

Isolation of carbapenem-resistant bacteria

All swab samples were vortexed thoroughly, then a loop of the corresponding suspension was plated onto Chromagar-carbapenem agar supplemented with meropenem 2 mg/L (Nam Khoa, Vietnam) to screen for non-susceptible *E. coli*, *K. pneumoniae* and *A. baumannii*. The plates were incubated at 35 ± 2°C for 20 h. Up to three suspected *E. coli* (reddish), *K. pneumoniae* (metallic blue) and *A. baumannii* (white) colonies from each sample were confirmed using MALDI-TOF (Bruker, Germany). *E. coli* ATCC 25922 was used as a negative control, two CRKP and CRAB were used as positive controls.

Antimicrobial susceptibility testing

Phenotypic AST was performed using disc diffusion and Etest (meropenem) methods for *A. baumannii*. For CRE and CRAB isolates VITEK 2 (bioMérieux, France) and Sensititre AST was used (Thermo Fisher Scientific, UK). The AST panel included 42 (CRE) and 26 antimicrobials (CRAB) (belonging to 13 classes and 10 classes, respectively) (Table S1, available as [Supplementary data](#) at JAC-AMR Online). CLSI breakpoints were used for susceptibility interpretation.³² *E. coli* ATCC 25922 was used for quality control. In this study, isolates that were intermediately resistant to carbapenems were regarded as resistant.

WGS

Genomic DNA was extracted from carbapenem-resistant isolates using Wizard genomic DNA extraction kit (Promega, US). Genome library preparation was carried out using Nextera XT library preparation kit and WGS was performed on the HiSeq2500 Illumina platform to generate 100 paired-end reads (Macrogen, Korea).

Data analyses

The prevalence of CREC, CRKP and CRAB in human/animal samples was compared between those individuals/animals using and not using

antimicrobials using Fisher's exact test. Acquired AMR and virulence genes as well as plasmid replicons were identified using SRST2 v0.2.0³³ with ARG-ANNOT antimicrobial resistance,³⁴ BIGSdb virulence genes (<https://bigsdb.web.pasteur.fr>) and PlasmidFinder³⁵ databases, respectively. We used SRST2 with the corresponding MLST scheme downloaded from PubMLST (<https://pubmlst.org/mlst>). SRST2 used Bowtie2³⁶ to map out raw reads against the reference database and SAMtools v1.3³⁷ to identify genes and alleles. We assembled all Illumina reads using the *de novo* assembler Unicycler v0.4.8 with the default settings.³⁸ Prokka v.1.5 was used to annotate the assembled contigs.³⁹

To investigate the phylogenetics of the *K. pneumoniae* ST16 isolate, we combined its genomic data with that from nine isolates obtained from a previous study in Vietnam.¹¹ Raw Illumina reads were mapped to the reference genome MGH78578 (CP000647.1) using RedDog pipeline v1.10b (<https://github.com/katholt/RedDog>). In brief, RedDog used Bowtie2 v2.2.3 to map raw reads and single nucleotide polymorphisms (SNPs) were identified with SAMtools v1.3. Gubbins v1.4.5 was used to remove recombinant regions from the resulting alignment file; SNPs identified in the recombinant regions were subsequently removed, resulting in a final alignment of 142 SNPs. Randomized Accelerated Maximum Likelihood (RaxML) was used to construct a maximum likelihood (ML) phylogenetic tree using GTR+G model of nucleotide substitution with 100 bootstrap replicates.⁴⁰

Sequence data of all CRE and CRAB isolates described in this study have been deposited in the European Nucleotide Archive (ENA) under study accession number PRJEB49509 (ERS8847840 to ERS8847852).

Results

Prevalence of CRE and CRAB in human and animals

A total of 652 human rectal swabs were collected from 381 households (19 subjects did not consent to rectal swabbing). About half (49.9%) human subjects were male, with a median age of 50 years (IQR 39–59). The prevalence of CRE isolates from human rectal swabs was 0.6%, including four CREC and one CRKP; CRAB was not found (Table 1). The prevalence of CRE

was higher among individuals who had used antimicrobials in the previous 90 days than among those who had not (4.2% versus 0.2%, Fisher exact test, $P=0.005$).

A total of 530 pooled faecal swabs were collected from animals in 400 households (237 chickens, 143 pigs and 150 ducks). The prevalence of carbapenem-resistant CRE/CRAB in chicken samples (2.1%) was greater than in pig/duck samples (0.7% each). In animals, a total of seven CRAB (six from chickens, one from ducks) and one CREC (from pig) were identified. Among chicken samples, the prevalence of CRAB was higher among flocks not recently treated with antimicrobials (3.4% versus 0.8%). In pigs and ducks, carbapenem-resistant bacteria were only detected in (one each) herd/flock that had not been treated with antimicrobials over the previous 7 days (Table 1).

Most carbapenem-resistant isolates came from different farms and samples, except one CREC and one CRKP isolate from the same human sample, and two distinct CRAB isolates from the same chicken sample by MLST (Table S2). CRE/CRAB bacteria were found in 6 out of 8 districts investigated, but 6/11 (55%) of the CRE/CRAB-positive samples were collected from Lai Vung district (Figure 1) (Tables S3 and S4).

Antimicrobial consumption and demographic features

Antimicrobial consumption and demographic features of CRE/CRAB-positive hosts are described in Tables S3 and S4. Three out of four human individuals whose rectal swab cultures were positive with CRE had a recent history of AMU within 90 days of the sampling date, including amoxicillin/clavulanic acid ($n=2$), cefuroxime ($n=1$), cefixime ($n=1$), and clarithromycin ($n=1$). The individual detected with both CREC and CRKP had pneumonia and was being treated with cefixime at the time of sample collection. In one CRAB-positive chicken farm raising fighting cockerels, tilmicosin and gentamicin had been used during the previous week.

Table 1. Prevalence of CRE/CRAB among animals and in-contact humans stratified by antimicrobial use

Antimicrobial use/source	No. samples	No. samples positive for CRE/CRAB (%)	Bacterial strains (ST)
Humans	652	4 (0.6)	
Used antimicrobials previous 90 days	71	3 (4.2)	01_EC_H ¹ (405), 03_EC_H (38), 04_EC_H (2705), 06_KP_H ¹ (16)
Not used antimicrobials previous 90 days	581	1 (0.2)	02_EC_H (1638)
Chickens	237	5 (2.1)	
Used antimicrobials previous 7 days	118	1 (0.8)	11_AB_C (762)
Not used antimicrobials previous 7 days	119	4 (3.4)	07_AB_C ¹ (762), 08_AB_C ¹ (NF), 09_AB_C (NF), 10_AB_C (NF), 12_AB_C (NF)
Ducks	150	1 (0.7)	
Used antimicrobials previous 7 days	56	0 (0.0)	–
Not used antimicrobials previous 7 days	94	1 (1.1)	13_AB_D (NF)
Pigs	143	1 (0.7)	
Used antimicrobials previous 7 days	29	0 (0.0)	–
Not used antimicrobials previous 7 days	114	1 (0.9)	05_EC_P (398)

AB, *A. baumannii*; EC, *E. coli*; KP, *K. pneumoniae*; identical superscripts indicate same subject; ST, sequence type; NF, not found.

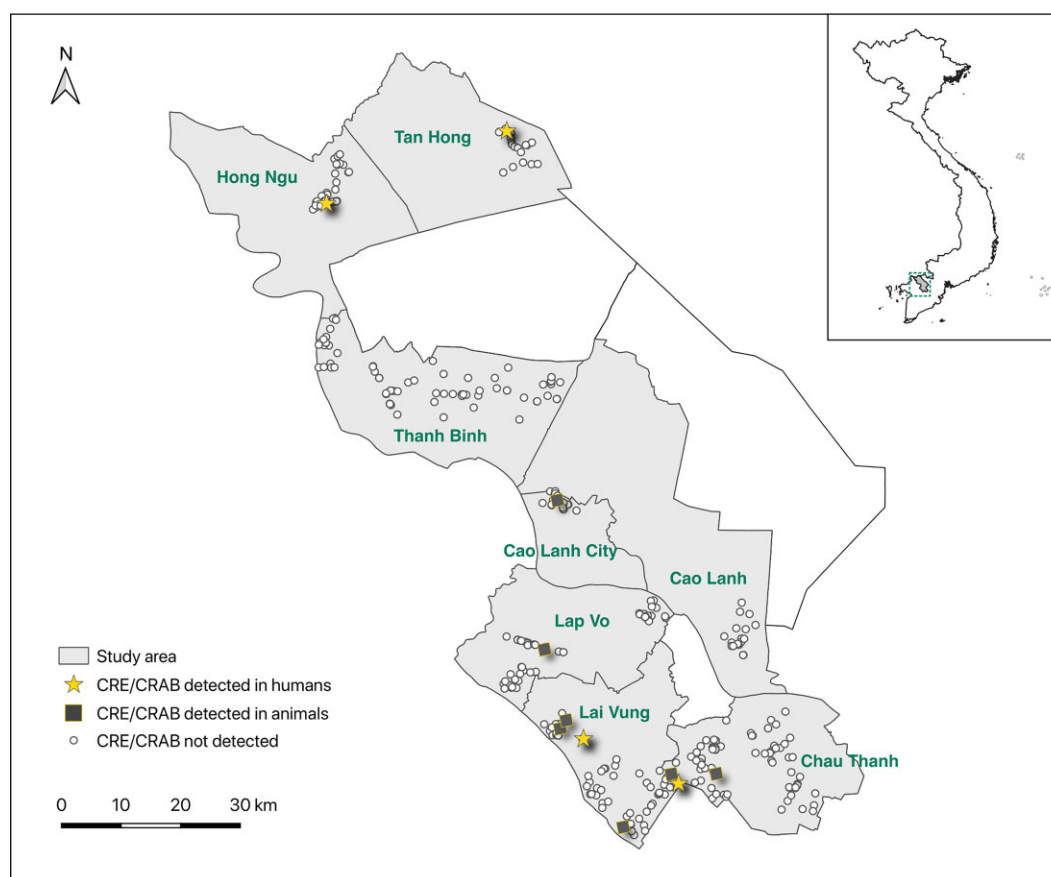


Figure 1. Map showing the collection sites and samples in Dong Thap province.

Sequence types (STs)

Based on the MLST profile, we identified five distinct *E. coli* STs: ST2705, ST1638, ST38, ST405 and ST398 (one for each isolate). The *K. pneumoniae* isolate was ST16. Out of the seven *A. baumannii* isolates, two (29%) were identified as ST762, and five (71%) were classified as STNF, (i.e. the ST was not identified based on the current MLST scheme). However, two out of five STNF isolates (from two chickens located on different farms) shared the same MLST profile (Table S2).

Phenotypic antimicrobial resistance

Among five CREC isolates, $\geq 80\%$ were resistant to 31/42 antimicrobials (8/13 classes) investigated; the resistance rates were 60% for azithromycin and minocycline, 40% for streptomycin and aztreonam, and 20% for nalidixic acid, ofloxacin, tigecycline and nitrofurantoin. All isolates were susceptible to colistin, amikacin and neomycin. The CRKP isolate was resistant to all antimicrobial agents (39/42) except colistin, neomycin and streptomycin (12/13 classes) (Table 2).

Seven CRAB isolates were tested for their susceptibility to 26 antimicrobials; however, breakpoints were only available for 17 (Table S1). 100% of the CRAB isolates were not susceptible to meropenem, cefotaxime, ceftazidime, ceftriaxone, piperacillin, and ticarcillin, but fully susceptible to cefepime, colistin, levofloxacin, ciprofloxacin, gentamicin, tobramycin, piperacillin/tazobactam,

tetracycline, doxycycline and co-trimoxazole (Table 2). For nine antimicrobials there were no available breakpoints. Overall, the MICs were 16 mg/L for cefpodoxime and ceftiofur (third-generation cephalosporins), ≥ 64 mg/L for amoxicillin and florfenicol, ≤ 0.25 mg/L for enrofloxacin, and ≤ 4 mg/L for oxytetracycline. Variable MICs were observed for azithromycin, neomycin and streptomycin.

Antimicrobial resistance genetic determinants

The genotypic AMR characterization of CREC/CRKP/CRAB isolates is displayed in Figure 2. *bla*_{NDM-1-like} genes were found in 4/5 CREC isolates (*bla*_{NDM-1} and *bla*_{NDM-5} were found in 2 isolates each); and *bla*_{OXA-181} in 1/5 isolates. One CREC isolate carried both *bla*_{NDM-5} and *bla*_{OXA-1} genes. β -Lactam resistance genes (*bla*_{AmpC1}, *bla*_{AmpC2}, *bla*_{AMPH} and *bla*_{MrdA}) were detected in all isolates, *qnrS*, *aadA*, and *sulI* were found in four isolates. Genes including *bla*_{TEM-1D} (β -lactam), *aac3-Iid*, *mphA*, *floR*, *sulII* and *tetA* were identified in three isolates.

The *bla*_{OXA-181} gene was detected in the CRKP isolate. CRKP also possessed β -lactam (*bla*_{SHV-148}, *bla*_{CTX-M-15}, *bla*_{TEM-1D} and *bla*_{AMPH}), fluoroquinolones (*qnrS1*, *qnrB-like*, *oqxAB*), aminoglycosides (*aac6Ib-cr*, *rmtB*) and other antimicrobial class resistance genes (*mphA*, *catA2*, *arr*, *sulI*, *tetA* and *dfrA27*).

CRAB isolates possessed variants of *bla*_{OXA-51-like} such as *bla*_{OXA-75} (2), *bla*_{OXA-208} (2), *bla*_{OXA-70} (1), *bla*_{OXA-91} (1) and *bla*_{OXA-203} (1), and *bla*_{OXA-24-like} such as *bla*_{OXA-72} (4), *bla*_{OXA-143}

Table 2. Summary phenotypic antimicrobial resistance of CREC/CRKP/CRAB isolates

ID: Sequence type:	CREC					CRKP			CRAB									
	MIC (mg/L)					R (isolates)			MIC (mg/L) or zone diameter ^a (mm)									
	01_EC_H1 405	02_EC_H 1638	03_EC_H 38	04_EC_H 2705	05_EC_P 398	Human (n=4)	Pig (n=1)	06_KP_H1 16	MIC	R	07_AB_C1 762	08_AB_C1 NF	09_AB_C1 NF	10_AB_C NF	11_AB_C 762	12_AB_C NF	13_AB_D NF	Duck (n=1)
Carbapenem																		
Ertapenem	≥8	≥8	≥8	≥8	≥8	4	1	≥8	1	1	2	1	1	2	2	4	4	1
Imipenem	≥16	≥16	≥16	0.5	≥16	3	1	2	1	1	≥16	3	3	≥32	≥8	≥16	≥32	1
Meropenem	≥16	≥16	≥16	2	≥16	4	1	≥16	1	1	≥16	3	3	≥32	≥8	≥16	≥32	1
β-Lactam																		
Cefepime ^a	≥64	16	≥64	≤1	8	3	1	≥64	1	1	21	20	20	22	21	21	18	0
Cefixime	≥4	≥4	≥4	≥4	≥4	4	1	≥4	1	1	14	16	13	14	13	14	12	1
Cefotaxime ^a	≥64	≥64	≥64	4	≥64	4	1	≥64	1	1	14	8	13	16	16	8	8	NA
Cefpodoxime	≥32	≥32	≥32	≥32	≥32	4	1	≥32	1	1	16	16	16	16	16	8	6	1
Ceftazidime ^a	≥64	≥64	≥64	4	≥64	3	1	≥64	1	1	14	13	14	14	14	14	13	1
Ceftiofur	≥32	≥32	≥32	4	≥32	4	1	≥32	1	1	16	16	16	16	8	16	16	NA
Ceftriaxone ^a	≥64	≥64	≥64	≤1	≥64	3	1	≥64	1	1	17	15	15	16	17	6	15	1
Cefoxitin	≥64	≥64	≥64	≥64	≥64	4	1	≥64	1	1								
Cefuroxime axetil	≥64	≥64	≥64	≥64	≥64	4	1	≥64	1	1								
Aztreonam	≥64	≤1	16	≤1	≤1	2	0	≥64	1	1	128	64	64	128	256	128	256	NA
Amoxicillin	≥256	≥256	≥256	≥256	≥256	4	1	≥256	1	1								
Ampicillin	≥32	≥32	≥32	≥32	≥32	4	1	≥32	1	1	15	18	18	17	16	17	14	1
Piperacillin ^a	≥128	≥128	≥128	≥128	≥128	4	1	≥128	1	1	32	32	32	32	32	32	≥128	1
Ticarcillin	≥128	≥128	≥128	≥128	≥128	4	1	≥128	1	1	32	32	32	32	32	32	≥128	1
AMC	≥32	≥32	≥32	≥32	≥32	4	1	≥32	1	1								
TZP	≥128	≥128	≥128	≥128	≥128	4	1	≥128	1	1	16	8	8	8	8	32	16	0
TIC/CLA	≥128	≥128	≥128	≥128	≥128	4	1	≥128	1	1								
Quinolones/ fluoroquinolones																		
Ciprofloxacin ^a	≥4	1	0.5	0.5	0.5	4	1	≥4	1	1	25	21	23	25	23	23	23	0
Enrofloxacin	≥32	2	0.5	0.5	0.5	4	1	≥32	1	1	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	NA
Levofloxacin ^a	≥8	1	1	1	1	4	1	≥8	1	1	26	25	25	25	25	25	25	0
Nalidixic Acid	≥32	16	16	4	16	1	0	≥32	1	1								
Ofloxacin	≥8	2	2	2	2	1	0	≥8	1	1								
Aminoglycosides																		
Amikacin	4	≤2	≤2	≤2	≤2	0	0	≥64	1	1								
Gentamicin ^a	≤1	≥16	8	≥16	≥16	3	1	≥16	1	1	19	19	19	20	21	20	17	0
Neomycin	1	1	≤0.5	1	1	0	0	≤0.5	0	0	≤0.5	≤0.5	≤0.5	2	1	1	1	NA
Streptomycin	16	≥512	8	≥512	16	2	0	≤4	0	0	16	8	≤4	16	32	32	8	NA
Tobramycin	≥16	8	8	8	8	4	1	≥16	1	1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	0
Phenicals																		
Chloramphenicol	16	≥64	8	≥64	≥64	3	1	≥64	1	1	64	64	64	64	64	64	128	NA
Fluorfenicol	32	≥256	8	256	256	3	1	32	1	1								
Tetracyclines																		
Doxycycline	16	32	32	16	32	4	1	32	1	1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	0

Continued

Table 2. Continued

ID: Sequence type:	CREC					CRKP			CRAB									
	01_EC_H1 405	02_EC_H 1638	03_EC_H 38	04_EC_H 2705	05_EC_P 398	Human (n=4)	Pig (n=1)	06_KP_H1 16	Human (n=1)	07_AB_C1 762	08_AB_C1 NF	09_AB_C NF	10_AB_C NF	11_AB_C 762	12_AB_C NF	13_AB_D NF	Chicken (n=6)	Duck (n=1)
	MIC (mg/L)					R (isolates)		MIC	R	MIC (mg/L) or zone diameter ^a (mm)								
Minocycline	2	≥16	≥16	4	≥16	2	1	≥16	1									
Oxytetracycline	16	512	512	512	>512	4	1	>512	1	≤4	≤4	≤4	≤4	≤4	≤4	≤4	NA	NA
Tetracycline ^a	2	≥16	≥16	≥16	≥16	3	1	≥16	1	21	21	20	19	19	20	19	0	0
Macrolides																		
Azithromycin	>64	>64	4	>64	8	3	0	>64	1	2	2	2	4	2	2	4	NA	NA
Sulphonamides																		
Co-trimoxazole ^a	≥320	≥320	≤20	≥320	≥320	3	1	≥320	1	25	24	24	24	25	25	23	0	0
Trimethoprim	≥16	≥16	≤0.5	≥16	≥16	3	1	≥16	1									
Nitrofurans																		
Nitrofurantoin	≤16	≤16	32	64	≤16	1	0	256	1									
Glycylcyclines																		
Tigecycline	≤0.5	2	≤0.5	≤0.5	≤0.5	1	0	4	1									
Polymyxins																		
Colistin	0.5	0.5	0.5	0.5	≤0.25	0	0	0.5	0	0.5	1	1	1	1	0.5	1	0	0

R, resistant; n, number of isolates; NA, breakpoints not available; bold type indicates not susceptible; TIC/CLA, ticarcillin/clavulanic acid; TZP, piperacillin/tazobactam; AMC, amoxicillin/clavulanic acid.
^aDisc diffusion method was used for MIC determination.

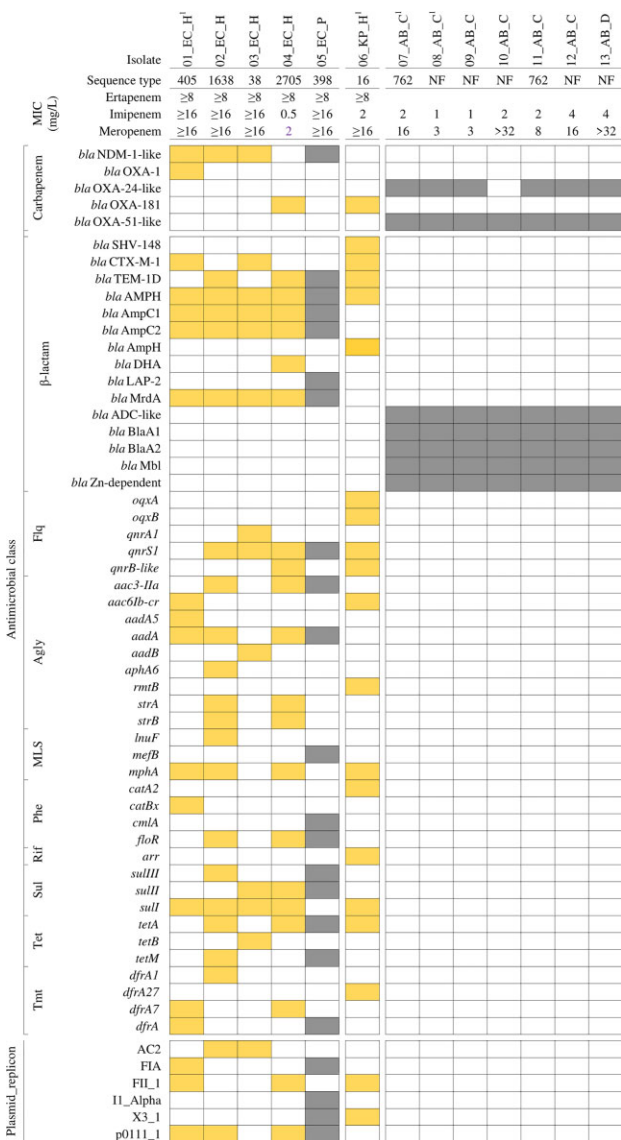


Figure 2. Distribution of carbapenem and other antimicrobial resistance genes in CREC/CRKP/CRAB isolates from humans and animals in Dong Thap province (Vietnam). Colour code: yellow, human isolate; grey, animal isolate. Genes are grouped by encoding resistant to β-lactams, fluoroquinolones (Flq), aminoglycosides (Agly), macrolide/lincosamide/streptogramin (MLS), phenicols (Phe), rifampicin (Rif), sulphonamides (Sul), tetracyclines (Tet), and trimethoprim (Tmt). Isolate species: EC, *E. coli*; KP, *K. pneumoniae*; AB, *A. baumannii*. Subscripts indicate individual samples/subjects. H, human; P, pig; C, chicken; D, duck.

(2). The highest MICs of meropenem were seen for strains containing *bla*_{OXA-208} (Table 3). All CRAB strains possessed a similar gene cassette conferring resistance to β-lactams: *bla*_{ADC}, *bla*_{A1}, *bla*_{A2}, *bla*_{Mbl}, and *bla*_{Zn-dependent}. None of the CREC/CRKP/CRAB isolates harboured *mcr* genes, which confer colistin resistance. Among Enterobacteriaceae, isolates with the NDM types had higher MIC values. For *A. baumannii*, higher MIC values were found for strains with OXA-208 (Table 3).

Phylogenetics of CRKP isolate

Our CRKP ST16 isolate clustered tightly with three (of nine) ST16 isolates recovered from patients affected by nosocomial outbreaks at a tertiary hospital in southern Vietnam in December 2019 (Figure 3). The four isolates carried an identical and extensive AMR gene profile including *bla*_{OXA-48}, *bla*_{SHV}, *bla*_{CTX-M-15}, *bla*_{AmpH}, *bla*_{TEM-1D}, *qnrS*, *aacAad*, *mphA*, *sulI*, *tetA*, and *dfrA7*, predicted to confer resistance to antimicrobials from the carbapenems, cephalosporins, β-lactams, quinolones, aminoglycosides, macrolides, tetracyclines and trimethoprim. Additionally, our CRKP ST16 isolate harboured the identical *bla*_{OXA-48}-carrying IncFII plasmid (coverage 90%, identity 99%) with previously identified in outbreak ST16 isolates (accession number: MT635909.1).¹¹

Discussion

This is, to the best of our knowledge, the first report describing carriage of CRE and CRAB in animals and humans living in close contact in rural (Mekong Delta) Vietnam. Our study confirms the presence of CRE in non-hospitalized human subjects and pigs at a relatively low prevalence (0.6%–0.7%). Although we did not find evidence of CRE in poultry species, CRAB was detected in 2.1% and 0.7% chickens and ducks. Carbapenem resistance was encoded by *bla*_{NDM-1}-like (4/5 CREC) and *bla*_{OXA-181} (1/5 CREC and 1/1 CRKP) genes. One CREC contained both *bla*_{NDM-1}-like and *bla*_{OXA-1}.

The observed prevalence of carriage of CRE (0.6%) was of similar magnitude to a previous study in rural Cambodia (~1%); however, in that study CRE was not identified in any of 285 livestock faecal samples. In that study, both CREC and CRKP isolates detected in humans harboured *bla*_{OXA-48}.²¹ Previous studies have detected *bla*_{OXA-48}-positive *E. coli* in 0.09% (1/1086) and 1.6% (3/183) healthy humans from Switzerland and Lebanon, respectively.^{41,42} In contrast, no CRE was detected in 433 and 320 non-hospital human samples from India or Spain.^{43,44} In China, carriage of CREC was 2.3% of 735 non-hospitalized humans, 3.9% of 305 chickens and 10.6% of 417 pigs (all encoded by *bla*_{NDM} carbapenemase genes).²⁰ Another study in Egypt revealed high levels of CRKP carriage in chickens with signs of respiratory diseases (15%) as well as in workers and veterinarians (10% of 49 faecal samples). The CRKP strains carried *bla*_{KPC}, *bla*_{OXA-48} and *bla*_{NDM} genes.⁴¹ A previous study demonstrated a higher prevalence of CRE among Vietnamese hospital patients on day of admission (13%),⁹ a much higher figure than our 0.6% among healthy individuals. It is likely that this difference reflects previous antimicrobial use or exposure to healthcare facilities.

The finding of a CRKP strain in a province identical to that found in a previous nosocomial outbreak in a crowded city in the country¹¹ suggests that transmission of CRKP from hospital facilities to the community may occur.

The finding of a CRKP strain in these rural settings identical to that found in a previous nosocomial outbreak in a hospital the country¹¹ and the high prevalence of colonization in hospital settings confirms transmission from hospital facilities to the community.

We only detected CRAB in poultry faecal samples, all of which harboured *bla*_{OXA-51}-like genes, and most (6/7) *bla*_{OXA-24}-like genes.

Table 3. Carbapenem resistance-encoding genes and MICs in CREC/CRKP/CRAB isolates

Sample ID	Sequence type (ST)	<i>bla</i> _{NDM-1} -like	<i>bla</i> _{OXA-1}	<i>bla</i> _{OXA-48} -like	<i>bla</i> _{OXA-51} -like	<i>bla</i> _{OXA-24} -like	MIC (mg/L)		
							MEM	IPM	ETP
01_EC_H ¹	405	<i>bla</i> _{NDM-5}	<i>bla</i> _{OXA-1}				≥16	≥16	≥8
02_EC_H	1638	<i>bla</i> _{NDM-1}					≥16	≥16	≥8
03_EC_H	38	<i>bla</i> _{NDM-1}					≥16	≥16	≥8
04_EC_H	2705			<i>bla</i> _{OXA-181}			2	0.5	≥8
05_EC_P	398	<i>bla</i> _{NDM-5}					≥16	≥16	≥8
06_KP_H ¹	16			<i>bla</i> _{OXA-181}			≥16	2	≥8
07_AB_C ¹	762				<i>bla</i> _{OXA-70}	<i>bla</i> _{OXA-72}	16	2	NT
08_AB_C ¹	NF				<i>bla</i> _{OXA-75}	<i>bla</i> _{OXA-143}	3	1	NT
09_AB_C	NF				<i>bla</i> _{OXA-75}	<i>bla</i> _{OXA-143}	3	1	NT
10_AB_C	NF				<i>bla</i> _{OXA-208}		>32	2	NT
11_AB_C	762				<i>bla</i> _{OXA-91}	<i>bla</i> _{OXA-72}	8	2	NT
12_AB_C	NF				<i>bla</i> _{OXA-203}	<i>bla</i> _{OXA-72}	16	4	NT
13_AB_D	NF				<i>bla</i> _{OXA-208}	<i>bla</i> _{OXA-72}	>32	4	NT

MEM, meropenem; IPM, imipenem; ETP, ertapenem; NT, not tested.

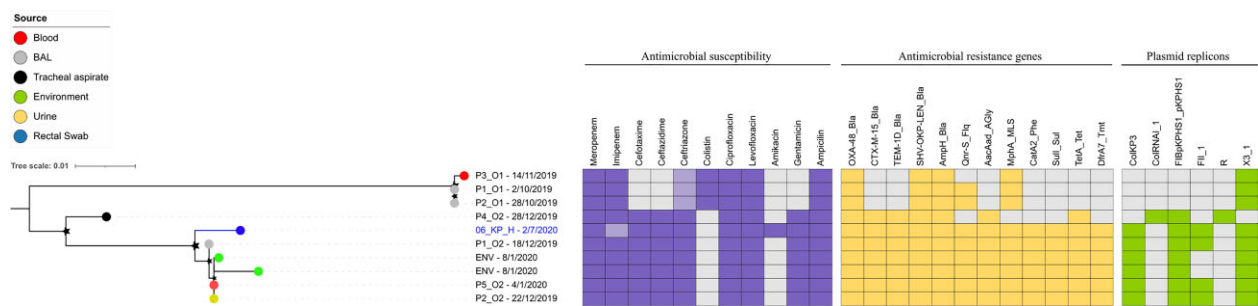


Figure 3. Phylogenetic structure of *K. pneumoniae* ST16 from a human carrier and bloodstream infections.

A study in Germany identified *bla*_{OXA-51}-like-producing *A. baumannii* in choanal swab samples of chickens (2.7%) and geese (7.5%).²⁶ CRAB was detected in 5.8% (3/52) and 11.2% (112/1000) poultry meat in Iran and Turkey, respectively.^{45,46} In contrast, a study in Switzerland demonstrated that poultry was the most frequently *A. baumannii*-contaminated type of meat (45.7% of 94 samples); however, none of them were carbapenem resistant.²⁷ Meat is suspected to be a potential source of MDR *A. baumannii*, presumably resulting from faecal contamination.⁴⁷ Although we did not investigate meat samples, our study suggests that poultry (including its meat) may potentially be a source of infection of CRAB and therefore this merits further study.

Although none of the four CRE-carrying individuals investigated had been recently treated with carbapenems, we found a strong association between antimicrobial use in the last 90 days and carriage of CRE. A previous study identified antimicrobial usage as the single most important explanatory factor for colonization with CRE.²⁸ Further interview data (data not shown) revealed that all CRE-positive individuals had visited health care facilities. It is not known to what extent individuals

may have been colonized in these settings. We did not, however, investigate to what extent farming practices and exposure to manure may have contributed to colonization with CRE in human subjects, as shown in the Cambodian study.²¹

Our data was suggestive of geographical clustering for CRAB and CREC, four chicken CRAB and two human CREC isolates came from the same district (Lai Vung).

Our study confirmed the presence of *bla*_{NDM-1}-like and *bla*_{OXA} genes in CREC strains. For meropenem and imipenem, the highest MICs (>16 mg/L) were observed among strains carrying *bla*_{NDM-1}-like genes. We did not, however, find evidence of colistin resistance among any of the tested strains. In addition to colistin, amikacin and neomycin (aminoglycosides) were the three antimicrobials to which all CREC strains were susceptible; in the case of CRKP, only neomycin, streptomycin and colistin had inhibitory activity.

Conclusions

We demonstrated faecal carriage of *E. coli*, *K. pneumoniae* and *A. baumannii* harbouring carbapenemase genes in humans and

animals in the Mekong Delta of Vietnam. The highest prevalence of colonization with CREC corresponded to human subjects previously treated with β -lactams and/or in contact with health care facilities. Our results suggest One Health genomic surveillance for CRE/CRAB to detect potential transmission from hospital settings; this could be implemented by longitudinal follow-up sampling of individuals and their animal contacts after being discharged from hospitals. It would also be important to investigate short- versus long-term fitness of carbapenemase gene-encoding plasmids in these individuals.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S4 are available as [Supplementary data](#) at JAC-AMR Online.

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