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Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making?

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Acquired carbapenemases pose one of the most pressing public health threats relating to antibiotic resistance. In most countries, the number of carbapenemase-producing bacteria from human clinical specimens is rising, and the epidemiological status of these multiresistant bacteria is progressively worsening. Furthermore, there is a growing number of reports of carbapenemases found either in bacteria isolated from non-human sources or in *Salmonella enterica* subsp. *enterica*, a zoonotic species. However, carbapenemases are not yet systematically sought in bacteria from non-human sources, reports of them are largely observational, and there is limited investigation of carbapenemase-positive bacteria in animals and possible links with people who may have acted as potential sources. Active surveillance and monitoring for carbapenem-resistant bacteria in the food chain and other non-human sources is urgently needed, with an enhanced and rigorous follow-up of all positive results. The carbapenems are currently our last good defence against multiresistant Gram-negative bacteria. Our ability to limit the rise and spread of carbapenemase producers, which occur only at basal levels in many countries at present, should serve as a key performance indicator for the success or failure of the efforts that have been called for by international organizations and governments to reduce the impact of antibiotic resistance.

Keywords: KPC, NDM, VIM, OXA-48, rivers, pets, food chain, *Salmonella enterica*

Introduction

Acquired carbapenemases pose one of the most pressing public health threats relating to antibiotic resistance. They confer resistance not just to carbapenems, but to almost all β-lactams, our most widely used class of antibiotic, and are encoded by genes that are transferable between bacteria. Furthermore, most carbapenemase-producing isolates of Enterobacteriaceae and non-fermenters (predominantly, but not exclusively, *Pseudomonas* spp. and *Acinetobacter* spp.) are resistant to multiple other classes of antibiotics, which severely limits therapeutic options even after the antibiogram of an infecting strain has been determined; the chance of empirically prescribing an appropriate antibiotic (or combination) is highly unlikely, which impacts on mortality.

Rates of acquired carbapenem resistance in most bacterial species and in most countries of the European Economic Area (EEA) are below the thresholds needed to register on the maps arising from surveillance schemes such as EARS-Net.[1] In consequence almost all countries in the EEA appear 'green and pleasant', but this masks a reality in which the actual number of carbapenemase-producing bacterial isolates is rising and the epidemiological status of these bacteria (sporadic versus local or regional spread versus national endemicity) is progressively worsening.[2] Here, it is important to mention that the carbapenem MICs for some carbapenemase-producing bacteria do not categorize them as resistant, irrespective of the clinical breakpoints used. Such non-susceptible isolates may not be considered in some prevalence studies.

We have had endemic carbapenemases (KPC and VIM types) in Greece and Cyprus for some time,[2,3] and the ease with which other countries can succumb is illustrated by Italy, where carbapenem resistance among blood culture isolates of *Klebsiella pneumoniae* rose from 1.3% in 2009 to 27% in 2011,[1] reflecting a nationwide outbreak of KPC producers.[4] Even more worryingly, about 13% of carbapenemases detected by Public Health England's Antimicrobial Resistance and Healthcare Associated Infections Reference Unit are in isolates of *Escherichia coli* (N. Woodford, unpublished data), which are the perfect vehicles for taking these enzymes

out of the hospital and establishing them in the community setting, where they will impact on primary care. The warnings about resistance from the Chief Medical Officer for England have come at a critical time.[5] We must act now to prevent carbapenemases becoming as established as extended-spectrum β-lactamases (ESBLs). Rates must be kept low because these are some of the most resistant bacteria that we face.

Spread of strains, plasmids and genes

For any Gram-negative resistance issue, especially situations involving the Enterobacteriaceae, one must consider not just the spread of resistant strains, but also the spread of their resistance genes between plasmids and the spread of those plasmids between strains, species and genera. Furthermore, any antibiotic use (whether appropriate or not) provides selective pressure for resistant strains, and of course that use is not limited to humans.[6] We need to consider the role of any non-human reservoirs of resistance genes and strains, which have the *potential* to be a public health threat. The problem is that although these reservoirs represent a theoretical hazard, quantifying the actual risk and the contribution to the burden of human disease is difficult. However, the potential for the transfer of bacteria between reservoirs, as well as both to and from man, is amply illustrated by considering non-typhoidal *Salmonella*, irrespective of its resistance.

About a decade ago, ESBL-producing *E. coli* with CTX-M enzymes emerged and spread internationally to become a substantial cause of community-acquired/community-onset urinary tract infections, bacteraemias and healthcare-associated infections. We have since discovered that ESBL-producing *E. coli* can also be found in food production animals, raw meat, companion animals and the environment, but after 10 years of intense global research and discussion, we still lack an indisputable assessment of the risk that these sources of resistant *E. coli* represent to human health. Rather, the evidence is fragmentary and suggests substantial differences between countries. For example, the *E. coli* strains, ESBLs and plasmids found in food animals and meat in the UK are distinct from those that cause the vast majority of human infections,[7] whereas closer associations between the food chain and human disease have been identified in the Netherlands.[8] The debate is still unresolved for ESBL-producing *E. coli*, and it is only just starting for carbapenemase-producing bacteria, for which the body of evidence is far less substantial.

Non-human carbapenem use

Carbapenem antibiotics have never been licensed for veterinary use in any country worldwide, as far as we are aware.[9] There are no legally 'safe' limits for carbapenems in meat because no maximum residue limit has been defined in Europe, so any food production animals treated with them could not enter the food chain, which effectively precludes their use in the agricultural sector. However, in the UK, vets can prescribe carbapenems to treat 'resistant' infections in companion animals, based on clinical judgement and as part of the cascade mechanism.[10] In the authors' experience, this is limited in the UK to the occasional use of imipenem by veterinary surgeons to treat dogs with highly resistant urinary tract infections. However, national figures on such usage are not collected, and the British Small Animal Veterinary Association advises that there is a strong argument that 'last-resort' antimicrobials, such as imipenem, should not be used for veterinary patients.[11]

By contrast, the prescribing of carbapenems is escalating in humans,[12] as a consequence of the rising numbers of ESBL producers and other multiresistant Gram-negative organisms. So the bulk of carbapenem prescribing occurs in human medicine, and this is currently the major sector reporting carbapenemase producers; but multidrug resistance means, of course, that many antibiotic classes, not just carbapenems and other β-lactams, have the potential to select indirectly for carbapenemase producers.

Carbapenemases from non-human sources

There is a growing number of reports of carbapenemases found either in bacteria isolated from non-human sources or in *Salmonella enterica* subsp. *enterica*, a zoonotic species (Table 1). The spread of OXA-48 and VIM-2 to *Salmonella* serovar Kentucky ST198-X1 has raised concerns as this strain is widely distributed in North Africa. *S. enterica* strains carrying carbapenem resistance genes are frequently resistant to fluoroquinolones, limiting treatment options, and also often display relatively low carbapenem MICs, making them difficult for laboratories to detect.[13] Where do such strains originate, and do they represent a public health risk?

The precise origins of the genes encoding most acquired carbapenemases remain undefined, but they have all escaped from environmental bacteria into species with clinical relevance. Exceptions and examples are (i) the OXA-48 family of enzymes, which occur naturally in *Shewanella* spp., a genus that inhabits lake sediments,[14,15] and (ii) OXA-23 carbapenemase, which is almost entirely restricted to *Acinetobacter baumannii* and originates from the environmental species *Acinetobacter radioresistens*.[16] For these reasons, finding carbapenemase genes by PCR[17] or metagenomic approaches in environmental samples has limited value if the identity of the host species is not determined. Reports of carbapenemases in sewage and waste water (Table 1) have often sampled downstream of hospitals, so the discovery of resistance in clinically relevant species is not surprising in this setting. We are contaminating the environment, releasing a concentrated supply of resistant bacteria that can spread their resistance genes into many different species.[18] The extent of this contamination can be dramatic in countries with poor sanitation, as exemplified by the widely publicized report of NDM carbapenemase in water samples taken from multiple sites around New Delhi.[19]

Other reports have documented the detection in environmental samples of carbapenemases that have rarely (IMI enzyme)[20] or never (BIC-1 enzyme)[21] been isolated from clinical isolates. By contrast, OXA-23 enzyme is undoubtedly a significant clinical concern, but detecting it in 'clinically inconsequential' *Acinetobacter* species from animals[22,23] may well represent further gene escape events from *A. radioresistens*. They seem unlikely to pose any substantial public health threat even to those in direct contact with the animals for four reasons: (i) the vast majority of human *Acinetobacter* infections are caused by *A. baumannii*; (ii) most non-hospitalized people would not be susceptible to infection by this genus; (iii) it is exceptional to find *A. baumannii* even as a colonist in people outside the hospital environment so gene transfer events to this species are unlikely; and (iv) the bulk of

Table 1. Acquired carbapenemases (by order of first report) in bacteria from non-human sources or in the zoonotic species *S. enterica*

Enzyme	Species	Source	Country	Year	Reference(s)
KPC (class A)	*S. enterica* subsp. *enterica* serovar Cubana	human (faeces)	USA	2003	35
	multiple genera	hospital sewage, effluent	Brazil	2011, 2013	18,36
	E. coli	river	Portugal	2012	37
	Citrobacter freundii, *Enterobacter cloacae*	hospital sewage	China	2012	38
IMI (class A)	*Enterobacter asburiae*	rivers	USA	2005	20
VIM (class B)	*Pseudomonas pseudoalcaligenes*, *Pseudomonas aeruginosa*	river, sewage	Portugal	2005, 2006	39,40
	PCR amplicons only	sewage, effluent	Germany	2009	17
	S. enterica subsp. *enterica* serovar Kentucky	human (faeces)	Morocco	2010	13
	E. coli , *S. enterica* subsp. *enterica* serovar Infantis	pig and poultry farms, flies, mice, manure	Germany	2012, 2013	27–29
	K. pneumoniae, *Helicobacter pylori*	river	Tunisia	2013	41
	K. pneumoniae	river	Switzerland	2013	42
IMP (class B)	PCR amplicons only	sewage, effluent	Germany	2009	17
	K. pneumoniae	river	Tunisia	2013	41
OXA-48 (class D)	PCR amplicons only	sewage, effluent	Germany	2009	17
	S. enterica subsp. *enterica* serovars Kentucky and Saintpaul	human (faeces)	Egypt	2010	13
	E. coli , *K. pneumoniae*	dogs	Germany	2013	26
NDM (class B)	multiple genera	water	India	2011	19
	K. pneumoniae	river	Vietnam	2012	43
	Acinetobacter lwoffii	poultry	China	2012	44
	A. baumannii	pig	China	2013	45
	E. coli	dogs/cat	USA	2013	25
	S. enterica subsp. *enterica* serovar Senftenberg	human (perirectal screen)	USA/India	2012	46
	S. enterica subsp. *enterica* serovar Westhampton	human (urine, faeces)	Reunion Island/ India	2012	47
	S. enterica subsp. *enterica* serovar Corvallis	black kite (wild raptor)	Germany	2013	31
	A. baumannii	water, hospital sewage	China	2013	48
OXA-23 (class D)	*Acinetobacter* 15TU	dairy cows	France	2012	49
	Acinetobacter sp.	horses	Belgium	2012	23
BIC-1 (class A)	*Pseudomonas fluorescens*	river	France	2012	21

carbapenem-resistant strains of this species belong to three successful and hospital-adapted international lineages.

The so-called 'big five' carbapenemases (KPC and OXA-48 non-metallo-carbapenemases as well as the IMP, NDM and VIM metallo-carbapenemases)[24] collectively cause the greatest clinical concern. So recent reports of 'enterobacteria' with NDM, VIM or OXA-48 enzymes from food production animals or companion animals are worrying, but the reasons for their occurrence have usually not been investigated or reported. The retrospective investigation that often takes place in such cases can be problematic and complicates the analysis, where the smoking gun has essentially ceased to smoke or is no longer at the scene. The significance of these facts is uncertain and it may be that the resistant bacteria were derived from human sources, either directly or indirectly. This seems especially likely for companion animals, although details of the owners have not been disclosed (recent hospitalization, etc.).[25,26] Transmission of a single *K. pneumoniae* strain with OXA-48 carbapenemase to several dogs in a veterinary clinic[26] illustrates that hand hygiene is as important here as in human medicine to prevent and control infections and to limit the spread of resistant bacteria.

On the other hand, the presence of *Salmonella* and *E. coli* isolates carrying VIM-1 carbapenemase has been reported in food-producing animals in Germany.[27–29] In this case, investigations showed that once that the bacteria are on the farm or in the farm environment, they can be maintained there, may be widely spread among animals (including insects and rodents) and can reach the environment via manure.[29,30] In addition, the role of wild birds as a reservoir for carbapenemase-encoding genes

should be taken into account, as *Salmonella* producing NDM-1 has been isolated from a black kite.[31]

A need for wider screening

There are currently no reports of carbapenemases in bacteria from retail meat or other foodstuffs, but the possibility needs to be considered. Would food that tested positive for carbapenemase producers be deemed fit for human consumption given the low prevalence in normal healthy people[32] and the potential for resistance genes to be transferred to species resident in the gut flora from bacteria ingested with improperly cooked meat or raw vegetables or in ready-to-eat food? Testing foodstuffs for carbapenem-resistant bacteria is not currently a legal requirement. The European Food Safety Authority has acknowledged that "the carbapenems…are assuming importance as 'last-resort' antimicrobials in the treatment of certain highly-resistant Gram-negative infections in humans, and their inclusion also needs to be considered when panels of antimicrobials are reviewed"[33] and is currently working on a scientific opinion on carbapenem resistance in food animal ecosystems.[34] In addition, European legislation is currently being revised and it seems likely that a significant extension in the monitoring of carbapenem resistance will be required for animals and food.[33] This move is critical to increase our knowledge and to allow a valid assessment of the extent of any potential threat; the few *ad hoc* reports of carbapenemases in animals are essentially meaningless in the absence of widespread, large-scale and proficient testing.

Concluding remarks

In summary, carbapenemases in bacteria from non-human sources are not yet being systematically sought; proposals to enhance and extend such monitoring are to be strongly welcomed. At the current time, reports of them are largely observational, and there is limited systematic investigation of the occurrence of carbapenemase-positive bacteria in animals and possible links with people (e.g. owners of animals, farmers, vets) who may have risk factors for infection or colonization (including prior exposure, hospitalization, antibiotic use, healthcare contact and/or travel overseas) and so may have acted as potential sources. Such active surveillance and monitoring for carbapenem-resistant bacteria in the food chain and other non-human sources is urgently needed, with an enhanced and rigorous follow-up of all positive results. The carbapenems are currently our last good defence against multiresistant Gram-negative bacteria. Our ability to limit the rise and spread of carbapenemase producers, which occur only at basal levels in many countries at present, should serve as a key performance indicator for the success or failure of the efforts that have been called for by international organizations and governments to reduce the impact of antibiotic resistance.

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References

1 European Centre for Disease Prevention and Control. *The EARS-Net Database*. http://www.ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx (4 September 2013, date last accessed).

2 Glasner C, Albiger B, Buist G *et al*. Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. *Euro Surveill* 2013; **18**: pii=20525.

3 Canton R, Akova M, Carmeli Y *et al*. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012; **18**: 413–31.

4 Giani T, Pini B, Arena F *et al*. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. *Euro Surveill* 2013; **18**: pii=20489.

5 Davies SC, Fowler T, Watson J *et al*. Annual Report of the Chief Medical Officer: infection and the rise of antimicrobial resistance. *Lancet* 2013; **381**: 1606–9.

6 Wallinga D, Burch DG. Does adding routine antibiotics to animal feed pose a serious risk to human health? *BMJ* 2013; **347**: f4214.

7 Joint Working Group of DARC and ARHAI. *ESBLs—A Threat to Human and Animal Health?* http://www.vmd.defra.gov.uk/pdf/ESBL_report.pdf (4 September 2013, date last accessed).

8 Leverstein-Van Hall MA, Dierikx CM, Cohen SJ *et al*. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin Microbiol Infect* 2011; **17**: 873–80.

9 World Organisation for Animal Health. *OIE List of Antimicrobials of Veterinary Importance*. http://web.oie.int/downld/Antimicrobials/OIE_list_antimicrobials.pdf (4 September 2013, date last accessed).

10 Veterinary Medicines Directorate. *Cascade Use of Authorised Medicines*. http://www.vmd.defra.gov.uk/mswd/cascade.aspx (4 September 2013, date last accessed).

11 British Small Animal Veterinary Association. *BSAVA Guide to the Use of Veterinary Medicines: Responsible Use of Antimicrobial Agents*. http://www.bsava.com/Advice/BSAVAGuidetotheUseofVeterinaryMedicines/Responsibleuseofantimicrobialagents/tabid/363/Default.aspx (4 September 2013, date last accessed).

12 Ashiru-Oredope D, Sharland M, Charani E *et al*. Improving the quality of antibiotic prescribing in the NHS by developing a new Antimicrobial Stewardship Programme: Start Smart–Then Focus. *J Antimicrob Chemother* 2012; **67** Suppl 1: i51–63.

13 Le Hello S, Harrois D, Bouchrif B *et al*. Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. *Lancet Infect Dis* 2013; **13**: 672–9.

14 Poirel L, Heritier C, Nordmann P. Chromosome-encoded Ambler class D β-lactamase of *Shewanella oneidensis* as a progenitor of carbapenem-hydrolyzing oxacillinase. *Antimicrob Agents Chemother* 2004; **48**: 348–51.

15 Potron A, Poirel L, Nordmann P. Origin of OXA-181, an emerging carbapenem-hydrolyzing oxacillinase, as a chromosomal gene in *Shewanella xiamenensis*. *Antimicrob Agents Chemother* 2011; **55**: 4405–7.

16 Poirel L, Figueiredo S, Cattoir V *et al*. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. *Antimicrob Agents Chemother* 2008; **52**: 1252–6.

17 Szczepanowski R, Linke B, Krahn I *et al*. Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. *Microbiology* 2009; **155**: 2306–19.

18 Picao RC, Cardoso JP, Campana EH *et al*. 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. *Diagn Microbiol Infect Dis* 2013; **76**: 80–5.

19 Walsh TR, Weeks J, Livermore DM *et al*. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* 2011; **11**: 355–62.

20 Aubron C, Poirel L, Ash RJ *et al*. Carbapenemase-producing Enterobacteriaceae, U.S. rivers. *Emerg Infect Dis* 2005; **11**: 260–4.

21 Girlich D, Poirel L, Nordmann P. Novel Ambler class A carbapenem-hydrolyzing β-lactamase from a *Pseudomonas fluorescens* isolate from the Seine River, Paris, France. *Antimicrob Agents Chemother* 2010; **54**: 328–32.

22 Dhanji H, Khan P, Cottell JL *et al*. Dissemination of pCT-like IncK plasmids harbouring CTX-M-14 ESBL among clinical *Escherichia coli* in the United Kingdom. *Antimicrob Agents Chemother* 2012; **56**: 3376–7.

23 Smet A, Boyen F, Pasmans F *et al*. OXA-23-producing *Acinetobacter* species from horses: a public health hazard? *J Antimicrob Chemother* 2012; **67**: 3009–10.

24 Woodford N. *Fighting the Rising Tide of Carbapenemases in Enterobacteriaceae. Be S.M.A.R.T. With Resistance, October 2012*. http://www.biomerieuxdiagnostics.com/upload/BE_SMART_Newsletter_8p_Num_5_FINAL.pdf (4 September 2013, date last accessed).

25 Shaheen BW, Nayak R, Boothe DM. Emergence of a New Delhi metallo-β-lactamase (NDM-1)-encoding gene in clinical *Escherichia coli* isolates recovered from companion animals in the United States. *Antimicrob Agents Chemother* 2013; **57**: 2902–3.

26 Stolle I, Prenger-Berninghoff E, Stamm I *et al*. Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. *J Antimicrob Chemother* 2013; **68**: 2802–2808.

27 Fischer J, Rodriguez I, Schmoger S *et al*. *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm. *J Antimicrob Chemother* 2012; **67**: 1793–5.

28 Fischer J, Rodriguez I, Schmoger S *et al*. *Salmonella enterica* subsp. *enterica* producing VIM-1 carbapenemase isolated from livestock farms. *J Antimicrob Chemother* 2013; **68**: 478–80.

29 Fischer J, Schmoger S, Baumann B *et al*. Carbapenemases are present in enterobacteria from non-domestic animals…more frequently than we thought. In: *Abstracts of the Twenty-third European Congress of Clinical Microbiology and Infectious Diseases. Berlin, Germany*, 2013. Abstract P1449. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.

30 Guerra B. An emerging problem for public health: carbapenemase-producing microorganisms are also present in food producing animals, their environment and wild birds. In: *Abstracts of the Fifth Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE), Ghent, Belgium*, 2013. p. 8.

31 Fischer J, Schmoger S, Jahn S *et al*. NDM-1 carbapenemase-producing *Salmonella enterica* subsp. *enterica* serovar Corvallis isolated from a wild bird in Germany. *J Antimicrob Chemother* 2013; **68**: 2954–2956.

32 Wickramasinghe NH, Xu L, Eustace A *et al*. High community faecal carriage rates of CTX-M ESBL-producing *Escherichia coli* in a specific population group in Birmingham, UK. *J Antimicrob Chemother* 2012; **67**: 1108–13.

33 European Food Safety Authority. Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food. *EFSA Journal* 2012; **10**: 2742. http://www.efsa.europa.eu/en/efsajournal/pub/2742.htm (4 September 2013, date last accessed).

34 The European Food Safety Authority. *Register of Questions. EFSA Self-Tasking Mandate on Carbapenem Resistance in Food Ecosystems, EFSA Q-2013–00010, M-2013-0004, 2013*. http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=ALL: (4 September 2013, date last accessed).

35 Miriagou V, Tzouvelekis LS, Rossiter S *et al*. Imipenem resistance in a *Salmonella* clinical strain due to plasmid-mediated class A carbapenemase KPC-2. *Antimicrob Agents Chemother* 2003; **47**: 1297–300.

36 Chagas TP, Seki LM, da Silva DM *et al*. Occurrence of KPC-2-producing *Klebsiella pneumoniae* strains in hospital wastewater. *J Hosp Infect* 2011; **77**: 281.

37 Poirel L, Barbosa-Vasconcelos A, Simoes RR *et al*. Environmental KPC-producing *Escherichia coli* isolates in Portugal. *Antimicrob Agents Chemother* 2012; **56**: 1662–3.

38 Zhang X, Lu X, Zong Z. Enterobacteriaceae producing the KPC-2 carbapenemase from hospital sewage. *Diagn Microbiol Infect Dis* 2012; **73**: 204–6.

39 Quinteira S, Ferreira H, Peixe L. First isolation of bla_{VIM-2} in an environmental isolate of *Pseudomonas pseudoalcaligenes*. *Antimicrob Agents Chemother* 2005; **49**: 2140–1.

40 Quinteira S, Peixe L. Multiniche screening reveals the clinically relevant metallo-β-lactamase VIM-2 in *Pseudomonas aeruginosa* far from the hospital setting: an ongoing dispersion process? *Appl Environ Microbiol* 2006; **72**: 3743–5.

41 Chouchani C, Marrakchi R, Henriques I *et al*. Occurrence of IMP-8, IMP-10, and IMP-13 metallo-β-lactamases located on class 1 integrons and other extended-spectrum β-lactamases in bacterial isolates from Tunisian rivers. *Scand J Infect Dis* 2013; **45**: 95–103.

42 Zurfluh K, Hachler H, Nuesch-Inderbinen M *et al*. Characteristics of extended-spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae isolates from rivers and lakes in Switzerland. *Appl Environ Microbiol* 2013; **79**: 3021–6.

43 Isozumi R, Yoshimatsu K, Yamashiro T *et al*. bla_{NDM-1}-positive *Klebsiella pneumoniae* from environment, Vietnam. *Emerg Infect Dis* 2012; **18**: 1383–5.

44 Wang Y, Wu C, Zhang Q *et al*. Identification of New Delhi metallo-β-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. *PLoS One* 2012; **7**: e37152.

45 Zhang WJ, Lu Z, Schwarz S *et al*. Complete sequence of the bla_{NDM-1}-carrying plasmid pNDM-AB from *Acinetobacter baumannii* of food animal origin. *J Antimicrob Chemother* 2013; **68**: 1681–2.

46 Rasheed JK, Kitchel B, Zhu W *et al*. New Delhi metallo-β-lactamase-producing Enterobacteriaceae, United States. *Emerg Infect Dis* 2013; **19**: 870–8.

47 Cabanes F, Lemant J, Picot S *et al*. Emergence of *Klebsiella pneumoniae* and *Salmonella* metallo-β-lactamase (NDM-1) producers on Reunion Island. *J Clin Microbiol* 2012; **50**: 3812.

48 Zhang C, Qiu S, Wang Y *et al*. Higher isolation of NDM-1 producing *Acinetobacter baumannii* from the sewage of the hospitals in Beijing. *PLoS One* 2013; **8**: e64857.

49 Poirel L, Bercot B, Millemann Y *et al*. Carbapenemase-producing *Acinetobacter* spp. in cattle, France. *Emerg Infect Dis* 2012; **18**: 523–5.