Journal of Antimicrobial Chemotherapy Advance Access published February 16, 2016

J Antimicrob Chemother
doi:10.1093/jac/dkv489

Journal of Antimicrobial Chemotherapy

Atypical epidemiology of CTX-M-15 among Enterobacteriaceae from a high diversity of non-clinical niches in Angola

T. G. Ribeiro[1], Â. Novais[1], L. Peixe[1] and E. Machado[1,2]*

[1]*UCIBIO/REQUIMTE, Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal;* [2]*FP-ENAS/CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal*

*Corresponding author. FP-ENAS/CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal. Tel: +351-225074630; Fax: +351-225074677; E-mail: emachado@ufp.edu.pt/elisabetemac@gmail.com

Received 3 September 2015; returned 26 October 2015; revised 9 December 2015; accepted 18 December 2015

Objectives: The objective of this study was to investigate the distribution and molecular epidemiology of ESBLs, acquired AmpCs and carbapenemases in Enterobacteriaceae from non-clinical niches in Angola, an under-researched sub-Saharan country.

Methods: Eighty-one samples were recovered from healthy persons ($n=18$), healthy animals ($n=33$) and their environments ($n=10$) or aquatic settings ($n=20$) in south Angola (2013). Samples were plated onto CHROMagar™ Orientation with/without antibiotics. Standard methods were used for bacterial identification, characterization of *bla* genes, antibiotic susceptibility testing and conjugation assays. Clonal analysis (XbaI-PFGE, MLST and *Escherichia coli* phylogroups), location of *bla* and plasmid characterization (S1-PFGE, I-CeuI-PFGE, replicon typing and hybridization) were also performed.

Results: ESBLs (almost exclusively CTX-M-15, 98%) were detected in 21% (45/216) of the isolates, recovered from diverse non-clinical niches and belonging to different Enterobacteriaceae species (mainly *E. coli*). Acquired AmpCs or carbapenemases were not found. The pandemic B2-ST131 *E. coli* clone was not identified, but some widespread clonal complexes (CCs) from A (CC10 and CC168), B1 (CC156) or D (CC38) phylogroups were detected. $bla_{\text{CTX-M-15}}$ was variably identified on typeable (29%; 100–335 kb; IncFII, $\text{IncFII}_{\text{K6}}$, IncHI2 and IncY) or non-typeable (16%; 70–330 kb) plasmids or on the chromosome (14%), while for 41% of the isolates its specific location was not determined.

Conclusions: This study reports, for the first time in Angola, an unexpected high occurrence of CTX-M-15 in diverse non-clinical niches and Enterobacteriaceae species, and uncovers novel plasmid replicons in under-researched geographical regions. The diffusion of $bla_{\text{CTX-M-15}}$ through such a high diversity of genetic backgrounds (clones, typeable/non-typeable plasmids and genetic environments) unveils an extraordinary ability for $bla_{\text{CTX-M-15}}$ acquisition and mobilization favoured by unrecognized ecological factors.

Introduction

The epidemiology of Enterobacteriaceae producing ESBLs, acquired AmpC β-lactamases (qAmpCs) and/or carbapenemases has mainly been investigated in developed countries, with studies showing their large-scale diffusion in different niches.[1–3] Currently, the role of trade globalization and human travel to endemic and/or low-income countries in the dispersion of antibiotic-resistant (AbR) bacteria is well recognized, pointing out the need to comprehensively analyse their epidemiology and ecology in these geographical regions.[4,5]

In Africa, available data on antimicrobial resistance indicate considerable resistance rates to extended-spectrum cephalosporins.[6] Nevertheless, studies analysing the occurrence and molecular epidemiology of ESBLs are limited to northern countries, certain species and/or the clinical setting, while qAmpCs or carbapenemases are sporadic.[7]

Angola is a sub-Saharan country with an emerging economy and close commercial or travel relationships with European, Asian and American countries (http://www.embangola.at/dados.php?ref=rela%E7%F5es-internacionais), for which few data on occurrence and epidemiological features of Enterobacteriaceae resistant to antibiotics are available.[8] Given that the climate, poor sanitation and uncontrolled antibiotic use might be key drivers for the emergence and dissemination of AbR bacteria, we aimed to investigate the distribution and molecular epidemiology of ESBLs, qAmpCs and carbapenemases in Enterobacteriaceae from non-clinical niches in Angola.

Table 1. Epidemiological data of CTX-M-15-producing Enterobacteriaceae isolates recovered from different origins in Angola (2013)

Species (no.)	*E. coli* phylogenetic group (no.)	PFGE type (no.)	ST/CC	Plasmid content: associated with $bla_{CTX-M-15}$ [size (kb) (Inc family)][a]	Plasmid content: other: size (kb)	Plasmid content: other: Inc family (no.)	Other AbR genes: bla_{TEM-1}	Other AbR genes: bla_{OXA-1}	Other AbR genes: *aac-6′-Ib-cr*	Origin (sample)	Region (commune)[b]	Coresistance to non-β-lactam antibiotics[c]
E. coli (*n*=34)	A_0	I	ST4977	—	70	Y	+	−	−	healthy person (28)	Benguela	TOB, STR, KAN, CIP, SUL, TET
	A_1	II	ST5044	—	—	—	−	−	−	healthy person (28)	Benguela	TOB, STR, KAN, CIP
	A_1	III	ST181/CC168	**330**	140	—	+	+	+	healthy person (29)	Benguela	GEN, TOB, STR, KAN, NET, CIP, SUL, TMP, TET, CHL
	A_0	IV	ST2325	**330**	—	—	+	+	+	healthy person (29)	Benguela	GEN, TOB, STR, KAN, NET, CIP, SUL, TMP, TET, CHL
	A_1	V	ST5091	**200**	110	FIA	+	+	+	healthy person (31)	Baía Farta	GEN, TOB, STR, KAN, NET, CIP, NAL, SUL, TMP, TET, CHL
	A_1	VI	ST5092	—	100	—	−	−	−	floor/walls (10/Farm A)	Benguela	STR, KAN, CIP, TMP, TET
	A_0 (2)	VII (2)	ST5093	—	—	Y; P; FIB	−	+	−	floor/walls (10/Farm A)	Benguela	(GEN), TOB, STR, (CIP), (NAL), SUL, TMP, TET, CHL
	A_1 (4)	VIII (4)	ST10/CC10	—	—	N (2)	−	−	−	pigs (16, 17/Farm D)	Benguela	(GEN), (TOB), STR, (KAN), NAL, SUL, TMP, TET
	A_1	IX	ST5094	**205** (FII36:A4:B1)	—	—	−	+	+	pigs (17/Farm D)	Benguela	GEN, TOB, STR, KAN, CIP, NAL, SUL, TMP, TET, CHL
	A_1	X	ST5095	**180** (FII36:A4:B1)	—	—	−	+	+	pigs (17/Farm D)	Benguela	GEN, TOB, STR, KAN, NET, CIP, NAL, SUL, TMP, TET
	A_1	XI	ST10/CC10	**80**	—	—	+	−	+	pigs (17/Farm D)	Benguela	STR, SUL, TMP, TET
	A_0	XII	ST5096	**130**	—	FIB	+	−	−	pigs (17/Farm D)	Benguela	STR, CIP, NAL, SUL, TMP, TET
	A_0 (2)	XIII (2)	ST5154	—	—	—	−	−	−	cows (14, 15/Farm D)	Benguela	GEN, TOB, (AMK), STR, KAN, CIP, NAL, TET
	A_0 (2)	XIV (2)	ST5097	**70**	—	—	+	−	−	cows (15/Farm D)	Benguela	(TOB), STR, (NET), NAL, SUL, TMP, TET
	A_1	XV	ST617/CC10	—	130	FIA	+	−	−	wastewater (59)	Benguela	GEN, TOB, STR, KAN, CIP, NAL, SUL, TMP, TET
	A_0	XVI	ST5093	—	—	Y; P	−	+	−	wastewater (60)	Benguela	TOB, SUL, TMP, TET, CHL
	A_1	XVII	ST617/CC10	—	—	FIB; FIA	−	+	−	wastewater (60)	Benguela	GEN, TOB, STR, NET, CIP, NAL, SUL, TMP, TET
	B_1	XVIII	ST156/CC156	—	—	FII	+	−	+	healthy person (36)	Benguela	GEN, TOB, STR, KAN, CIP, NAL, SUL, TMP, CHL
	B_1	XIX	ST1727	—	—	—	−	−	−	cows (14/Farm D)	Benguela	TOB, STR, KAN
	B_1 (2)	XX (2)	ST448/CC448	**215** (FII36:A4:B1)			−	+	+	treated water for human consumption (62)	Lobito	GEN, TOB, STR, KAN, NET, CIP, NAL, SUL, TMP, TET, CHL
	B_1	XXI	ST167/CC10	**180** (FII36:A4:B1)	100	Y	+	−	+	wastewater (59)	Benguela	GEN, TOB, AMK, STR, KAN, NET, CIP, NAL, SUL, TMP, TET, CHL
	B_2	XXII	ST372	**330** (HI2)	—	—	+	+	+	healthy person (29)	Benguela	GEN, TOB, STR, KAN, NET, CIP, NAL, SUL, TMP, TET, CHL
	D_2 (5)	XXIII (5)	ST38/CC38	—[d]	100	FII	−	−	−	water for animal consumption (43, 44/Wild Animal Park)	Dombe Grande	STR, (CIP), (NAL)

K. pneumoniae (*n*=4)	NA	XXIV (3)	ST730	**100** (Y)	200; 377	FIIk; I1	+	–	–	chickens (8; Farm A); water for animal consumption (57/Farm A)	Benguela	STR, (TOB), (KAN), CIP, (NAL), SUL, TMP, TET, CHL
	NA	XXV	ST215	**200** (FIIk$_6$)	—	—	+	+	+	pigs (17/Farm D)	Benguela	GEN, TOB, STR, KAN, NET, CIP, SUL, TMP, TET
K. oxytoca (*n*=1)	NA	XXVI	NA	**310** (HI2)	90; 170	Y	+	+	+	urban sewer line wastewater (58)	Catumbela	GEN, TOB, STR, NET, CIP, NAL, SUL, TMP, TET, CHL
E. hormaechei (*n*=2)	NA	XXVII	NA	**335** (HI2)	—	—	+	+	+	healthy person (29)	Benguela	STR, NAL, SUL, TMP, TET
	NA	XXVIII	NA	—	90	—	+	+	+	river (38)	Catumbela	GEN, TOB, STR, KAN, NET, NAL, SUL, TMP, TET, CHL
E. asburiae (*n*=1)	NA	XIX	NA	—	60	—	–	+	+	river (38)	Catumbela	GEN, TOB, KAN, TMP, TET
C. freundii (*n*=1)	NA	XXX	NA	—[d]	70	—	+	–	–	wastewater (59)	Benguela	STR, CIP, NAL, SUL, TMP, TET, CHL
C. werkmanii (*n*=1)	NA	XXXI	NA	**100** (Y)	—	—	+	+	+	wastewater (59)	Benguela	GEN, TOB, STR, KAN, NET, CIP, NAL, SUL, TMP, TET, CHL

AMK, amikacin; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; NET, netilmicin; STR, streptomycin; SUL, sulphonamides; TET, tetracyclines; TMP, trimethoprim; TOB, tobramycin; NA, not applicable.

[a]Plasmids carrying *bla* genes are shown in bold and transferability of the $bla_{CTX\text{-}M\text{-}15}$ gene is indicated by underlining.

[b]Samples were collected from Benguela Province, which comprises nine municipalities (Benguela, Lobito, Bocoio, Balombo, Ganda, Cubal, Caimbambo, Baía Farta and Chongoróí), divided into >30 communes.

[c]The variable presence of a given resistance phenotype is indicated in parentheses.

[d]$bla_{CTX\text{-}M\text{-}15}$ is located on the chromosome.

Materials and methods

Sample collection

Eighty-one samples were collected from different non-clinical origins of the Benguela Province of Angola in 2013 (Figure S1, available as Supplementary data at *JAC* Online), as detailed below.

Healthy persons

Eighteen rectal swabs were taken from randomly selected healthy persons without exposure to antibiotics or hospitals in the 3 months preceding sampling. All participants provided written informed consent.

Animals and their environment

Thirty-six samples were collected from healthy food-producing animals (n=28, rectal swabs) and their environments (n=8) at Farms A and B (10–20 animals) and Farms C and D (>200 animals) [13 chickens (Farms A and C), 9 cows (Farms B and D), 6 pigs (Farm D), 3 waters for animal consumption (100 mL; Farms A–C), 3 feed samples (25 g; Farms A, C and D) and 2 swabs from floor/walls (Farms A and C)], as well as seven samples from wild animals (2 monkeys and 3 goats; faeces) and waters for animal consumption (n=2; 100 mL) at a Wild Animal Park.

Aquatic environments

Twenty samples were collected from river (n=2) or lagoon (n=1) waters (close to agricultural/residential areas, domestic animal production farms and/or septic tanks), urban sewer line wastewaters (n=3), a wastewater treatment station (n=2) and waters used for human consumption (n=12; 7 treated and 5 untreated) (100 mL).

Sample processing

Faecal samples from food-producing animals of the same type and farm were pooled in lots of three totalling 10 samples (9 pools and 1 individual). Sixty-three samples were processed as described previously[9] and cultured on CHROMagar™ Orientation with and without cefotaxime (1 mg/L), ceftazidime (1 mg/L) or imipenem (1 mg/L), followed by selection of isolates representing different colony morphotypes of presumptive Enterobacteriaceae per plate.

Detection and characterization of bla genes

ESBL, qAmpC or carbapenemase genes were identified by PCR and sequencing.[9–11] ESBL or carbapenemase production was additionally discarded by phenotypic tests (http://www.eucast.org/).[12]

Bacterial identification, antimicrobial susceptibility testing and conjugation assays

Isolates carrying acquired *bla* genes were identified by MALDI-TOF MS (Bruker Daltonik, Leipzig, Germany). *Citrobacter freundii* complex and *Enterobacter cloacae* complex were identified at species level by sequencing of genotypic markers.[13,14] Antibiotic susceptibility testing and conjugation assays (24, 30, 37 and 42°C) were performed as described previously.[10,15]

Molecular characterization of isolates carrying acquired bla genes

Escherichia coli phylogroups were identified[16] and clonal relatedness was established by XbaI-PFGE[10] and MLST for representative *E. coli* and *Klebsiella pneumoniae* isolates (different PFGE types) (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/documents/primersColi_html; http://www.pasteur.fr/recherche/genopole/PF8/mlst/primers_Kpneumoniae.html).

The location of *bla* was investigated by hybridization of S1-, I-CeuI- or XbaI-digested (when negative with S1/I-CeuI) genomic DNA.[15] Plasmids carrying *bla* were characterized by replicon typing and subtyping (IncF plasmids; http://pubmlst.org/plasmid/primers/incF.shtml).[15,17,18] The $bla_{CTX\text{-}M\text{-}15}$ genetic environment was also investigated.[9,19]

Results and discussion

High occurrence of ESBLs, with dominance of CTX-M-15 among different species and non-clinical niches

The bla_{ESBL} genes were identified in isolates ($n=45$) of different species and almost all sample types (30.2%; 19 of 63 samples) (Table 1), while qAmpC or carbapenemase genes were not found. Thus, we report for the first time the dispersion of ESBL-producing bacteria in a wide diversity of non-clinical niches in Angola. The human faecal carriage of ESBL producers (22.2%; 4 of 18 samples) was higher than in most African (6.7%–21.7%) (Table S1) or developed (0.6%–20.3%) countries,[1] despite differences in sample size. Similarly, the occurrence of ESBLs among food-producing animals and their environments (25%–50%) or aquatic samples (30%) was higher than expected (Table 1 and Table S1). The detection of ESBL producers among healthy persons and aquatic samples reflects a worrying level of environmental contamination, which in poor living conditions poses serious risk of transmission.[5] Despite the paucity of data regarding antibiotic consumption and resistance in Angola,[6] the high rates of clinical bacteria resistant to extended-spectrum cephalosporins recently reported[20] suggests a similar scenario in clinical settings.

All but one ESBL producer (1 *K. pneumoniae*/SHV-12) encoded CTX-M-15 (98%, 44/45; Table 1). CTX-M-15 producers were identified as *E. coli* ($n=34$), *K. pneumoniae* ($n=4$), *Enterobacter hormaechei* ($n=2$), *Enterobacter asburiae* ($n=1$), *C. freundii* ($n=1$), *Citrobacter werkmanii* ($n=1$) and *Klebsiella oxytoca* ($n=1$) recovered from diverse sample types, and were frequently resistant to non-β-lactams (Table 1). The wide dispersion of CTX-M-15 in non-clinical settings has been well documented in developed countries,[1,2] but its almost exclusive detection in such a diversity of species and niches in Angola contrasts with the situation in other African countries (Table S1).[7,18] This atypical scenario could reflect a recent penetration of $bla_{CTX\text{-}M\text{-}15}$ into this geographical area and/or the local emergence of $bla_{CTX\text{-}M\text{-}15}$ driven by unrecognized factors.

CTX-M-15 producers from animals were identified on different farms, some of which import animals from Portugal (e.g. chickens/Farm A), where ESBLs other than CTX-M-15 are frequent.[9,19] To the best of our knowledge, this is the first report of CTX-M-15-producing *K. pneumoniae* among healthy food-producing animals.

Identification of international E. coli clones from phylogroups A, B1 and D

CTX-M-15-producing *E. coli* were clonally diverse ($n=34$; 23 PFGE types and 20 STs) and belonged to phylogroup A ($n=23$; 68%), B1 ($n=5$; 15%), D ($n=5$; 15%) or B2 ($n=1$; 3%) distributed in different niches (Table 1). The higher prevalence of CTX-M-15 among *E. coli* belonging to phylogroup A might reflect that CTX-M-15 is well established in human/animal commensal strains in sub-Saharan countries (Table S1).[8] The pandemic B2-ST131 *E. coli*[1,2] was absent (Table S1), whereas widespread clonal complexes (CCs) from A (one CC168 and seven CC10), B1 (one CC156) or D (five CC38) phylogroups were frequent (Table 1). These clones are widely represented in collections of ESBL, qAmpC and/or carbapenemase producers from non-clinical niches in developed and non-developed countries (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/GetTableInfo_html).[2,18,19] The remaining isolates ($n=20$) belonged to ST448 or STs identified here for the first time (Table 1).

CTX-M-15-producing *K. pneumoniae* belonged to ST730 ($n=3$; one PFGE type) or ST215 ($n=1$) (Table 1). The international *K. pneumoniae* clonal groups CG15 or CG258, highly represented in clinical isolates in Africa, were absent.[21] *E. hormaechei* were clonally diverse ($n=2$; two PFGE types) (Table 1).

Location of $bla_{CTX\text{-}M\text{-}15}$ in variable typeable and non-typeable plasmids or in the chromosome of different species

$bla_{CTX\text{-}M\text{-}15}$ was transferred by conjugation in 20% of the isolates (9/44; 24 and 37°C), being unequivocally identified on plasmids of variable sizes (~70–335 kb) ($n=20/44$, 45%) or on the chromosome ($n=6/44$, 14%), while in 41% ($n=18/44$) of the isolates its specific location was not determined (Table 1).

Plasmids carrying $bla_{CTX\text{-}M\text{-}15}$ were identified as 180–215 kb IncFII (F36:A4:B1) in diverse *E. coli* ($n=5$), 310–335 kb IncHI2 in *K. oxytoca*, *E. hormaechei* and *E. coli* ($n=1$ each), 200 kb $IncFII_{K6}$ in *K. pneumoniae* ($n=1$) or 100 kb IncY in diverse *K. pneumoniae* ($n=3$) or *C. werkmanii* ($n=1$) (Table 1). For seven *E. coli*, $bla_{CTX\text{-}M\text{-}15}$ hybridized in non-typeable plasmids (~70–330 kb) (Table 1), uncovering novel replicon types in this region. The association of $bla_{CTX\text{-}M\text{-}15}$ with the F36:A4:B1 plasmid variant was previously identified in clinical isolates (Central African Republic) and pets (France).[22,23] $bla_{CTX\text{-}M\text{-}15}$ was chromosomally located in 14% (five *E. coli* and one *C. freundii*) of the isolates, a situation increasingly reported.[16] Negative results in S1/I-CeuI hybridization assays were obtained ($n=18$, 41%; *E. coli*, *E. hormaechei* and *E. asburiae*), although plasmids (60–130 kb) were detected for some isolates (data not shown). However, positive hybridization signals were observed in XbaI-digested genomic DNA (29–669 kb bands; Figure S2), as reported previously.[16] The absence of conjugative transfer and the band sizes (25% >250 kb) suggest a $bla_{CTX\text{-}M\text{-}15}$ chromosomal location, although we cannot discard non-typeable plasmids.

Diversity of $bla_{CTX\text{-}M\text{-}15}$ genetic environments

Most isolates harboured $bla_{CTX\text{-}M\text{-}15}$ flanked upstream by IS*Ecp1* or IS*26* ($n=36$ or $n=4$, respectively) and downstream by *orf477* ($n=44$), resembling common genetic platforms.[19] In two isolates, IS*3* was 116 bp after the 3′ end of IS*Ecp1*, corresponding to a novel configuration (GenBank accession number KT192055). CTX-M-15 producers harboured variably $bla_{TEM\text{-}1}$ (48%), $bla_{OXA\text{-}1}$ (41%) or *aac(6′)-Ib-cr* (39%) (Table 1). The simultaneous presence of $bla_{TEM\text{-}1}$, $bla_{OXA\text{-}1}$ and *aac(6′)-Ib-cr*, or $bla_{TEM\text{-}1}$ and *aac(6′)-Ib-cr* were observed mostly in isolates harbouring $bla_{CTX\text{-}M\text{-}15}$ located on plasmids (Table 1).

Conclusions

In this study, one of the few conducted in Africa embracing such a high diversity of samples, we report a high and almost exclusive occurrence of CTX-M-15 in diverse Enterobacteriaceae species

and non-clinical niches in Angola. The variability observed in $bla_{CTX\text{-}M\text{-}15}$ genetic environments, genetic locations and clones suggests an extraordinary ability for acquisition and mobilization of $bla_{CTX\text{-}M\text{-}15}$ by multiple genetic backgrounds, which is not comparable to that reported in developed countries. Moreover, our study unveils possible novel plasmid backgrounds involved in the spread of $bla_{CTX\text{-}M\text{-}15}$ in natural reservoirs in under-researched geographical regions.

Acknowledgements

We are very grateful to Hélder Gonçalves, Erineia Gonçalves and Walter Bonfim, for support in sample recruitment, and to all contributing farmers and healthy volunteers.

Funding

This work received financial support from the European Union (FEDER funds) through Programa Operacional Factores de Competitividade – COMPETE, and from National Funds [Fundação para a Ciência e a Tecnologia (FCT)] through project UID/Multi/04378/2013. The work also received funds from the European Union (FEDER funds) under the framework of Quadro de Referência Estratégica Nacional (QREN) NORTE-07-0124-FEDER-000066. T. G. R. and Â. N. were supported by fellowships from FCT (SFRH/BD/75752/2011 and SFRH/BPD/104927/2014, respectively).

Transparency declarations

None to declare.

Supplementary data

Table S1, Figure S1 and Figure S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Woerther PL, Burdet C, Chachaty E *et al*. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev* 2013; **26**: 744–58.

2 Ewers C, Bethe A, Semmler T *et al*. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. *Clin Microbiol Infect* 2012; **18**: 646–55.

3 Guerra B, Fischer J, Helmuth R. An emerging public health problem: acquired carbapenemase-producing microorganisms are present in food-producing animals, their environment, companion animals and wild birds. *Vet Microbiol* 2014; **171**: 290–7.

4 van der Bij AK, Pitout JD. The role of international travel in the worldwide spread of multiresistant Enterobacteriaceae. *J Antimicrob Chemother* 2012; **67**: 2090–100.

5 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.

6 Leopold SJ, van Leth F, Tarekegn H *et al*. Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: a systematic review. *J Antimicrob Chemother* 2014; **69**: 2337–53.

7 Storberg V. ESBL-producing Enterobacteriaceae in Africa – a non-systematic literature review of research published 2008–2012. *Infect Ecol Epidemiol* 2014; **4**: 20342.

8 Albrechtova K, Kubelova M, Mazancova J *et al*. High prevalence and variability of CTX-M-15-producing and fluoroquinolone-resistant *Escherichia coli* observed in stray dogs in rural Angola. *Microb Drug Resist* 2014; **20**: 372–5.

9 Machado E, Coque TM, Cantón R *et al*. Antibiotic resistance integrons and extended-spectrum β-lactamases among Enterobacteriaceae isolates recovered from chickens and swine in Portugal. *J Antimicrob Chemother* 2008; **62**: 296–302.

10 Freitas F, Machado E, Ribeiro TG *et al*. Long-term dissemination of acquired AmpC β-lactamases among *Klebsiella* spp. and *Escherichia coli* in Portuguese clinical settings. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 551–8.

11 Bogaerts P, Rezende de Castro R, de Mendonça R *et al*. Validation of carbapenemase and extended-spectrum β-lactamase multiplex endpoint PCR assays according to ISO 15189. *J Antimicrob Chemother* 2013; **68**: 1576–82.

12 Pires J, Novais Â, Peixe L. Blue-Carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. *J Clin Microbiol* 2013; **51**: 4281–3.

13 Ribeiro TG, Novais Â, Branquinho R *et al*. Phylogeny and comparative genomics unveil independent diversification trajectories of *qnrB* and genetic platforms within particular *Citrobacter* species. *Antimicrob Agents Chemother* 2015; **59**: 5951–8.

14 Andrade LN, Novais Â, Branquinho R. *et al*. Species reassignment of *Enterobacter cloacae* complex isolates by *in silico* phylogenetic analysis. In: *Abstracts of the Sixth Congress of European Microbiologists, 2015*. Abstract FEMS-2692, p. 749. Federation of European Microbiological Societies, Maastricht, The Netherlands.

15 Rodrigues C, Novais Â, Machado E *et al*. Detection of VIM-34, a novel VIM-1 variant identified in the intercontinental ST15 *Klebsiella pneumoniae* clone. *J Antimicrob Chemother* 2014; **69**: 274–5.

16 Rodríguez I, Thomas K, Van Essen A *et al*. Chromosomal location of $bla_{CTX\text{-}M}$ genes in clinical isolates of *Escherichia coli* from Germany, The Netherlands and the UK. *Int J Antimicrob Agents* 2014; **43**: 553–7.

17 Johnson TJ, Bielak EM, Fortini D *et al*. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant Enterobacteriaceae. *Plasmid* 2012; **68**: 43–50.

18 Ben Sallem R, Ben Slama K, Rojo-Bezares B *et al*. IncI1 plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ or $bla_{CMY\text{-}2}$ genes in *Escherichia coli* from healthy humans and animals in Tunisia. *Microb Drug Resist* 2014; **20**: 495–500.

19 Rodrigues C, Machado E, Peixe L *et al*. IncI1/ST3 and IncN/ST1 plasmids drive the spread of $bla_{TEM\text{-}52}$ and $bla_{CTX\text{-}M\text{-}1/\text{-}32}$ in diverse *Escherichia coli* clones from different piggeries. *J Antimicrob Chemother* 2013; **68**: 2245–8.

20 Agostinho MH. IACS – international experiences. In: *Oral Communications of the International Congress on Preventing and Fighting Healthcare-Associated Infections, Coimbra, 2014*. www.apih.pt/congresso_2014/18_iacs_angola.pdf.

21 Breurec S, Guessennd N, Timinouni M *et al*. *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. *Clin Microbiol Infect* 2013; **19**: 349–55.

22 Rafaï C, Frank T, Manirakiza A *et al*. Dissemination of IncF-type plasmids in multiresistant CTX-M-15-producing Enterobacteriaceae isolates from surgical-site infections in Bangui, Central African Republic. *BMC Microbiol* 2015; **15**: 15.

23 Dahmen S, Haenni M, Châtre P *et al*. Characterization of $bla_{CTX\text{-}M}$ IncFII plasmids and clones of *Escherichia coli* from pets in France. *J Antimicrob Chemother* 2013; **68**: 2797–801.