Journal of Antimicrobial Chemotherapy
DOI: 10.1093/jac/dkh464
Advance Access publication 14 October 2004

Reply

P. M. Bennett[1]*, C. T. Livesey[2], D. Nathwani[3], D. S. Reeves[4], J. R. Saunders[5] and R. Wise[6]

[1]Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD; [2]Central Veterinary Laboratory, New Haw, Surrey KT15 3NB; [3]Ninewells Hospital, Tayside University Hospitals, Dundee DD1 9SY; [4]Journal of Antimicrobial Chemotherapy, Editorial Office, Birmingham B1 2JS; [5]School of Biological Sciences, University of Liverpool, Liverpool L69 7ZB; [6]Department of Medical Microbiology, City Hospital NHS Trust, Dudley Road, Birmingham B18 7QH, UK

Keywords: antibiotic resistance, resistance genes, genetically modified plants

*Corresponding author. Tel: +44-117-928-7897;
Fax: +44-117-928-7896; E-mail: Peter.M.Bennett@bristol.ac.uk

Sir,

We read the correspondence from Goodyear[1] with interest. Whereas we basically agree with the points raised regarding animal husbandry, these reservations do not alter materially the central arguments in the BSAC Working Party Report,[2] that the three resistance genes are widespread as the result of natural spread between bacteria, that the most likely route for a resistance gene into a new bacterium is from one that possesses it, and that rescue of a resistance gene from plant DNA is likely to be a very infrequent event, if it can happen at all in the absence of significant sequence homology. In terms of risk, what is important is quantification. If the gene(s) is already widespread, as a consequence of natural transfer, then the additional risk arising from an unlikely, i.e. very low frequency, event can be considered of little consequence, because it will add little to the risk, in the overall scheme of things.

However, the addition of any new adventitious pathway for transfer of bacterial antibiotic resistance genes is, in principle, undesirable, because it may be a more effective pathway into bacteria that do not yet carry the genes than is currently believed to be the case. Hence, when possible, bacterial antibiotic resistance genes should not be incorporated into plant genomes in the course of construction of GM cultivars.

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Journal of Antimicrobial Chemotherapy
DOI: 10.1093/jac/dkh444
Advance Access publication 7 October 2004

Detection of CTX-M-1 and TEM-52 β-lactamases in *Escherichia coli* strains from healthy pets in Portugal

Daniela Costa[1], Patricia Poeta[1], Laura Briñas[2], Yolanda Sáenz[2], Jorge Rodrigues[1,3] and Carmen Torres[2]*

[1]Departamento de Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro, Vila Real; [3]Centro de Estudos de Ciências Animais e Veterinárias, Vila Real, Portugal; [2]Area de Bioquímica y Biología Molecular, Universidad de La Rioja, Madre de Dios 51, 26006 Logroño, Spain

Keywords: extended-spectrum β-lactamases, healthy animals, Enterobacteriaceae

*Corresponding author. Tel: +34-941-299750;
Fax: +34-941-299721; E-mail: carmen.torres@daa.unirioja.es

Sir,

In recent years, the dissemination of *Escherichia coli* strains harbouring extended-spectrum β-lactamases (ESBLs) in clinical settings has caused a great deal of concern. Most ESBLs are derived from the classical TEM-1, TEM-2 and SHV-1 enzymes by amino acid substitutions in their sequences.[1] A new type of ESBL, CTX-M enzymes, is increasingly being reported among human clinical *E. coli* strains;[2] although only two references exist about detection of this type of β-lactamase in animal *E. coli* strains,[3,4] they have never been reported in pets before. The first detection of a CTX-M-containing *E. coli* strain in Portugal was reported very recently.[5] It was recovered from a healthy human. Until now such strains had not been reported from animals in Portugal. The objective of this work was to study the intestinal colonization by ESBL-containing *E. coli* strains in healthy pets in Portugal.

Faecal samples of 75 healthy pets (39 dogs and 36 cats) that had not received previous antibiotic treatment, were recovered in Portugal during 2003 and tested for the presence of ESBL-containing *E. coli* strains. Samples were seeded in Levine agar supplemented with cefotaxime (2 mg/L), and colonies with typical *E. coli* morphology were selected and identified by classical biochemical methods. Susceptibility testing for 17 antibiotics was carried out by agar dilution and disc diffusion methods and those *E. coli* strains that showed broad-spectrum cephalosporin (cefotaxime or ceftazidime) resistance were selected (one per animal), and studied further. The screening of ESBL production was also analysed by the double disc test (cefotaxime and ceftazidime with or without clavulanic acid). The presence of TEM, SHV, OXA, CTX-M, FOX and CMY β-lactamase-encoding genes was studied by PCR and sequencing[3,6] in all the broad-spectrum cephalosporin-resistant *E. coli* strains recovered.

Table 1. Characteristics of the four ESBL-containing *E. coli* strains recovered from healthy pets in Portugal

E. coli	Animal source	MIC (mg/L)								Phenotype of resistance for non-β-lactams[a]	ESBL screening test	ESBL encoding genes detected
		AMP	TIC	AMC	FOX	CTX	CAZ	ATM	IPM			
E14	dog	>256	>128	16	4	32	16	4	0.06	STR–TET	+	$bla_{\text{TEM-52b}}$
E39	dog	>256	>128	16	8	16	16	4	0.06	STR	+	$bla_{\text{TEM-52b}}$
E55	dog	>256	>128	16	2	16	8	2	0.125	STR–TET	+	$bla_{\text{TEM-52b}}$
E42	dog	>256	>128	64	2	128	2	16	0.06	STR–TET–CHL–SUL	+	$bla_{\text{CTX-M-1}}$

AMP, ampicillin; TIC, ticarcillin; AMC, co-amoxiclav; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; IPM, imipenem; STR, streptomycin; TET, tetracycline; CHL, chloramphenicol; SUL, sulfamethoxazole.

[a] Non-β-lactam antibiotics tested: gentamicin, tobramycin, amikacin, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, sulfamethoxazole, chloramphenicol, streptomycin and tetracycline.

Mutations in the promoter–attenuator region of the chromosomal *ampC* gene were also studied by PCR and sequencing.[3]

Broad-spectrum cephalosporin-resistant *E. coli* strains were detected in five of the 75 faecal samples analysed (6.6%, four dogs and one cat). Four of the five strains, obtained from four unrelated dogs, gave positive ESBL screening test results and were resistant to cefotaxime and/or ceftazidime. The $bla_{\text{TEM-52b}}$ gene was identified in three of these four strains and the $bla_{\text{CTX-M-1}}$ gene in the remaining one, these strains being negative for the other β-lactamase genes tested by PCR. The characteristics of these *E. coli* strains are included in Table 1. The three $bla_{\text{TEM-52b}}$-containing strains were also resistant to streptomycin, and two of them also to tetracycline. The $bla_{\text{CTX-M-1}}$-containing strain was resistant to streptomycin–tetracycline–sulfamethoxazole–chloramphenicol and also harboured the *int*I gene, associated with type I integrons (detected by PCR).

The remaining broad-spectrum cephalosporin-resistant *E. coli* strain that was recovered from a healthy cat, showed a negative ESBL screening test and all PCR assays for TEM, SHV, OXA, CTX-M, FOX and CMY β-lactamase-encoding genes were negative. Mutations at the −42, −18, −1 and +58 positions of the promoter–attenuator region of the chromosomal *ampC* gene were detected by PCR and sequencing. This strain showed a pattern of multi-resistance, which included nalidixic acid, ciprofloxacin, tetracycline, streptomycin, gentamicin, tobramycin, trimethoprim/sulfamethoxazole and chloramphenicol, and this strain also harboured the *int*I gene.

To our knowledge, this is the first time that ESBL-encoding genes have been detected in healthy pets and also the first time that CTX-M β-lactamases have been detected in *E. coli* strains from animal origin in Portugal. More studies should be carried out in the future to track the evolution of this type of β-lactamase in different environments.

Acknowledgements

We thank the veterinary Hospital Montenegro of Porto (Portugal) for providing us with the faecal samples for this study. This work has been supported in part by a grant from the Fondo de Investigaciones Sanitarias of Spain (FIS 01/973).

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Journal of Antimicrobial Chemotherapy
DOI: 10.1093/jac/dkh447
Advance Access publication 7 October 2004

Antibiotics in media for isolation of *Campylobacter* spp. do not enhance resistance

Lilian Pumbwe and Laura J. V. Piddock*

Antimicrobial Agents Research Group, Division of Immunity and Infection, University of Birmingham, Birmingham B15 2TT, UK

Keywords: induction, gene expression, susceptibility

*Corresponding author. Tel: +44-121-414-6966;
Fax: +44-121-414-3599; E-mail: l.j.v.piddock@bham.ac.uk

Sir,

Campylobacter spp. have become the major cause of gastroenteritis in the Western world. However, isolation and culture of these bacteria from different source samples is difficult as they are fastidious and slow growing, and are thus out-competed by