

Short Report

Screening of clinical, food, water and animal isolates of *Escherichia coli* for the presence of blaCTX-M extended spectrum beta-lactamase (ESBL) antibiotic resistance gene loci

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ABSTRACT

A small study was carried out in order to examine the molecular presence of bla CTX-M gene phylogenetic groups in *E. coli* (n=317) isolated from food (n=54), water (n=7), animal sources (n=69), using consensus bla CTX-M primers and PCR, in addition to human faecal isolates (n=69) and VTEC O157:H7 (n=64). None of the clinically significant faecal VTEC O157:H7 isolates were shown to carry blaCTX-M type phylogenetic groups, nor were such phylogenetic groups observed in any of the food, water and animal isolates. One community faecal isolate (1/69; 1.4%), dating from 1997, carried this phylogenetic group. As recent work has indicated that a significant proportion of such phylogenetic groups are carried in community isolates of *E. coli* with little or no hospital contact, it is important that surveillance is increased to identify potential source(s) and reservoirs of such resistance in the community. Further prospective surveillance is thus required to help elucidate the origins of such phylogenetic group in the community. The significance of this study is that the ESBL-producing *E. coli* associated with local hospital outbreaks is not commonly found in local food, water or animal sources. In addition, given that ESBL-producing *E. coli* is now a significant organism, both in hospitals and nursing homes in Northern Ireland, this report demonstrates that such organisms were present in the community, as early as 1997.

Keywords: esbl, CTX-M β -lactamases, community infections, food, animal, molecular epidemiology, PCR.

INTRODUCTION

Extended spectrum β -lactamase (esbl) producing organisms were first described in Germany in 1983 from *Klebsiella pneumoniae*, following the introduction of the new oxyiminocephalosporins, including cefotaxime, aztreonam and ceftazidime.¹ These enzymes hydrolyze oximino- β -lactams, including the third generation cephalosporins, thereby conferring antibiotic resistance in those organisms which carry the esbl resistance determinant gene loci.² Such loci are carried in several genera with the *Enterobacteriaceae*, including *Klebsiella*, *Enterobacter*, *Escherichia* and *Salmonella*.¹ For recent reviews on esbls and *Enterobacteriaceae*, please see Rupp and Fey³ and/or Shah *et al.*⁴ and Bonnet⁵ for a review on CTX-M mediated resistance.

Recently Woodford *et al.*⁶ have described the emergence of a bla CTX-M-15 esbl in *E. coli* from 42 centres in the UK, including Northern Ireland, where overall, 70 (24%) were reported to originate from community patients, many whom had limited hospital contact and where 12 centres had community isolates. Given the widespread distribution of this bla CTX-M type phylogenetic group, particularly from community isolates, it is important to identify the distribution of such phylogenetic groups in *E. coli* in the community, which may be a reservoir and potential sources of bla CTX-M

type phylogenetic groups, promoting cephalosporin resistance in *E. coli* associated with community acquired urinary tract infections (UTIs).

Therefore, it was the aim of this small study, to employ molecular methods to screen animal, food and water isolates of *E. coli* for the presence of bla CTX-M type phylogenetic groups, as well as to identify the presence of these phylogenetic groups in a comprehensive collection of community acquired faecal VTEC O157:H7 isolates from throughout Northern Ireland.

MATERIALS & METHODS

Source of *E. coli* isolates

E. coli isolates (n=317) as detailed in Table 1, were examined

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in this study. All isolates were confirmed phenotypically as *E. coli*, by employment of a combination of biochemical assays and the API20E Identification scheme (Biomérieux Ltd., UK), using standard laboratory identification methods. In addition, all verocytotoxigenic *E. coli* O157:H7 were confirmed serologically and by employment of a multiplex PCR assay of virulence gene determinants (VT1+VT2+*eae* gene loci), as previously described.⁷

DNA extraction, PCR amplification & amplicon detection

E. coli isolates (table 1) were subcultured onto Columbia agar base (CM331, Oxoid Ltd., Basingstoke, England), supplemented with defibrinated horse blood 5% [v/v] (Oxoid). All DNA isolation procedures were carried out in a Class II Biological Safety Cabinet in a room geographically separate from that used to set up reaction mixes and also from the "post-PCR" room in order to minimise the production of false positive results and in accordance with Good Molecular Diagnostic Practice (GMDP), as defined in the guidelines of Millar *et al.*⁸ PCR amplification of the *bla*_{CTX-M} gene loci was performed in accordance with a previously published method². During each run molecular grade water was included randomly as negative controls and appropriate DNA template from a reference isolate of *Enterobacter cloacae* (CTX-M 9) and a wild characterized *E. coli* (CTX-M 15) obtained from the Northern Ireland outbreak,⁶ were included as positive controls. In addition, the MA1/MA2 primer combination were challenged with seven non-esbl producing organisms, including *E. coli* (n=2), *Enterobacter cloacae* (n=1), *Klebsiella pneumoniae* (n=2) and *Klebsiella terrigena* (n=2). In addition, 16 wild type clinical esbl-producing *E. coli* isolates from Northern Ireland, which were confirmed as CTX-M 15 subtype, were also included, as positive controls, as well as an esbl-producing *Klebsiella oxytoca* isolate.

Following amplification, aliquots (15µl) were removed from each reaction mixture and examined by electrophoresis (80V, 45min) in gels composed of 2% (w/v) agarose (Gibco,UK) in TAE buffer (40mM Tris, 20mM acetic acid, 1mM EDTA, pH 8.3), stained with ethidium bromide (5µg/100ml). Gels were visualised under UV illumination using a gel image analysis system (UVP Products, England) and all images archived as digital (*.bmp) graphic files.

RESULTS AND DISCUSSION

Employment of the MA1/MA2 CTX-M consensus primer pair was successful at identifying the esbl gene locus in all esbl-producing isolates, whereas no PCR amplicon was produced on examination of the non-esbl producing organisms examined, including non-esbl producing *E. coli* (data not shown). Examination of the 317 *E. coli* isolates, as detailed in Table 1, produced only one positive result, which was isolated from a faecal specimen of a female patient in the community, where no significant growth or faecal pathogens were detected. None of the VTEC O157:H7 isolates examined were positive for the presence of an esbl gene locus.

CTX-M-producing *E. coli* have been described recently as a rapidly developing problem in the UK, where the study of Woodford *et al.*⁶ demonstrated the presence of CTX-M-15 in *E. coli* originating from 42 centres throughout the UK. Another report in the UK by Munday *et al.*⁹ demonstrated

that surveillance of 1000 faecal samples collected and screened at York Hospital during the last quarter of 2003, resulted in 17 (1.7%) CTX-M phylogenetic groups being identified, including CTX-M-9 (n=9), CTX-M-15 (n=5) and CTX-M-14 (n=3). Both these studies have indicated that such phylogenetic group occur in community isolates of *E. coli*, where there has been no or very limited contact with the hospital environment.

Locally in Northern Ireland, a recent report by Loughrey *et al.*¹⁰ demonstrated the presence of ESBL-producing *E. coli* in 120/307 (39%) faecal specimens from 13 long-term care facilities. This report also showed that 60 (50%) of 120 ESBL *E. coli* -positive residents had no hospital admissions since January 2004. The majority of ESBL-producing *E. coli* had phenotypes consistent with production of a CTX-M enzyme. Isolates assigned presumptively to strain A by PCR accounted for 59/120 (49%) ESBL-producing *E. coli*. Although distinct from strain A, most of the other 61 isolates also produced a group 1 CTX-M ESBL; these isolates had varying antibiograms, suggesting multiple strains. In the eastern district of Belfast, 50/175 samples were ESBL-producing *E. coli* -positive, and 38 (76%) of these were strain A; in the other districts 70/132 samples were positive, but only 22 (31%) were strain A. The proportion of strain A isolates varied widely in different nursing homes, ranging from 0/11 ESBL-producing *E. coli* in one centre to 9/9 in another. Epidemic strain A was the predominant ESBL-producing *E. coli* strain among nursing home residents in Belfast and this organism was found in many residents with no history of recent hospital admission.

Although the epidemiology of esbl-producing *E. coli* with the CTX-M phylogenetic groups have been more clearly defined in the hospital setting, the origins of esbl-producing *E. coli* isolates remain unclear in the community. Therefore, any attempts through surveillance studies, to help define these origins should be encouraged.

Recently, two independent epidemiological studies in non-hospitalized patients demonstrated various risk factors for acquisition of an esbl-producing organism in the community. In the first study by Colodner *et al.*¹¹, 311 non-hospitalized patients with community-acquired UTIs, showed that (i) previous hospitalization in the past 3 months, (ii) antibiotic treatment in the past 3 months, (iii) age over 60 years, (iv) diabetes, (v) male gender, (vi) *Klebsiella pneumoniae* infection, (vii) previous use of third-generation cephalosporins, (viii) previous use of second-generation cephalosporins, (ix) previous use of quinolones and (x) previous use of penicillin, were significant risk factors for the acquisition of such an infection. In the second study by Borer *et al.*¹², 187 *Enterobacteriaceae* bacteremias were detected, of which 119 were community-acquired (63.6%), of which six cases were due to an esbl-producing organism. This study demonstrated that patients suffering from community acquired bacteraemia with an esbl were older and where urinary catheterization and bed-ridden conditions were significant risk factors and where such patients were more likely to suffer from complications and had a higher mortality. Although these studies identify several risk factors for developing an esbl-associated infection, there was no discussion in either study, as to where the esbl-producing

TABLE 1:

Description (origin, date) and presence of extended spectrum β -lactamase *bla* CTX-M phylogenetic groups in *E. coli* isolates examined in this study.

| Description of isolates | Source | Number of isolates examined | Date of isolation of cultures | No. PCR esbl +ve (% +ve) | Comments |
|------------------------------|---|---|-------------------------------|--------------------------|--|
| Human [clinical] | | | | | |
| Faeces | Human faeces submitted by GPs in the community and in-patient hospital wards | 69 | 1997 | 1 (1.4%) | CTX-M PCR +ve specimen originated from female patient in the community. |
| VTEC O157:H7 | Human faeces | 10 | 1997 | 0 | VTEC isolates |
| | | 18 | 1998 | 0 | referred to |
| | | 15 | 1999 | 0 | NIPHL* from |
| | | 11 | September – December 2003 | 0 | primary diagnostic laboratories |
| | | 10 | January – September 2004 | 0 | throughout N. Ireland |
| Animal [clinical] | | | | | |
| Clinical isolates | Equine | 69 | January – July 2004 | 0 | |
| Environmental <i>E. coli</i> | Water Well water (n=4) Swimming pool (n=1) Bore hole water (n=1) Chlorinated tap water (n=1) | 7 | January – July 2004 | 0 | Isolated from water submitted by Environmental Health Officers from throughout Northern Ireland |
| Environmental <i>E. coli</i> | Food (total) Shellfish Cooked meats Rice Sauces Cooked restaurant meal Pastry with cream filling Vegetables/salads | 54 28 9 2 2 10 2 1 | April – June 2004 | 0 | Isolated from foodstuffs submitted by Environmental Health Officers from throughout Northern Ireland |

organisms originated in the community. Therefore it is important to be able to identify the origins of esbl-organisms in susceptible patient populations in the community.

Recent reports have demonstrated the presence of *bla* CTX-M phylogenetic groups in animals. Bri as *et al.*¹³ demonstrated the presence of a CTX-M-14 from *E. coli* isolated from faecal material of health chickens, and Shiraki *et al.*¹⁴ demonstrated the presence of CTX-M-2 in *E. coli* from bovine faecal specimens and suggested that the acquisition of such phylogenetic group in the bovine *E. coli* isolates may have originated from cattle through the use of cephalosporins such as ceftiofur and that cattle could be a reservoir of CTX-M-2-producing *E. coli*. These concluded that continuous and strategic surveillance of antimicrobial-resistant bacteria in

livestock is essential to suppress further dissemination of these bacteria into society at large.

Munday *et al.*⁹ demonstrated that there was dissemination of CTX-M type esbls into the *Enterobacteraceae* by a variety of mechanisms of horizontal gene transfer. Furthermore, Liebana *et al.*¹⁵ suggested that β -lactam resistance in animal isolates can be generated *de novo* and demonstrated the presence of an AmpC-like esbl in *Enterobacteraceae* from turkeys, chickens, pigs and cattle. Therefore, it may be postulated that *bla* CTX-M loci in meat contaminated with viable organisms containing such phylogenetic groups may be one source of such phylogenetic groups for susceptible patients in the community.

Please correct to "In our limited study of *E. coli* isolates obtained from foods, waters and animal sources, in 2004, during which period, we were actively finding CTX-M-15 phylogenetic groups in clinical *E. coli* from blood culture, urine and sputum specimens, we were not able to detect the presence of any CTX-M esbl *E. coli*, from any non-human source. Shellfish were particularly targeted as they were collected from inshore marine waters, where the catchment area included agricultural run-off and sewage treatment works. No CTX-M phylogenetic groups were observed in *E. coli* from any food, water or animal isolate examined and likewise none of the VTEC faecal isolates showed any evidence of bla CTX-M involvement.

As recent work has indicated that a significant proportion of such phylogenetic groups are carried in community isolates of *E. coli* with little or no hospital contact,^{6,9} it is important that surveillance is increased to identify potential source(s) and reservoirs of such resistance in the community, particularly in food animals and pets. Further prospective surveillance is thus required to help elucidate the origins of such phylogenetic groups in *E. coli* in the community.

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The authors have no conflict of interest.

REFERENCES

- Philippon AG, Arlet G, Lagrange PH. Origin and impact of plasmid-mediated extended-spectrum beta-lactamases. *Eur J Clin Microbiol Infect Dis.* 1994; **13**(Suppl 1): S17-S29.
- Saladin, M., Cao VT, Lambert T, Donay JL, Herrmann JL, Ould-Hocine Z, et al. Diversity of CTX-M beta-lactamases and their promoter regions from *Enterobacteriaceae* isolated in three Parisian hospitals. *FEMS Microbiol Lett.* 2002; **209**(2): 161-8.
- Rupp ME, Fey PD. Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*: considerations for diagnosis, prevention and drug treatment. *Drugs.* 2003; **63**(4): 353-65.
- Shah AA, Hasan F, Ahmed S, Hameed A. Extended-spectrum beta-lactamases (ESBLs): characterization, epidemiology and detection. *Crit Rev Microbiol.* 2004; **30**(1): 25-32.
- Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother.* 2004; **48**(1): 1-14.
- Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, et al. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β -lactamases in the UK. *J Antimicrobiol Chemother.* 2004; **54**(4): 735-43.
- Crothers JL, Moore JE, Millar BC, Watabe M, Rooney PJ. Determination of verocytotoxin and *eae* gene loci by multiplex PCR in *Escherichia coli* O157:H7 isolated from human faeces in Northern Ireland: a four-year study of trends, 1997-2000. *Br J Biomed Sci.* 2004; **61**(1): 1-7.
- Millar BC, Xu J, Moore JE. Risk assessment models and contamination management: implications for broad-range ribosomal DNA PCR as a diagnostic tool in medical bacteriology. *J Clin Microbiol.* 2002; **40**(5): 1575-80.
- Munday CJ, Whitehead GM, Todd NJ, Campbell M, Hawkey PM. Predominance and genetic diversity of community- and hospital-acquired CTX-M extended-spectrum β -lactamases in York, UK. *J Antimicrob Chemother.* 2004; **54**(3): 628-33.
- Loughrey A, Rooney P, O'Leary M, McCalmont M, Warner M, Karisik E, et al. Prevalence of an epidemic ESBL-producing *Escherichia coli* strain in LTCFs in Belfast. [Abstract] *Clin Microbiol Infect.* 2007; **13**(Suppl 1): S373.
- Colodner R, Rock W, Chazan B, Keller N, Guy N, Sakran W, et al. Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. *Eur J Clin Microbiol Infect Dis.* 2004; **23**(3): 163-7.
- Borer A, Gilad J, Menashe G, Peled N, Riesenberg K, Schlaeffer F. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* strains in community-acquired bacteremia in Southern Israel. *Med Sci Monit.* 2002; **8**(1): CR44-7.
- Briñas L, Moreno MA, Zarazaga M, Porrero C, Sáenz Y, García M. Detection of CMY-2, CTX-M-14, and SHV-12 beta-lactamases in *Escherichia coli* fecal-sample isolates from healthy chickens. *Antimicrob Agents Chemother.* 2003; **47**(6): 2056-8.
- Shiraki Y, Shibata N, Doi Y, Arakawa Y. *Escherichia coli* producing CTX-M-2 beta-lactamase in cattle, Japan. *Emerg Infect Dis.* 2004; **10**(1): 69-75.
- Liebana EM, Gibbs M, Clouting C, Barker L, Clifton-Hadley FA, Pleydell E et al. Characterization of beta-lactamases responsible for resistance to extended-spectrum cephalosporins in *Escherichia coli* and *Salmonella enterica* strains from food-producing animals in the United Kingdom. *Micro Drug Resist.* 2004; **10**(1): 1-9.