JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *E. COLI* CONTAMINATION OF CHICKEN MEAT IN THE IRISH RETAIL MARKET

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ARTICLE INFO

Received 18. 3. 2014
Revised 18. 3. 2014
Accepted 19. 3. 2014
Published 1. 4. 2014

Short communication

OPEN ACCESS

ABSTRACT

Animals represent potential reservoirs for the dissemination of antimicrobial resistance. Twenty domestically produced chicken meat samples were collected from 19 retail outlets in Ireland, inoculated into Bolton broth and cultured on modified charcoal cefoperazone deoxycholate (mCCDA) and Preston agars. Selected representative coliforms included 16 *E.coli* and 4 *Pseudomonas aeruginosa*. All *E.coli* isolates were confirmed as ESBL producers, 15 isolates harbored a $bla_{\text{CTX-M group-1}}$ gene, and none belonged to the *E.coli* 025b:H4-ST131 clonal group. Pulsed field gel electrophoresis (PFGE) analysis identified 13 distinct pulsed field profiles and comparison with more than 300 human clinical isolates of ESBL producing *E. coli* did not reveal any similarities. ESBL producing *E. coli* were detected on retail meats in the Irish market place. Although no similarity was apparent between poultry and human isolates this does not preclude a role for ESBL-producing *E.coli* in meat in dissemination of antimicrobial resistance.

Keywords: Extended spectrum beta-lactamase, *E.coli*, food safety, molecular epidemiology

INTRODUCTION

Extended spectrum beta-lactamases (ESBLs) confer resistance to the expanded spectrum cephalosporins, and limit severely the options available for treatment of serious infection. Infection with ESBL producing *Enterobacteriaceae* (ESBL-PE) results in significant increases in morbidity, mortality and healthcare costs (**Schwaber and Carmeli, 2007**). The rapid dissemination of these enzymes, in particular the CTX-M beta-lactamases has been linked with their association with particular epidemic clones (e.g. *E.coli* 025b:H4-ST131) and plasmids (e.g. IncFII). Animals are increasingly recognised as potential reservoirs for ESBL-PE, with several reports in recent years in both food producing and companion animals **(Boyle *et al.*, 2010; Horton *et al.*, 2011; Marshall, Levy, 2011; Randall *et al.*, 2011; Wieler *et al.*, 2011; Borjesson *et al.*, 2013**). Contamination of retail meats with ESBL-PE has also been documented in a number of countries **(Leverstein-van Hall *et al.*, 2011; Overdevest *et al.*, 2011**). The presence of ESBL-PE in meat also represents a methodological problem in relation to use of Bolton broth for isolation of *Campylobacter* spp. from meat because ESBL producing *E. coli* is associated with the capacity to grow through the cefoperazone used as a selective component in Bolton broth (**Moran *et al.* 2011**). The aim of this study was to exploit the observation that Bolton broth now acts as selective agent for ESBL producing *E. coli* to assess retail chicken meat in the Irish market place for the presence of ESBL-PE and to investigate links to human isolates.

MATERIAL AND METHODS

Twenty domestically produced chicken meat samples were collected from 19 retail outlets in Ireland in the course of a national survey to assess frequency of campylobacter contamination in poultry. Samples were inoculated into Bolton broth, homogenised and after overnight incubation were cultured on modified charcoal cefoperazone deoxycholate agar (mCCDA) and Preston agar in accordance with standard procedures for isolation of *Campylobacter* from chicken, BS EN ISO 10272-1:2006. In addition to selecting isolates of *Campylobacter*, representative colonies with morphology suggestive of coliform bacteria were also selected for further examination. Colonies thus selected from the 20 samples were *Escherichia coli* in 16 cases and *Pseudomonas aeruginosa* in 4. Isolates were screened for ESBL production by the combination disk method of the Clinical Laboratory Standards Institute (CLSI) using cefpodoxime (**Clinical Laboratory Standards Institute, 2011**). All isolates were screened for susceptibility to the following antimicrobial agents in accordance with CLSI disk diffusion methods: ampicillin (10µg); cefpodoxime (10µg); cefotaxime (30µg); ceftazidime (30µg); cefoxitin (30µg); amoxicillin-clavulanate (20µg -10µg); piperacillin-tazobactam (100µg -10µg); ertapenem (10µg); meropenem (10µg); nalidixic acid (30µg); ciprofloxacin (5µg); gentamicin (10µg); kanamycin (30µg); streptomycin (10µg); chloramphenicol (30µg); sulphonamides (300µg); tetracycline (30µg); trimethoprim (5µg); minocycline (30µg). All ESBL producing *E. coli* were screened for the presence of $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, and $bla_{\text{OXA-1}}$ by PCR as previously described **(Essack, *et al.* 2001; Woodford *et al.*, 2004**). A duplex PCR assay using primers specific to the *pab*B (region specific to the 025b-ST131 clone) and *trp*A genes was performed on all as previously described (**Clermont *et al.*, 2009**). Pulsed field gel electrophoresis (PFGE) was performed on all ESBL producing *E. coli* by the PulseNet protocol with *Xba*I (**Swaminathan *et al*, 2001**). The pulsed field profiles (PFPs) generated were compared with over 300 PFPs of human clinical isolates of ESBL producing *E. coli* collected throughout Ireland. Analysis of PFPs was performed using the Dice coefficient with clustering by the unweighted pair group method with arithmetic averaging (UPGMA).

RESULTS AND DISCUSSION

All 16 *E. coli* isolates were confirmed as ESBL producers, were resistant to ampicillin, cefotaxime, sulphonamides, and trimethoprim, and susceptible to piperacillin-tazobactam and amikacin (Table 1).

Table 1 Antimicrobial susceptibility testing, PCR and PFGE analysis of *E. coli* isolates

Isolate Number	Source	Antibiogram[1]	PCR analysis	PFP
667	Whole chicken	ACpdCtxAtmTmSuTe	$bla_{\text{CTX-M-group-1}}$, bla_{TEM}	M
705	Whole chicken	ACpdCtxTmCSSuTe	$bla_{\text{CTX-M-group-1}}$, bla_{TEM}	I
671	Chicken breast	ACpdCtxAtmNaGTmSuTe	$bla_{\text{CTX-M-group-1}}$, bla_{TEM}	K
666	Whole chicken	ACpdCtxAtmNaCipAmcTmSuTe	$bla_{\text{CTX-M-group-1}}$, bla_{TEM}	E
714	Skinless chicken fillet	ACpdCtxAtmNaTmSu	$bla_{\text{CTX-M-group-1}}$	L
679	Whole chicken	ACpdCtxKAmcTmSSuTe	$bla_{\text{CTX-M-group-1}}$	C1
678	Chicken fillet	ACpdCtxAtmAmcTmSSuTe	$bla_{\text{CTX-M-group-1}}$	F
715	Skinless chicken fillet	ACpdCtxCazKAmcTmSSuTe	bla_{TEM}	J
723	Whole chicken	ACpdCtxAtmNaCipTmSu	$bla_{\text{CTX-M-group-1}}$, bla_{TEM}	H
658	Chicken fillet	ACpdCtxAtmAmcTmSuTe	$bla_{\text{CTX-M-group-1}}$	C
721	Chicken fillet	ACpdCtxAtmTmSuTe	$bla_{\text{CTX-M-group-1}}$, bla_{TEM}	B
690	Whole chicken	ACpdCtxTmSuTe	$bla_{\text{CTX-M-group-1}}$	D
665	Whole chicken	ACpdCtxAtmAmcTmSu	$bla_{\text{CTX-M-group-1}}$	A
669	Whole chicken	ACpdCtxAtmAmcTmSSuTe	$bla_{\text{CTX-M-group-1}}$, bla_{TEM}	B1
716	Chicken leg	ACpdCtxTmSuTe	$bla_{\text{CTX-M-group-1}}$	D1
707	Whole chicken	ACpdCtxNaMnTmSSuTe	$bla_{\text{CTX-M-group-1}}$	G

[1]A = Ampicillin, Cpd = Cefpodoxime, Ctx = Cefotaxime, Caz = Ceftazidime, Atm = Aztreonam, Amc = Amoxicillin – Clavulanic Acid, Na = Nalidixic acid, Cip = Ciprofloxacin, G = Gentamicin, K = Kanamycin, C = Chloramphenicol, S = Streptomycin, Su = Sulphonamides, T = Tetracycline, Tm = Trimetoprim, Mn = Minocycline.

None of the *P. aeruginosa* isolates were confirmed as ESBL producers. PCR confirmed that 15/16 *E.coli* isolates harbored a $bla_{\text{CTX-M group-1}}$ gene, 8 harbored a bla_{TEM} gene, 7 harbored both bla_{TEM} gene and $bla_{\text{CTX-M-group-1}}$ genes (**Essack *et al.*, 2001; Woodford *et al.* 2006; Woodford *et al.*, 2004**) and none belonged to the *E.coli* 025b:H4-ST131 clonal group (**Clermont *et al.*, 2009**). Pulsed field gel electrophoresis (PFGE) analysis identified 13 distinct pulsed field (PFPs) among the 16 *E. coli* isolates (Figure 1). Comparison of PFP's with a large database of PFPs of more than 300 human clinical isolates of ESBL producing *E. coli* from Ireland not reveal any indistinguishable or similar isolates.

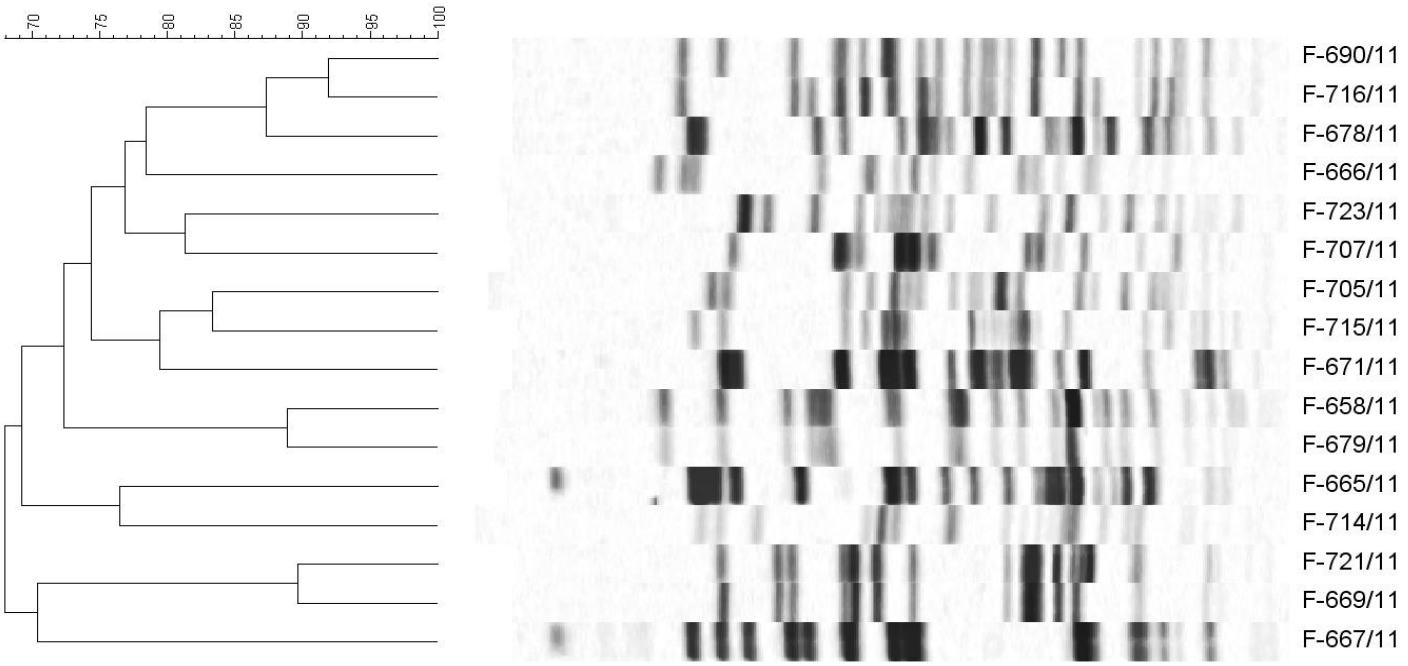

Figure 1 Dendogram showing relatedness of ESBL producing *E. coli* isolated from retail chicken meat

Carriage of ESBL-PE by food-producing and companion animals and contamination of retail meats may contribute to increases in human infections associated with these organisms. According to data from the European Antimicrobial Surveillance Network (EARS-Net) the incidence of *E. coli* associated with blood stream infection that produce ESBLs in Ireland has increased from 1.2% in 2002 to 9.9% in quarter 3 of 2013 (http://www.hpsc.ie/hpsc/A-Z/MicrobiologyAntimicrobialResistance/EuropeanAntimicrobialResistanceSurveillanceSystemEARSS/EARSSSurveillanceReports/2011Reports/File,13096,en.pdf). A small number of studies have examined relationships between ESBL-PE isolated from human, animal and food sources. Randall *et al*. (2011) recently assessed the prevalence of ESBL producing *E. coli* from poultry in Great Britain and found CTX-M-1, CTX-M-15, CTX-M-3 and TEM-52 in chicken broiler samples, and CTX-M-1, CTX-M-14, CTX-M-15, CTX-M-55 and TEM-52 in turkey samples. Analysis of PFGE patterns and MLST profiles did not reveal a relationship between poultry and human isolates (**Randall *et al.*, 2011**). Conversely, two separate reports from the Netherlands have indicated links between human and animal isolates of ESBL-PE **(Leverstein-van Hall *et al.*, 2011; Overdevest *et al.*, 2011**). Leverstein-van Hall *et al.* (2011) found that human and poultry *E. coli* isolates shared the same ESBL genes ($bla_{\text{CTX-M-1}}$ and $bla_{\text{TEM-52}}$), and plasmids (IncI I) and were of the same sequence type (ST10, ST58, ST117) **(Leverstein-van Hall *et al.*, 2011**). Overdevest *et al* (2011)

reported similarities (gene type, sequence type) between ESBL-producing *E. coli* isolated from meat samples, human rectal swabs and blood culture specimens (**Overdevest *et al.*, 2011**). These findings are interesting as human consumption of antibiotics is low but agricultural use of antibiotics is high in the Netherlands compared with other European countries **(Goossens *et al.* 2005; Grave *et al.* 2010**). Although the major ESBL type (CTX-M-group 1) identified among isolates of ESBL-producing *E.coli* recovered from chicken meat samples in this study is also dominant among human isolates of ESBL-PE in Ireland (**Morris *et al.*, 2009**) no relationship could be identified by PFGE between the *E. coli* carrying the resistance determinant and those *E. coli* from humans. Conversely in a recent study of ESBL-producing *Salmonella* Kentucky isolated from poultry samples in Ireland relationships with human and environmental isolates were apparent (**Boyle *et al.*, 2010**). *Escherichia coli* O25b:H4-ST131 is a very successful pandemic uropathogenic clone associated predominantly with community acquired antimicrobial resistant infection and has been implicated in a number of outbreaks of human infection. The close association between the extended-spectrum β-lactamase (ESBL) CTX-M-15 and *E. coli* ST131 has been implicated in the international dissemination of this enzyme (**Bush, 2010; Rogers *et al.* 2011**). This clonal group was not identified among *E. coli* isolated from retail chicken meat samples in this study, but a number of studies have reported *E. coli* ST131 in animal and retail meat samples **(Mora *et al.*, 2010; Pomba *et al.* 2009**).

CONCLUSION

ESBL-producing *E.coli* was isolated from 16 of 20 domestically produced chicken meat samples on retail sale in Ireland using the standard method for detection of *Campylobacter* spp. (BS EN ISO 10272-1:2006). Although there is no similarity between the *E. coli* isolated from poultry and those from our large human database this does not preclude a role for ESBL producing *E. coli* in meat in dissemination of plasmid encoded resistance.

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