PRESENCE OF ANTIMICROBIAL RESISTANT *Escherichia coli* IN RETAIL RAW MEATS ON THE ISLAND OF IRELAND.

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Abstract – Six hundred samples of retail raw meats were purchased across the island of Ireland and consisted of equal numbers of beef, chicken and pork. The samples were enriched overnight in tryptone soya broth supplemented cefotaxime (0.5mg/l), ciprofloxacin (0.06 mg/l) or meropenem (0.25mg/l). The concentrations were designed to be one 'step', or binary concentration change, above the ecological cut off values for *E. coli* presented on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) website. Broths were streaked onto TBX and after incubation one presumptive antimicrobial resistant *E. coli* (AREC) was selected from each positive plate, for further study. Pure cultures were confirmed as *E. coli* using MALDI-TOF, and had their sensitivity to a panel of 13 antimicrobials determined by disc diffusion.

In total, 600 meat samples yielded 496 isolates of *E. coli* of which 467 (94%) were resistant to one or more antimicrobial tested whilst 143 (27%) were ESBL producers (chicken 130, pork 12, beef 1). Ciprofloxacin resistance was seen in 110 (22%) AREC, of which 16 were also ESBL producers. Two isolates were resistant to ertapenem. Based on resistance to 2 or more classes of antimicrobial agents, 442 (89%) isolates were multi-drug resistant (MDR).

Key Words – *E. coli*, retail, meats, antimicrobial, resistance.

I. INTRODUCTION

Antimicrobial resistance is recognised globally as a major public health concern and antimicrobial resistance arises as a result of exposure of bacteria to antimicrobial agents. Antimicrobial agents have been used in human medicine, veterinary medicine, and agriculture for several decades and the appropriate and inappropriate use of these agents has resulted in the emergence and dissemination of antimicrobial resistant bacteria. This project aimed to assess the potential hazard presented by antibiotic resistant *Escherichia coli* (AREC), particularly those producing extended spectrum β lactamase (ESBL), in foods derived from animal production systems, and sold on the island of Ireland (IoI), and to define the potential risks posed by these organisms to the consumer. Sampling was at retail level with beef, chicken and pork selected for study. To maximize the number of AREC recovered samples were enriched in non-selective broth supplemented with an antimicrobial present at twice the ecological cut-off value (ECOFF) value defined on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) website [1].

Previous studies on the IoI seeking to isolate *Campylobacter* spp. from raw chicken had shown that ESBL *E. coli* could be readily isolated from enrichment broth (Bolton) which was supplemented with a third generation cephalosporin, cefoperazone [2].

II. MATERIALS AND METHODS

All samples were purchased by trained sampling officers and shipped to the laboratory with 24h of purchase. TinyTag Transit 2 temperature loggers were used to confirm all samples remained < 8°C <0°C during transport. A population based sampling plan was used to select stores at which samples would be purchased. Sample temperatures were measured *in situ* using an infra-red thermometer (Fluke Foodpro infra-red thermometer, Fluke UK Ltd, Norwich, UK).

From each sample three aliquots of 10g were excised and added to 100ml of tryptone soya broth (TSB) supplemented with:

1. Cefotaxime (CTX): 0.5mg/l
2. Ciprofloxacin (CP): 0.064 mg/l
3. Meropenem (MP): 0.25mg/l

The broths were incubated overnight at 37˚C, then streaked onto tryptone bile x-glucuronide medium (TBX) and again incubated overnight at 37˚C. Appropriate negative and positive controls were used. From each positive sample one typical *E. coli* colony was selected and streaked to purity on tryptone soy agar (TSA) and again incubated overnight at 37˚C. Purified cultures were checked for the ability to produce indole at 44˚C, and the ability to ferment lactose. Presumptive *E. coli* were harvested into 1ml of nutrient broth with 10% glycerol and stored at -80˚C.

Prior to determining the antimicrobial resistance profiles of the isolates the speciation was confirmed by MALDI-TOFF. Isolates were then characterized using standard disk diffusion technique, in terms of clinical anti-microbial resistance, using a panel of 13 compounds: ampicillin, chlorampenicol, ertapenem, streptomycin, sulphonamides, tetracycline, trimethoprim, nalidixic acid, kanamycin, ciprofloxacin, ceftazidime, gentamycin and cefotaxime. All ESBL producing *E. coli* (n = 143) were screened for the presence of $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, and $bla_{\text{OXA-1}}$ by PCR using specific primers and protocols as previously described [3,4].

III. RESULTS AND DISCUSSION

Overall 496 AREC were obtained from the three meats sampled in this study, with 77.6% being obtained from chicken and 15.9% from pork. Beef yielded only 6.5% of isolates, Table 1. ESBL production was confirmed in 143 (29%) of the *E. coli* isolates, with 130 being isolated from chicken, 1 from beef and 12 from pork. All of the ESBL producers were susceptible to cefoxitin, ertapenem and meropenem and resistant to ampicillin, cefpodoxime and cefotaxime.

Ciprofloxacin resistance was found in 110 (22%) isolates with 86 isolated from chicken, 4 from beef and 20 from pork. Thirteen (1 pork, 1 beef, 11 chicken) of these were also ESBL producers.

Table 1. Antimicrobial resistant *E. coli* (AREC) isolated from retail meats

	Number of AREC isolates			
Antibiotic	**Chicken**	**Pork**	**Beef**	**Total**
Cefotaxime	179	15	9	203
Ciprofloxacin	192	62	21	275
Meropenem	14	2	2	18
Total	**385**	**79**	**32**	**496**

Although 18 isolates were obtained from broths containing meropenem only 6 showed resistance to ertapenem (10µg) and none were resistant to meropenem (10µg), using the disk assay.

It is noteworthy that a significant proportions, 27%, of the *E. coli* isolates were identified as ESBL producers and/or resistant (21%) to ciprofloxacin. ESBL producing *Enterobacteriacae* are a major public health problem both in the hospital and community settings [5]. Infection with an ESBL producing organism results in increases in healthcare costs, length of hospital stay, morbidity and mortality. Data suggest that the incidence of invasive infection with ESBL producing *Enterobacteriaceae* is increasing year on year. The proportion of *E. coli* associated with invasive infection that were ESBL producers increased from 1.1% in 2004 to 10.6% in quarters 1-2 of 2014 [6] The availability of antimicrobial agents for the treatment of infection caused by ESBL producing *E .coli* is limited due to the fact that these isolates are frequently co-resistant to multiple antimicrobial classes. Most recent EARS-Net data reveals that the proportion of *E. coli* causing blood stream infection in Ireland that were multi-drug resistant (MDR) increased from 5.7% in 2004 to 15.1% in quarters 1-2 of 2014. Therefore the increasing proportion of *E. coli* associated with infection, including invasive infection, that are resistant to the flouroquinolones (e.g. ciprofloxacin) is a major concern. Again, an increasing proportion of *E. coli* associated with invasive infection are reported as fluoroquinolone resistant, increasing from 13.4% in 2004 to 26.8% in 2013. The successful dissemination of ESBL producing *E. coli* and ciprofloxacin resistant *E. coli* associated with human infection has been attributed in part to the widespread dissemination of particular clonal groups (e.g. *E. coli* O25b:

ST131) [7] and epidemic plasmids (e.g. IncFI) [8]. Therefore, at least a proportion of the isolates obtained will be subject to further study.

IV. CONCLUSION

Most samples of retail chicken, over 96%, yielded antimicrobial resistant *E. coli* whilst pork yielded less that 16%, and beef less than 6%. ESBL *E. coli* carriage was highest in chicken, confirming that this meat is the greatest cause for concern in terms of being a vehicle for anti-microbial resistant *Escherichia coli* to enter households.

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