Prevalence of extended-spectrum-\(\beta\)-lactamase-producing 
*Escherichia coli* from pigs at slaughter in the UK in 2013

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Objectives: To determine the prevalence and types of extended-spectrum \(\beta\)-lactamase (ESBL)-producing *Escherichia coli* occurring in pigs at slaughter in the UK in 2013.

Methods: Caecal samples from 637 pigs, sampled via a UK-wide monitoring programme in 2013, were enriched overnight in buffered peptone water, before plating to CHROMagar CTX and Oxoid Brilliance ESBL agar. Presumptive ESBL-producing *E. coli* from both media were tested for ESBL phenotype using MAST ES\(\beta\)L ID discs. Isolates with an ESBL phenotype were examined for the presence of \(\text{bla}_{\text{CTX-M}}, \text{bla}_{\text{OXA}}, \text{bla}_{\text{SHV}}\) and \(\text{bla}_{\text{TEM}}\) genes using a multiplex PCR. All \(\text{bla}_{\text{CTX-M}}\) and \(\text{bla}_{\text{SHV}}\) genes identified by PCR were sequenced.

Results: A total of 23.4% (95% CI 19.2–27.6) of pigs were positive for ESBL-producing *E. coli*; 22% (95% CI 17.8–26.1) of the pigs carried *E. coli* producing CTX-M enzymes [comprising enzyme types 1 (18.7% of pigs), 3 (0.2%), 14 (0.5%), 15 (0.5%), 27 (0.5%), 32 (0.5%) and 55 (0.3%)] and 2.2% (95% CI 0.8–3.6) of the pigs carried *E. coli* producing SHV-12. Five pigs carried both CTX-M- and SHV-12-producing *E. coli* as different isolates.

There were no statistically significant differences observed between the two medium types in terms of the proportions of each CTX-M enzyme type isolated.

Conclusions: In this UK study, 23.4% of pigs were found to be positive for ESBL-producing *E. coli* using selective culture media. The use of two different commercially available ESBL isolation media was found to improve the detection of ESBL-producing *E. coli*.

Keywords: cephalosporins, resistance, CTX-M, SHV, ESBLs

Introduction

In recent years healthy farm animals have been found to be reservoirs of extended-spectrum \(\beta\)-lactamase (ESBL)-producing Enterobacteriaceae, particularly *Escherichia coli*.1 Such isolates have been found in farm animals in the UK2–4 and in other countries.5–11 The most common type of ESBL gene in *E. coli* from farm animals is those encoding enzymes of the CTX-M group,1 which confer resistance to third- and fourth-generation cephalosporins.12 Cephalosporins are important for the treatment of infections in animals and humans,13 and bacteria that produce ESBL enzymes pose a possible public health risk via ingestion of contaminated meat products.14

Several recent studies have investigated the prevalence of ESBL-producing bacteria in pigs. In Switzerland, 15.2% of 59 pigs were positive for Enterobacteriaceae carrying group 1 or group 9 CTX-M genes.15 In a study of 80 pig farms in Spain,11 29 *E. coli* isolates from different farms recovered on selective media were cephalosporin resistant, of which 72% produced ESBLs including SHV-12 (41%), CTX-M-1 (10%), CTX-M-9 (10%) and CTX-M-14 (10%) with the remaining 28% having a non-ESBL phenotype. In a study in Korea, 21.5% of 558 faecal samples from healthy pigs showed the presence of CTX-M-\(\beta\)-lactamase-producing *E. coli*.9 In two pig production operations in China, the prevalence of ESBL-producing *E. coli* increased from 2.2% during August 2002 to 10.7% in February 2007.10

In a longitudinal study on three single-sited farrow-to-finish ESBL-positive pig farms in Denmark, the prevalence of CTX-M-positive *E. coli* in pigs varied from 12% during finishing to 58% just before weaning.16 Studies have also shown that cefotiofur and cefquinome can both select for CTX-M-producing strains in pigs,17 whilst there is evidence that a voluntary ban in 2010 on the use of cephalosporins in pigs in Denmark significantly reduced the occurrence of ESBLs in pigs at slaughter by 2011.18
The aim of this study was to determine the occurrence of ESBL-producing E. coli in pigs at slaughter in the UK and to compare the ability of two different commercially available ESBL media for isolation of ESBL-producing E. coli.

Materials and methods

Overall aims and study outline

This study was part of a larger monitoring programme that was established in order to estimate the prevalence of Salmonella enterica, Toxoplasma gondii, hepatitis E virus, Yersinia spp., porcine reproductive and respiratory syndrome virus and ESBL-producing E. coli in UK pigs at slaughter and to investigate antibiotic resistance in Campylobacter coli. The results for these other microorganisms will be published elsewhere. The study design was consistent, where possible, with the technical specifications for the previous EU monitoring scheme for Salmonella in slaughter pigs (Commission Decision 2006/668/EC).

Sampling

Staff trained in standard sampling procedures collected the samples from 637 pigs at 14 high-throughput abattoirs (which together process ~80% of slaughtered pigs in the UK) between January and May 2013. The pigs originated from 444 farms with between 1 and 10 pigs sampled per farm. For 324 of the farms, only a single pig was sampled, and for a further 79, 25 and 10 farms only two, three and four pigs were sampled, respectively. Samples were collected along the processing line from each carcase, including the caecum at the evisceration point. The number of carcases sampled at each abattoir was proportional to their annual throughput. The date of sampling and pig to be sampled on each occasion was randomly assigned. As the study was due to run from mid-January to mid-April, the number of pigs sampled per month was as follows: January, 102; February, 205; March, 198; April, 130; and May, 2. Differences to mid-April, the number of pigs sampled per month was as follows:

- January, 102; February, 205; March, 198; April, 130; and May, 2.
- Randomly assigned.

Isolation of ESBL-producing E. coli from caecal contents

One gram of caecal contents from each pig was added to 9 mL of buffered peptone water and incubated at 37°C for 24 h (±4 h). A loopful (10 μL) of this enrichment broth was plated to CHROMagar CTX (CHROMagar, France) and ESBL Brilliance agar (Oxoid, UK), and these plates were subsequently incubated at 37°C for 48 h (±4 h). Presumptive ESBL-producing E. coli were not investigated by month because of the limited number of months examined.

Characterization of presumptive ESBL-producing E. coli

Suspect ESBL-producing E. coli were confirmed as E. coli using the presence of ESBL, OXA and SHV genes using a multiplex PCR. These isolates were tested for the presence of blaCTX-M, blaOXA, blaSHV and blaTEM genes using a multiplex PCR. All isolates positive for blaCTX-M or SHV genes were subjected to sequencing of those genes to determine the CTX-M or SHV enzyme types as previously described.

Statistical methods

A two-sample test of proportions was performed to determine whether there was a statistically significant difference between CHROMagar CTX and Oxoid Brilliance ESBL agar in the proportion of isolates identified as presumptive ESBL positive, CTX-M positive, SHV positive and SHV or CTX-M positive. The same statistical test was used to investigate differences in the CTX-M enzyme types isolated from the two media.

Results

Caecal samples positive for ESBL-producing E. coli

A total of 370 E. coli with an ESBL phenotype were isolated from 149 pigs from both media and these isolates were tested for the presence of blaCTX-M, blaOXA, blaSHV and blaTEM genes using a multiplex PCR (Table 1). There were multiple isolates (up to seven) from most of the suspect ESBL-positive pigs. From the different pigs, 63.7% had one or two isolates tested, 16.1% had three isolates tested and the remainder had four to seven isolates tested. These multiple isolates per pig represented isolates from the different media, but also in some instances multiple isolates from one medium representing variances in colony type.

In all, 149/637 (23.4%, 95% CI 19.2–27.6) pig caecal samples were positive for E. coli strains with an ESBL phenotype when results from both media were considered (Table 1). Of the 637 pigs, 22.0% (95% CI 17.8–26.1) were positive for E. coli with blaCTX-M, blaOXA, blaSHV and blaTEM genes; five pigs were positive for both blaCTX-M and blaOXA genes but within different isolates of E. coli. Thus, all of the isolates with an ESBL phenotype from the pigs tested were determined to have either a blaCTX-M or a blaOXA ESBL gene.

A total of 16 E. coli isolates from eight pigs were also positive for blaOXA genes (results not shown). As all of these OXA-positive isolates were also CTX-M-positive, the OXA enzyme type was not determined. The CTX-M enzyme type for these OXA-positive E. coli isolates was CTX-M enzyme type 1 or 15.

Table 1. Proportion of 637 pigs in the UK in 2013 positive for presumptive ESBL-producing E. coli, CTX-M, SHV and/or SHV E. coli by type of isolation medium

<table>
<thead>
<tr>
<th>Type of isolation medium</th>
<th>Medium positive a</th>
<th>CTX-M positive</th>
<th>SHV-12 positive b</th>
<th>SHV-12 or CTX-M positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHROMagar CTX</td>
<td>127/637 (19.9%)</td>
<td>127/637 (19.9%)</td>
<td>0/637 (0%)</td>
<td>127/637 (19.9%)</td>
</tr>
<tr>
<td>Oxoid Brilliance ESBL agar</td>
<td>132/637 (20.7%)</td>
<td>120/637 (18.8%)</td>
<td>14/637 (2.2%)</td>
<td>132/637 (20.7%)</td>
</tr>
<tr>
<td>Both media combined</td>
<td>149/637 (23.4%)</td>
<td>140/637 (22.0%)</td>
<td>14/637 (2.2%)</td>
<td>149/637 (23.4%)</td>
</tr>
</tbody>
</table>

aNumber of samples from pigs showing growth of presumptive ESBL-producing E. coli on each medium/total examined.

bOf the 14 pigs that had SHV-12-positive E. coli, 5 also had CTX-M-positive E. coli.
A total of 55 pigs were also positive for *E. coli* with *bla*\(_{\text{TEM}}\) genes (results not shown), but all of these isolates were also positive for *bla*\(_{\text{CTX-M}}\) or *bla*\(_{\text{SHV}}\) genes. No *bla*\(_{\text{TEM}}\) genes were sequenced as all isolates with an ESBL phenotype carried *bla*\(_{\text{CTX-M}}\) or *bla*\(_{\text{SHV}}\) ESBL genes.

It was not possible to calculate the with-in-herd prevalence, since the pigs tested were from 444 different farms, and for almost three-quarters of these farms, only one pig per farm was tested.

**CTX-M enzyme types**

A total of 352/370 isolates from both media were positive by PCR for the *bla*\(_{\text{CTX-M}}\) gene. Of these isolates, a total of 154 (74 from Oxoid Brilliance ESBL agar and 80 from CHROMagar CTX) were sequenced to determine their CTX-M enzyme type (Table 2). These CTX-M-sequenced isolates represented at least one isolate from all of the 140 CTX-M-positive pigs. The CTX-M enzyme types in relation to all 637 pigs were types 1 (18.7% of pigs), 3 (0.2% of pigs), 14 (0.5% of pigs), 15 (1.4% of pigs), 27 (0.5% of pigs), 32 (0.5% of pigs) and 55 (0.3% of pigs), giving a total of 22% of pigs positive for CTX *E. coli*. One pig was positive for two different CTX-M enzyme types (1 and 27).

**Performance of the different agars**

There were seven more CTX-M-positive pigs identified from CHROMagar CTX than from Oxoid Brilliance ESBL agar, but the 14 SHV-positive pigs were only detected on Oxoid Brilliance ESBL agar (Table 1). The difference in isolation of CTX-M and SHV-producing *E. coli* between different media was only significant for the SHV isolates (*P* = 0.0002). The SHV-positive isolates were later found to grow on CHROMagar CTX, although at 48 h, colony size was small and could be missed at 24 h of incubation. Overall, by combining results from both media, more ESBL-positive pigs were detected than if just one of the media had been used. There were no statistically significant differences observed between the two media types in terms of the proportions of each CTX-M enzyme type isolated (Table 2).

**Discussion**

Previous studies focusing on ESBL-producing Enterobacteriaceae or *E. coli* in pigs have shown varying prevalence estimates and ESBL types in different countries.\(^9\)\(^15\)\(^16\) These may be true differences reflecting variation between countries, but may also, at least in part, be due to the use of different isolation methodologies and media. For example, when two commercial ESBL media were compared,\(^22\) CHROMagar CTX (as used in this study) inhibited more AmpC isolates than the other medium, which could lead to fewer positives, but better specificity. In another study, Oxoid Brilliance ESBL agar (as used in this study) was found to perform better than another commercial ESBL medium or MacConkey agar + a ceftazidime disc.\(^23\)

In this study, 23.4% (95% CI 19.2 –27.6) of 637 pigs gave rise to *E. coli* with an ESBL phenotype isolate on selective media, with 22.0% (95% CI 17.8 –26.1) of pigs confirmed to be CTX-M positive and 22.2% (95% CI 0.8 –3.6) of pigs confirmed to be SHV positive (some pigs were positive for both CTX and SHV isolates). This is a similar percentage of positive pigs to that observed in a study in Korea\(^9\) and a slightly higher percentage than that in a study in Switzerland.\(^15\) In the study in Switzerland, the isolation medium after enrichment was Oxoid Brilliance ESBL agar,\(^15\) which was one of the media used in this study. However, if one takes the results of this study from Oxoid Brilliance ESBL agar only, then the percentage of pigs positive for ESBL isolates was 20.7%, which is not that dissimilar to the 15.2% of pigs positive in the Swiss study.

Whilst in a Danish study\(^16\) it was possible to determine the percentages of pigs positive for ESBL-producing *E. coli* at different life stages (e.g. pre- and post-weaning), this was not possible in this study, since the animals were simply recorded as being <6, 6–12 or >12 months old. The Danish study involved sampling on farms, which makes recording the life stages of pigs possible, whilst this study involved sampling in abattoirs, and as such it was less straightforward to gather data on the life stage of all the pigs, which were generally of the same approximate age. In addition, within-herd prevalence could not be calculated because of the small numbers of animals per farm. The geographical distribution of the pigs according to source farm corresponded well with the distribution of the overall UK pig population. It was not possible to investigate geographical variation in the prevalence of ESBL-producing *E. coli* in detail due to the small number of pigs from several regions.

This was the first UK survey of ESBL-producing *E. coli* in pigs. The robust study design, with randomized sampling of a large number of pigs from a wide geographical area and a large number of farms at abattoirs that slaughter ~80% of all UK pigs, has provided a representative estimate of prevalence in UK pigs at slaughter.

In this study the most common CTX-M type detected in *E. coli* was CTX-M-1 and 18.7% of all pigs tested were positive for this CTX-M enzyme type. *E. coli* with group 1 CTX-M types have also been isolated from pigs in Switzerland\(^15\) and Denmark.\(^16\) Conversely, CTX-M-14 was the most common in the Korean study,\(^9\) whereas this was found in only 0.5% of pigs in this study.
study. Other CTX-M types isolated from pigs in Korea were CTX-M-3, CTX-M-15, CTX-M-27, CTX-M-55 and CTX-M-65.9 All of these CTX-M types were also seen in this survey with the exception of CTX-M-65, but with the addition of CTX-M-1 and CTX-M-32. Fourteen pigs in this study were positive for SHV-12, which has been reported as a major ESBL type in pigs from Spain.11

Other studies of ESBL-producing Escherichia coli in farm animals in the UK have included chickens from ~2008 and turkeys from ~2007.3 In these studies, 3.6% of individual broiler caecal samples, 5.2% of turkey meat production farms and 6.9% of turkey breeder farms were positive for ESBL-producing E. coli.

The use of two media for isolation of ESBLs did increase the number of ESBL-positive pigs identified. In particular, the SHV-12 strains were only isolated from the Oxoid Brilliance ESBL agar (although subsequent testing showed these did grow on CHROMagar CTX, but as small colonies at 48 h), whereas there were marginally more CTX-M-positive pigs from CHROMagar CTX.

The results reported here are from the first UK-wide study of ESBL-producing E. coli in UK pigs at slaughter. Overall, 22.0% of UK pigs were found to harbour CTX-M ESBL-producing E. coli (mainly enzyme type 1), which is similar to the percentage of pigs colonized with ESBL-producing E. coli in similar studies performed in some other countries.

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Transparency declarations
None to declare.

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