Development of a screening protocol for ESBLs in food

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Back to top

Background

Consumers expect that the meat they purchase will be safe to eat. Whilst adequate cooking will kill harmful bacteria that could be present on meat, if products are mishandled, such bacteria can potentially infect individuals who prepare the food or others indirectly via contaminated surfaces or other food products that become contaminated.

In recent years, there has been considerable concern about the bacteria *Escherichia coli (E. coli)* with a certain type of antibiotic resistance profile being present in farm animals such as cattle, chickens, pigs and turkeys. Such bacteria can sometimes subsequently be found in some meat, particularly chicken meat. This type of antibiotic resistance primarily under investigation in this study is caused by enzymes called extended-spectrum beta-lactamases (ESBLs), which confer resistance to a group of antibiotics known as the cephalosporins, which are important antibiotics used to treat human infections. Whilst many strains of *E. coli* bacteria live harmlessly in the intestines of most mammals, some strains can cause mild to serious diseases in humans (for example gastro-enteritis, urinary tract infections and systemic infections of the blood and internal organs). If the *E.coli* strains are resistant to antibiotics the infections become more difficult to treat effectively, and the bacteria can be considered more dangerous.

Whilst there have been many studies that have investigated the presence of ESBL-positive bacteria in meat, the methods used in these studies have varied, although there have been some common aspects. Also, to date, there has been a lack of data to validate the sensitivity of the methods used. For example, will such tests be able to detect low numbers of ESBL-producing bacteria from meats, when there might be lots of other bacteria present that could confuse the results? To this end, the purpose of this study was to develop and validate methods, including a loop-mediated isothermal amplification (LAMP) assay, to isolate ESBL-producing bacteria from meats using different agars and to detect ESBL-producing bacteria from meats using genetic methods.

Back to top

Research Approach

The three main objectives were:

- Evaluation of methods for the extraction, detection and confirmation of ESBLs from food.

- Comparison of procedures for bacterial speciation and genetic screening approaches.
- Selection, evaluation and trial of a method of screening food for ESBLs.

Back to top

Results

Laboratory studies were performed to determine that the different isolation and detection tests were both sensitive (able to isolate and detect low numbers of ESBL-producing bacteria on meats) and specific (correctly isolated and detected only ESBL-producing bacteria). These laboratory studies included a total of 10 different ESBL-producing strains added at different concentrations to chicken, beef, pork, lamb and turkey meats as well as limited work with ready meals. As a result of these laboratory studies an agar isolation method was developed capable of detecting as few as 10 ESBL-producing bacteria per gram in all meat types for all the ESBL-producing bacteria tested (lower levels were not tested). This agar method was shown to be an improvement on some previously used agar methods. The genetic methods performed in a similar manner to the agar tests, with good sensitivity and specificity.

Both agar methods and genetic methods have different advantages and disadvantages. For example, the genetic methods can be quicker and give some indication of the type of ESBL gene present, whilst the agar methods are able to isolate the bacteria of interest for further work if needed. When both agar and genetic tests are used together, they present a powerful combination.

Once the agar and genetic tests were developed and tested, a Standard Operation Procedure (SOP) was written and passed on to Industry, along with training in all the methodologies involved, so that they could perform a small scale trial of the methods.

The SOPs were passed to Leatherhead Food Research for independent evaluation and trialling. The test evaluation work comprised of three sections:

- In house validation – using confirmed ESBL-producing strains and non-target strains to produce data on sensitivity, specificity and repeatability
- Methodology evaluation – a total of 300 abattoir caecal and neck flap poultry samples were used to assess the performance of the screening methods and calculate relevant test statistics (measures of how well the test performs) in detecting ESBLs under field conditions
- A small ring trial of the SOP – 10 blind samples were analysed by Leatherhead Food Research and two independent operators to assess the transferability and robustness of the SOP and experimental procedure. Proof of principle of the method was also established for an additional test matrix, using 30 retail meat samples. All of this work was completed with satisfactory results.

Isolation and detection of ESBL–producing Enterobacteriaceae from meat and other foodstuffs is an important part of monitoring for food safety as some such organisms have potential to cause disease in humans. The LAMP assays developed in this study combined with use of chromogenic agars have the potential to provide robust, rapid detection, isolation and preliminary characterisation of ESBL-producing bacteria in meat**.**

To our knowledge, this is the first time that the use of LAMP assays combined with chromogenic agars has been evaluated for testing for ESBLs from food samples. A dual approach that combines both genetic and phenotypic methods has additional strengths over one method used alone.

Research report

England, Northern Ireland and Wales

PDF
Gweld Evaluation of a screening protocol for the detection of Extended Spectrum Beta Lactamases of Enterobacteriace in food as PDF(Open in a new window) (4.22 MB)