Detection of ESBL E. coli in chicken carcasses

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1 Aim and scope

*Escherichia coli* count in chicken carcasses by counting colonies in a solid medium following incubation under aerobiosis at 44°C in accordance with ISO 16649-2, followed by isolation of cephalosporin resistant colonies. This SOP was written at the request of the FASFC.

2 Definitions and abbreviations

**ESBL:** Extended Spectrum Beta Lactamase

B-lactamases are enzymes that inactivate β-lactam antibiotics by cleaving the annular structure within the antibiotic. β-lactamases contain various groups of enzymes each of which is able to inactivate a particular range of molecules. ESBL confer resistance to most of the β-lactam antibiotics but are not active against cephamycins and carbapenems. Most ESBLs are inhibited by clavulanic acid.

**AmpC** β-lactamases give resistance against all β-lactam antibiotics except carbapenems and are not inhibited by clavulanic acide, but by boric acid.

**Carbapenemases** give resistance against practically all available β-lactam antibiotics.

The wide variety of β-lactamases, each of which has its own activity, together with their presence in different combinations make it difficult to interpret results.

3 Principle of the method

The method is based upon ISO16649-2 (detection limit of 10 cfu/g) and on information of CLSI, 2009 (Performance Standards for antimicrobial susceptibility testing. 19th Informational supplement M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA). E. coli strains presenting resistance against 3rd generation cephalosporin may further be confirmed by means of the double disk method.

4 Safety

See SOP I/07/004/N : Biosafety guidelines.

5 Sampling

Samples are taken by official authorities or strains are sent as pure cultures on nutrient agar.

6 Receipt, distribution, storage and destruction of samples

SOP 03/F/12 : Sample management

7 Testing

7.1 Consumables

- TBX (BioRad ref 3555309)
- Cefotaxime CTX Ref: C7039, Sigma (1g) stock of 10 mg/ml in water
- Diluent: BPW
7.2 Devices and laboratory equipment
- Incubator at 44°C +/- 1°C

7.3 Environmental requirements
See SOP I/07/004/N : Biosafety guidelines

7.4 Preparing solutions
Dissolve 10 mg cefotaxime in 1 ml HPLC water in order to obtain a CTX stock solution of 10 mg/ml. Filter sterilize (0.45µm)

7.5 Preparing culture media
- TBX is bought ready-to-use.
- TBX+ CTX (1mg/l) : pour 100 µl of the CTX stock solution CTX (10mg/ml) into 100 ml TBX that was cooled down to 47°C +/- 2°C. Fill Petri dishes with approximately 15 ml of TBX + CTX medium.

7.6 Working method

7.6.1 Preparing samples
Transfer in a sterile way 10 g +/- 0.5 g of sample into a stomacher bag. Transfer the contents of one flask of dilution solution (BPW, 90ml +/- 5%) into the stomacher bag. Stomacher for 1 to 2 minutes. This is the mother solution.

7.6.2 E. coli count according to ISO 16649-2
- Mother solutions and dilutions :
Maka a 10-fold dilution series in BPW (18ml) using 2 ml of the previous solution. Mix thoroughly by vortexing before taking an amount of the dilution and/or preparing the next dilution.
- Plating :
Transfer 1 ml of the mother solution and the dilutions of samples into a sterile Petri dish using a sterile pipette with a capacity of no more than 5ml. Transfer approximately 15ml cooled TBX (47 °C +/- 2°C) to the dish. Carefully mix the inoculums with the culture medium. Leave to cool until the medium has solidified.
- Incubation
Incubate the Petri dishes upside down: 24 h +/- 2 h at 44 °C +/- 1 °C
- Reading
Count all blue-green colonies on the selected dilutions. Choose, if possible the plates containing 15 to 150 (no more) colonies. When there are less than 15 colonies, an estimated number shall be used.

7.6.3 Isolating cephalosporin resistant E. coli colonies.
At the same time as the E. coli count ((see previous section) shall be plated 1 ml of each dilution on TBX + 1 mg/l Cefotaxime (CTX).
Detection of ESBL *E. coli* in chicken carcasses

- Incubation
  Incubate the Petri dishes upside down: **24 h +/- 2 h at 44 °C +/- 1 °C**
- Reading
  When typical (blue-green) *E. coli* are present, the sample shall be considered positive for the presence of ESBL *E. coli*. For the confirmation step by means of phenotypic profiling of the \( \beta \)-lactamase type (BLSE-AmpC) may be used 2 colonies of these plates.

### 7.7 Decontaminating and cleaning laboratory equipment

See SOP 22/F/06 ‘Entretien, nettoyage et désinfection du matériel’

### 8 Result validation (depending on measuring programme)

- Blank: there is not even one colony to be counted
- Positive inspection for *E. coli* ATCC25922, presence of typical colonies on the TBX medium
- Positive inspection for resistant cephalosporin: *E. coli* NCTC 13351, presence of typical colonies on the TBX+ CTX medium

### 9 Calculations

Not applicable

### 10 Method validation

The method used is a validated method.

Validation tests were carried out at the Food-borne pathogens laboratory of the WIV/ISP.

### 11 Test reports

The reports including results shall be written using Starlims

### 12 Quality control

- **First line control:**
  First line control for ESBL detection of *E. coli* strains: see section 8.

- **Third line control**
  Control frequency shall be defined by the instances organizing ring tests, i.e. the EUR-L Antibiotic Resistance. Normally, an EQAS ring test is set up each year for the MIC determination of *E. coli* strains in which shall also be implemented an ESBL detection. The absence of third line control shall be compensated for by second line tests.
13 Standards and References


ISO 16649-2: Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli -- Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide

SOP 03/F/12 : Gestion des échantillons / Sample management
SOP 03/N/23 : Kalibratie van micropipetten - in uitbesteding / Calibrating micropipettes – outsourcing in progress
SOP 11/F/08 : Gestion des frigos, congélateurs, incubateurs et bains-marie/ Management of refrigerators, incubators and bains-marie
SOP 11/F/05/21 : Gestion des souches bactériennes / Management of bacterial strains
SOP 22/F/17 : Réception, inscription et distribution primaire des échantillons / Taking receipt, registering and primary distribution of samples
SOP I/07/004/F : Règles générales et recommandations pour les examens microbiologiques / General rules and recommendations for microbiological tests
SOP 22/F/06 : Entretien, nettoyage et désinfection du matériel / Maintenance, cleaning and disinfection of equipment
SOP 22/F/4009 : Traitement de l’échantillon / Sample handling
SOP 11/F/05/44 : Gestion du rapport avec LIMS11 / Report management in LIMS11
DOC 11/VM/05/xx: Validatiedossier ‘Bepaling van ESBL E. coli in kippenkarkassen’. En cours / Validation file “Determination of ESBL E.coli in chicken carcasses” – in progress
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