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*Original paper*

# Characterisation of ESBL producing *E. coli* strains isolated from chicken meat

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**Abstract** The dissemination of antibiotic-resistant bacteria through the food chain is a matter of concern. Food-producing animals are the primary reservoir of zoonotic pathogens and the rate of detection of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* has increased in last years. The aim of this study was to analyze the genotypic and phenotypic properties of ESBL-producing *E. coli* strains from chicken meat and establishing a relationship between isolates. ESBL-producing isolates mentioned in the present study were found to be resistant to multiple drugs, such as extended-spectrum cephalosporins, gentamicin, chloramphenicol, tetracycline, nalidixic acid, ciprofloxacin and sulfamethoxazole. Additionally, our results clearly showed that there are some similarities between genes and bacterial properties in isolates coming from the different populations.

**Keywords** *ESBL*, *Escherichia coli*, antimicrobial resistance

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# Introduction

Antimicrobial resistance in bacteria has become a major public health issue and the capability of multidrug resistance is a matter of concern. Antimicrobial agents are indispensable in the control of bacterial infections, not only in humans, but also in animals and plants. Some authorities regard food producing animals as the primary source of antibiotic resistance genes present in human food-borne pathogens, whereas others consider that the imprudent use of antibiotics in humans is the major source of the problem (FOUNOU & al., [16]). In case of zoonotic bacteria like *E. coli*, the use of antimicrobials in animals may determine drug-resistant bacterial populations, which represent a potential threat to the consumer. When a pathogen has already acquired an antibiotic resistance, it is difficult to control it clinically, even in the absence of gene transfer or development of drug resistance (ENGBERG & al., [5]; SORUM, [15]).

Extended-spectrum β-lactamase is an enzyme that allows bacteria to become resistant to a wide variety of penicillins and cephalosporins. Bacteria that contain this enzyme are known as ESBLs or ESBL-producing bacteria. ESBL-producing *Enterobacteriaceae* are resistant to strong antibiotics including extended spectrum cephalosporins. The occurrence of the ESBL-producing bacteria by *Enterobacteriaceae* (e.g. *E.coli*) makes the infections with these organisms harder to treat because they acquire resistance to penicillins and cephalosporins (first, second and third generation). Resistance to third-generation cephalosporins in *E. coli* increased significantly in the last years. Among ESBLs, the CTX-M-type enzymes are most common and their number has increased rapidly during the last years (CANTÓN & al., [3]; CARATTOLI, [4]).

The aim of this study was to analyze the genotypic and phenotypic properties of ESBL-producing *E. coli* isolates from chicken meat. Another objective was to establish a relationship between these strains. In order to assess the genomic resistance two of the most ESBL-producing genes, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM}$ were selected.

# Materials and Methods

### *1. Phenotypic detection of ESBLs*

The following antimicrobial agents were tested for bacterial resistance: trimethoprim, tigecycline, tetracyclines, sulfamethoxazol, nalidixic acid, meropenem, gentamicin, colistin, ciprofloxacin, chloramphenicol, ceftazidim, cefotaxime, azithromycin, ampicillin, imipenem, ertapenem, cefoxitin, cefepim, cefotaxime and clavulanic acid, ceftazidim and clavulanic acid, temocilin. The determination of Minimum Inhibitory Concentration (MIC) was done using the broth dilution method, as recommended by Clinical and Laboratory Standards Institute (CLSI) [18, 19]. "The Sensititre susceptibility plates for *Salmonella* and *Escherichia coli*" by Trek Diagnostic System [20] were used. The microorganisms were tested for their ability to produce visible growth in microtitre plate wells holding identical volumes of broth with a defined inoculum in the presence of an antimicrobial agent solution in incrementally two-fold increasing concentrations. The tests were performed under standardized conditions in order to obtain reproducible results.

Nine strains of presumptive ESBL-producing *E. coli* isolated from broiler skin samples were tested for antimicrobial susceptibility. Isolation of *E.coli* was performed in accordance to the European Reference Laboratory for Antimicrobial resistance recommendations (https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/397_esbl-ampc-cpeprotocol-version-meat-v6-16-02-18.pdf). *E. coli* confirmation was carried with biochemical tests.

For evaluating the phenotypic antibio-resistance, the colonies were taken and dissolved completely in 5 ml de-mineralized water, so to have a value of 0.5 on the McFarland scale, representing approximately 1-2 x 108 cfu/ml. From this suspension were transferred 50 µl to 11 ml of cation adjusted Muller-Hinton broth. The mix was homogenized and then inoculated. The inoculum was approximately 5 x 105 cfu/ml. Subsequently, all wells were covered with an adhesive seal and the samples were incubated at 370°C for 24 hours. After incubation, the results were read using the SENSITITRE manual. The MIC is recorded as the lowest concentration of antimicrobial agent that inhibits visible growth. The growth appears as turbidity or as a deposit of cells at the bottom of the well (SENSITITRE).

The epidemiological cut-off values are established by EUCAST (the European Committee on Antimicrobial Susceptibility Testing); these values and the range tested for each antimicrobial agent are presented in table 1.

### *2. Characterization of ESBLs-producing isolates*

**Bacterial DNA extraction**

The DNA was extracted from a single typical colony cultured on Columbia with 5% sheep blood agar using InstaGene Matrix (BIORAD, Marnes-la-Coquette, 140 France) according to the manufacturer's instructions.

**Table 1.** Range of antimicrobials tested for Escherichia coli and cut-off values applied for the classification of strains as resistant

| Antimicrobial agent | Abbreviation | Range tested (µg/ml) | Cut-off value (µg/ml) R > |
|---|---|---|---|
| Trimethoprim | TMP | 0.25-32 | 2 |
| Tigecycline | TGC | 0.25-8 | 1 |
| Tetracyclines | TET | 2-64 | 8 |
| Sulfamethoxazol | SMX | 8-1024 | 64 |
| Nalidixic acid | NAL | 4-128 | 16 |
| Meropenem | MERO | 0.03-16 | 0.12 |
| Gentamicin | GEN | 0.5-32 | 2 |
| Colistin | COL | 1-16 | 2 |
| Ciprofloxacin | CIP | 0.015-8 | 0.06 |
| Chloramphenicol | CHL | 8-128 | 16 |
| Ceftazidim | TAZ | 0.5-8 | 0.5 |
| Cefotaxime | FOT | 0.25-4 | 0.25 |
| Azithromycin | AZI | 2-64 | 16 |
| Ampicillin | AMP | 1-64 | 8 |
| Imipenem | IMI | 0.12-16 | 0.5 |
| Ertapenem | ETP | 0.015-2 | 0.06 |
| Cefoxitin | FOX | 0.5-64 | 8 |
| Cefepim | FEP | 0.06-32 | 0.12 |
| Cefotaxime + clavulanic acid | F/C | 0.06/4-64/4 | 0.25/4 |
| Ceftazidim + clavulanic acid | T/C | 0.12/4-128/4 | 0.5/4 |
| Temocilin | TRM | 0.5-128 | 32 |

**PCR sequence detection**

The detection of blaCTX-M-1 and blaTEM genes was performed by Polimerase Chain Reaction (PCR) according to Lalzampuia et al. [11] protocol with minor modifications. The primer's design are displayed in table 2. The amplification reactions were performed in a final volume of 25 µL containing 1 U Maxima Hot Start Taq DNA Polymerase (THERMO SCIENTIFIC, California, United States), 1 × PCR buffer, 0.2 mM dNTPs, 1,5 mM MgCl2, 0.2 µM of each primers and 2 µL DNA template. The amplification reaction was carried out in an BIORAD C1000 instrument (BIORAD, California, USA) and involved an initial denaturation phase at 95°C for 5 min, followed by 30 amplification cycles (95°C for 30 s, 57°C for 30 s and 72°C for 30 s) and a final elongation phase at 72°C for 7 min. The amplification reactions were performed in a final volume of 25 µL containing 1 U Maxima Hot Start Taq DNA Polymerase (THERMO SCIENTIFIC, California, United States), 1 × PCR buffer, 0.2 mM dNTPs, 1,5 mM MgCl2, 0.2 µM of each primers and 2 µL DNA template. The amplification reaction was carried out in an BIORAD C1000 instrument (BIORAD, California, USA) and involved an initial denaturation phase at 95°C for 5 min, followed by 30 amplification cycles (95°C for 30 s, 57°C for 30 s and 72°C for 30 s) and a final elongation phase at 72°C for 7 min.

**Table 2.** Details of the oligonucleotide primers used in the present study

| Genes | Primer sequences | Expected amplicon size (bp) | References |
|---|---|---|---|
| *Bla* *CTX-M-1* | 5'-CCCATGGTTAA AAAACACTGC-3' | 950 | HORTON & al., [7] |
| | 5'-CAGCGCTTTTG CCGTCTAAG-3' | | |
| *Bla* *TEM* | 5'-ATAAAATTCTT GAAGACGAAA-3' | 1080 | WEILL & al., [17] |
| | 5'-GACAGTTACCA ATGCTTAATC-3' | | |

### *3. Sequencing of $bla_{CTX-M-1}$ and $bla_{TEM}$ genes*

The PCR product of $bla_{CTX-M-1}$ and $bla_{TEM}$ genes was isolated with 1.5% agaroze gel electrophoresis and purified using the QIAquick Gel Extraction Q3 Kit (QIAGEN, Germany) following the manufacturer's protocol. The DNA sequencing was performed using the same primers shown in table 2 and the BigDye Terminator reaction mix v 3.1 (APPLIED BIOSYSTEMS INC., Foster City, CA, USA) with PCR conditions as recommended by the manufacturer. The sequencing reactions were purified with BigDyeXTerminator according to the APPLIED BIOSYSTEMS recommended protocol and then loaded on the 3500 Genetic Analyzer (APPLIED BIOSYSTEMS). The consensus sequences were obtained using BioEdit software Version 7.2 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html, January 2018). The $bla_{CTX-M-1}$ and $bla_{TEM}$ sequences were submitted to BLAST (ftp://ftp.ncbi.nlm.nih.gov/blast) for identification and genetic comparison. A phylogenetic tree was built using the MEGA 7 Molecular Evolutionary Genetics Analysis version 7.0 for large datasets (KUMAR, & al., [10]). Analysis version 7.0 for large datasets (KUMAR, & al., [10]).

## Results and discussions

### *1. Susceptibility testing results*

All strains isolated and confirmed as presumptive ESBL-producing have proved to be resistant to antimicrobials from the 3rd generation of cephalosporin, respectively to ceftazidim and cefotaxime. Moreover, these strains present antibio-resistance to substances from other antimicrobials class and because of their resistance to more than four substances, they were considered multidrug resistant. Table 3 presents the outcome of antimicrobial resistance screening. The vertical lines in bold mark the microbiological cut-off values. White fields mark the range of dilution tested for each antimicrobial agent.

### *2. Detection of genes responsible for ESBL resistance*

All isolates were tested with PCR for the presence of $bla_{TEM}$ and $bla_{CTX\text{-}M\text{-}1}$. This approach made it possible to detect the genetic background for many of the isolates (Table 4). The ESBL-producing profiles were established based on their resistance to cefotaxim, ceftazidim, cefepime, cefoxitin, combined with the synergy test with clavulanate. Out of nine presumptive ESBL-producing strains tested, in four were identified the genes $bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM}$ or both of them. These results confirmed the ESBL-producing profile of these strains. On the other hand, the absence of $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM}$ in others strains is not sufficient to rule out the ESBL profile of them, as long as there are several *bla* genes responsible for that.

Prevalence of $bla_{TEM}$ and $bla_{CTX\text{-}M\text{-}1}$ gene recorded in this study is in agreement with the studies conducted previous by ENSOR & al., [6]. CTX-M may be increased due to the wide use of third generation cephalosporins, especially ceftriaxone and cefotaxime or may be associated with high mobilization of the encoding genes (BARGUIGUA & al., [1]). BARLOW & al., [2] reported that the $bla_{CTX\text{-}M}$ genes have been mobilized to plasmid almost 10 times more frequently than other class A b-lactamases.

ESBL-producing isolates in the present study were found to be resistant to multiple drugs, such as extended-spectrum cephalosporins, TET, SMX, GEN, CHL, AMP, NAL, CIP. PFEIFER & al., [12] concluded that the therapy

**Table 3.** Antimicrobial resistance in Escherichia coli isolates (n = 9) from chicken meat
Distribution (%) of MIC values (µg/ml)

| Substance abreviation | Resistance (%) | Distribution (%) of MIC values (µg/ml) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.01 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 |
| TMP | 33.3 | | | | | 4 | 2 | | | | | | | 3 | | | | |
| TGC | 0 | | | | | 2 | 7 | | | | | | | | | | | |
| TET | 66.7 | | | | | | | | 3 | | | | | 6 | | | | |
| SMX | 55.5 | | | | | | | | | | 3 | 1 | | | | | | 5 |
| NAL | 77.8 | | | | | | | | | 1 | | | | 1 | 1 | 6 | | |
| MERO | 0 | | 9 | | | | | | | | | | | | | | | |
| GEN | 22.2 | | | | | | 3 | 3 | 1 | | | | | 2 | | | | |
| COL | 0 | | | | | | | 9 | | | | | | | | | | |
| CIP | 88.9 | | 1 | | | 2 | 1 | | | 1 | 1 | 3 | | | | | | |
| CHL | 44.4 | | | | | | | | | | 4 | | 3 | | 1 | 1 | | |
| TAZ | 100 | | | | | | | 2 | | 1 | 2 | 4 | | | | | | |
| FOT | 100 | | | | | | | | | 1 | 8 | | | | | | | |
| AZI | 11.1 | | | | | | | | | 4 | 3 | 1 | | 1 | | | | |
| AMP | 100 | | | | | | | | | | | | | | 9 | | | |
| IMI | 0 | | | | | 8 | 1 | | | | | | | | | | | |
| ETP | 11.1 | 1 | 4 | 3 | 1 | | | | | | | | | | | | | |
| FOX | 77.8 | | | | | | | | | 1 | 1 | 1 | | 3 | 3 | | | |
| FEP | 100 | | | | | 6 | | | | | 3 | | | | | | | |
| F/C | 55.5 | | | 2 | 1 | | | | | 1 | 4 | 1 | | | | | | |
| T/C | 66.7 | | | | 1 | 1 | 1 | | | 3 | | 3 | | | | | | |
| TRM | 0 | | | | | | | | | 1 | 3 | 3 | 2 | | | | | |

with an individual antibiotic could lead to a selective pressure high enough to establish a resistant gene pool in the bacterial population at farm-level. On the other hand, the use of non-b-lactam antimicrobials can also make some selection pressure for ESBL resistance genes because the resistance determinants against cephalosporins, aminoglycosides, tetracycline, and sulfonamides are often located on the same plasmid (SCHMID & al., [14]; JACOBY & SUTTON, [8]).

### 3. *Sequencing of bla$_{CTX-M-1}$ AND bla$_{TEM}$ genes*

BLAST alignment revealed a high similarity between the *bla* genes found in *E. coli* strains tested and *bla* genes variants from *Klebsiella pneumoniae, Salmonella enterica, E. coli* and *Shigella dysenteriae* strains (GenBank Accession Numbers MF953243.1 and CP030132.1 for *K. pneumoniae*, CP030839.1 for *Salmonella*, LS999562.1 and CP027135.1 for *E. coli* and CP024467.1 for *S. dysentariae* respectively). These results are presented in the figures 1 and 2.

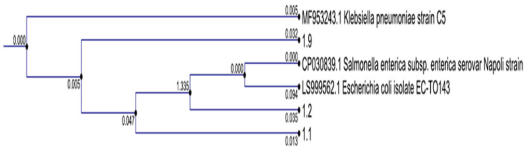


**Figure 1.** Phylogenetic tree of ESBL *E. coli* strains carrying *bla$_{CTX-M-1}$* gene

The evolutionary history was inferred using the Neighbor-Joining method (SAITOU & NEI [13]). The evolutionary distances were computed using the Kimura 2-parameter method (KIMURA [9]) and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA7 (KUMAR, & al., [10]).

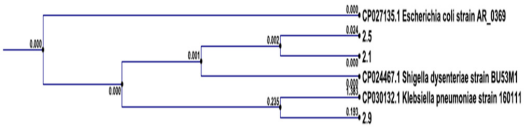


**Figure 2.** Phylogenetic tree of ESBL *E. coli* strains carrying *bla$_{TEM}$* gene

The results obtained confirmed that the *bla* genes are highly conserved and are involved in the antimicrobial resistance of different strains. The phylogenetic grouping was used to obtain some preliminary information on the genetic background of these bacteria and the pathogenicity of the *E. coli* strains. Additional resistance to substances from other antimicrobial class is explained due to the localization of resistance genes within multi-drug resistance regions on conjugative plasmids, which might be transferred together with the ESBL genes between bacterial isolates.

Abusive usage or cephalosporin overdosing during the poultry production may account for the relatively high ESBL prevalence. Long-term surveillance and prudent use of antimicrobials in food producing animals ensures the reduction or prevention of ESBL- producing *E. coli* dissemination of, as well as its transmission to humans through the retail food supply chain.

ESBL producing *E. coli* strains have been reported worldwide and in Romania, but these were focused on clinical strains (PORUMBEL & al. [21]). However, the incidence and characterization of foodborne ESBL-producing *E. coli* strains have been rarely reported in Romania. This

**Table 4.** Correlation between the phenotype antimicrobial resistance and bla genes

| Strain number | ESBL-group resistance | | | | | Additional resistance | Category | bla genes |
|---|---|---|---|---|---|---|---|---|
| | AMP | TAZ | FOT | FOX | FEP | | | |
| 1 | >64 | 4 | >4 | 4 | 8 | TMP, TET, SMX, NAL, CIP, CHL | ESBL phenotype | *bla$_{CTX-M-1}$, bla$_{TEM}$* |
| 2 | >64 | 1 | >4 | 8 | 8 | NAL, CIP | ESBL + pAMPc phenotype | *blaCTX-M-1* |
| 3 | >64 | >8 | >4 | >64 | 0.25 | NAL, CIP | ESBL + pAMPc phenotype | - |
| 4 | >64 | 8 | >4 | 64 | 0.25 | TET, NAL, CIP | ESBL + pAMPc phenotype | - |
| 5 | >64 | 4 | 4 | 64 | 0.25 | TET, SMX, NAL, GEN, CIP, CHL | ESBL + pAMPc phenotype | *bla$_{TEM}$* |
| 6 | >64 | 16 | >4 | >64 | 0.25 | - | ESBL + pAMPc phenotype | - |
| 7 | >64 | >8 | >4 | >64 | 0.25 | TMP, TET, SMX, NAL, CIP, CHL | ESBL + pAMPc phenotype | - |
| 8 | >64 | >8 | >4 | 64 | 0.25 | TET, SMX, NAL, GEN, CIP | ESBL + pAMPc phenotype | - |
| 9 | >64 | 4 | >4 | 16 | 8 | TMP, TET, SMX, NAL, CIP | ESBL + pAMPc phenotype | *bla$_{CTX-M-1}$, bla$_{TEM}$* |

work represent only a step in the improvement of our knowledge on the genetic determinants of antimicrobial resistance in isolates strains from chicken meat.

## Conclusions

The ESBL-producing isolates in the present study were found to be resistant to multiple drugs, such as extended-spectrum cephalosporins, gentamicin, chloramphenicol, tetracycline, nalidixic acid, ciprofloxacin and sulfamethoxazole. This aspect is very worrying given that the combining antibiotic therapy could fail as long as the multidrug resistance is more and more spreading.

Additionally, the results clearly showed that there are some similarities between genes and bacterial properties in isolates from the different populations.

ESBL producers are expected to increase in the future, in both live animals and food, and a more prudent use of antimicrobials in general may be necessary, together with the implementation of international measures to control zoonotic pathogens and limit the global emergence of these resistance characteristics.

## References


1. A. BARGUIGUA, E. F. OTMANI, M. TALMI, F. BOURJILAT, F. HAOUZANE, K. ZEROUALI, M. TIMINOUNI, Characterization of ESBL producing Escherichia coli and Klebsiella pneumoniae isolates from community in Morocco. *J Med Microbiol* 60, 1344-1352 (2011).
2. M. BARLOW, R.A. REIK, S.D. JACOBS, M. MEDINA, M.P. MEYER, High rate of mobilization for blaCTX-M. *Emerg Infect Dis* 14, 423-28 (2008).
3. R. CANTÓN, J.M. GONZÁLEZ-ALBA, J.C. GALÁN, CTX-M enzymes: origin and diffusion. *Front Microbiol* 3, 110 (2012).
4. A. CARATTOLI, Plasmids and the spread of resistance. *Int J Med Microbiol* 303, 298-304 (2013).
5. J. ENGBERG, F.M. AARESTRUP, D.E. TAYLOR, P. GERNER-SMIDT, I. NACHAMKIN – Quinolone and macrolide resistance in Campylobacter jejuni and C. coli: resistance mechanisms and trends in human isolates. *Emerg Infect Dis*, 7, 24-34, (2001).
6. V.M. ENSOR, M. SHAHID, J.T. EVANS, P.M. HAWKEY, Occurrence, prevalence and genetic environment of CTX-M beta lactamases in Enterobacteriaceae from Indian hospitals. *J Antimicrob Chemother* 58, 1260-1263 (2006).
7. R.A. HORTON, L.P. RANDALL, E.L. SNARY, H. COCKREM, S. LOTZ, H. WEARING, D. DUNCAN, A. RABIE, I. MCLAREN, E. WATSON, R.M. LA RAGIONE, N.G. COLDHAM Fecal carriage and shedding density of CTX-M extended-spectrum-b-lactamase-producing Escherichia coli in cattle, chickens, and pigs: implications for environmental. *Appl. Environ Microbiol*, 77, 3715-3719 (2011).
8. G.A. JACOBY and L. SUTTON, Properties of plasmids responsible for production of extended-spectrum b-lactamases. *Antimicrob Agents Chemother*, 35, 164-169 (1991).
9. M. KIMURA, A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol,* 16, 111-20, (1980).
10. S. KUMAR, G. STECHER, K.TAMURA, MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*, 33, 1870-4 (2016).
11. H. LALZAMPUIA, T.K. DUTTA, I. WARJRI, R. CHANDRA, PCR-Based Detection of Extended-Spectrum b-Lactamases (blaCTX-M-1 and blaTEM) in Escherichia coli, Salmonella spp. and Klebsiella pneumoniae isolated from Pigs in North Eastern India (Mizoram), *Indian J Microbiol*., 53(3), 291-296 (2013).
12. Y. PFEIFER, A. CULLIK, W.WITTE, Resistance to cephalosporins and carbapenems in gram-negative bacterial pathogens. *Int J Med Microbiol*, 300, 371-379 (2010).
13. N. SAITOU, M. NEI, The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, 4, 406-25 (1987).
14. A. SCHMID, S. HORMANSDORFER, U. MESSELHAUSSER, A. KASBOHRER, C. SAUTER-LOUIS, R. MANSFELD, Prevalence of extended-spectrum b-lactamase–producing Escherichia coli on Bavarian dairy and beef cattle farms. *Appl Environ Microbiol*., 79, 3027-3032 (2013).
15. H. SORUM, Antibiotic resistance in food-related bacteria – a result of interfering with the global web of bacterial genetics. *Int J Food Microbiol*, 78, 43-56, (2002).
16. L.L. FOUNOU, R.C. FOUNOU, S.Y. ESSACK. Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. *Front Microbiol*. 2016; 7:1881. Published 2016 Nov 23. doi:10.3389/fmicb.2016.01881
17. F.X. WEILL, M. DEMARTIN, L. LAETITIA FABRE, A.D. GRIMONT PATRICK, Extended-spectrum-b-lactamase (TEM-52)-producing strains of *Salmonella enterica* of various serotypes isolated in France. *J Clin Microbiol* 42, 3359-3362 (2004).
18. *** Clinical and Laboratory Standards Institute. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline. M45-A. Clinical and Laboratory Standards Institute, Wayne, PA, (2006).
19. *** Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 16th informational supplement. M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA, (2006).
20. *** Sensititre susceptibility plates for *Salmonella* and *E.coli* (2009).
21. I. PORUMBEL, M. CHELARIU, I. GHEORGHE, C. CURUTIU, M. POPA, C. DELCARU, M. GROSU, R. GRIGORE, S. BERTESTEANU, V. LAZAR, T. VASSU DIMOV. *Rom Biotechnol Lett,* 23(3): 13572-13580 (2018)