

Presence of *Escherichia coli* strains producing extended spectrum β - lactamases (ESBL) in raw meat.



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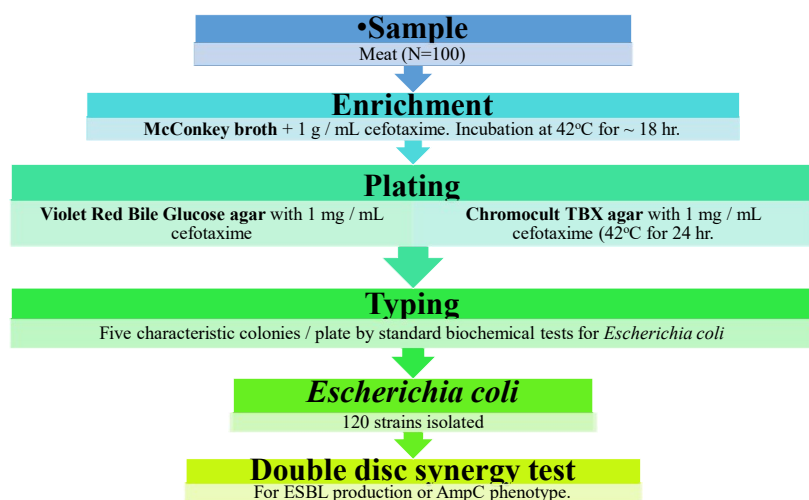
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Introduction

- Antimicrobial resistance is currently a serious concern for scientists and policy makers.
- Of interest is the observed resistance of Enterobacteriaceae to cephalosporins and monobactams
- The broad-spectrum β -lactamases (ESBLs) are hydrolytic enzymes produced among others by Enterobacteriaceae, that confer resistance to penicillins, cephalosporins; and aztreonam.
- ESBL-producing organisms are of clinical significance since they are associated with severe infections (bacteraemias, intra-abdominal infection, urinary tract infections, and respiratory tract infections).
- ESBL *Escherichia coli* has been isolated from healthy productive animals or from food of animal origin.

Aim of this study

- To examine the presence of ESBL producing *Escherichia coli* strains in fresh meat and the ESBL producing genes present.



Double disc synergy test antibiotics	
Cefoxitin	30 mg
Cefepime	
Ceftazidime / clavulanic acid	30/10 mg
Cefotaxime	30 mg
Cefotaxime / clavulanic acid	30/10 mg

	Amplicon Primer sequence (5' to 3')	Size (bp)
<i>bla</i> _{SHV}	CTT TAT CGG CCC TCA CTCAA AGG TGC TCA TCA TGG GAA AG	237
<i>bla</i> _{TEM}	CGC CGC ATA CAC TAT TCT CAG AAT GA ACG CTC ACC GGC TCC AGA TTT AT	445
<i>bla</i> _{CTX-M}	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593
<i>bla</i> _{OXA}	ACA CAA TAC ATA TCA ACT TCG C	813

PCR verification	
Method for <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M} , <i>bla</i> _{SHV} and <i>bla</i> _{OXA} genes (Fang et al (2008))	94 isolates

PCR conditions			
	Stage	T (°C)	Time (secs)
30 cycles	Initial denaturation	85	900
	Denaturation	94	30
	Annealing	62	90
	Extension	72	60
	Final extension	72	600

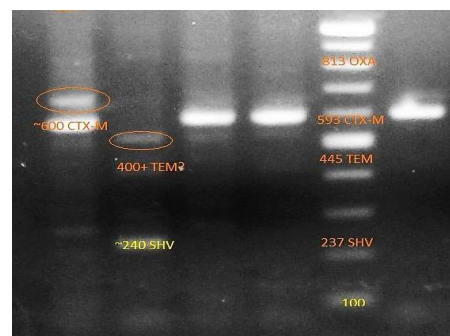


Table 1. Phenotypic characterization according to the double disc synergy test

	Isolates (n=86)
True ESBL	86 (71.67%)
AmpC	22 (18.33%)
Unknown etiology	10 (8.33%)

Table 2. Genetic profile of the selected isolates examined

	Isolates (n=86)
<i>bla</i> _{CTX-M}	30 (31.91%)
<i>bla</i> _{TEM}	8 (8.51%)
<i>bla</i> _{TEM} & <i>bla</i> _{CTX-M}	22 (23.4%)
<i>bla</i> _{SHV}	0
<i>bla</i> _{OXA}	0

Comparison of results with selected publications

Author(s)	Location	Findings
Egervärn et al. (2014)	Imports to Sweden from EU (including Greece)	61% of the meat samples tested positive for ESBL or AmpC strains
Tham et al. (2012)	Mediterranean countries	• ESBL strains were not isolated in any of the food samples • No selective enrichment used
Doi et al. (2010)	USA	78.12% of the meat samples tested positive for ESBL or AmpC
Ahmed et al. (2009)	Japan	• <i>bla</i> _{TEM-1} : 17.3% • <i>bla</i> _{CMY-2} : 23.2%

References
 Agerso Y., Aarestrup F.M., Pedersen K., et al. (2012). Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. *J Antimicrob Chemother* 67(3): 582-588.
 Ahmed A.M., Shimabukuro H., Shimamoto T. (2009). Isolation and molecular characterization of multidrug-resistant strains of *Escherichia coli* and *Salmonella* from retail chicken meat in Japan. *J Food Sci* 74: M405-M410.
 Doi Y., Paterson D.L., Egea P., et al. (2010). Extended-spectrum and CMY-type beta-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. *Clin Microbiol Infect* 16: 33-38.
 Egervärn M., Björksson S., Byfors S., et al. (2014). *Escherichia coli* with extended-spectrum beta-lactamases or transferable AmpC beta-lactamases and *Salmonella* on meat imported into Sweden. *Int J Food Microbiol* 171: 8-14.
 Fang H., Ataker F., Hedin G., Dornbusch K. (2007). Molecular epidemiology of extended-spectrum beta-lactamases among *Escherichia coli* isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *J Clin Microbiol* 46(2): 707-12.
 Jensen L.B., Hasman H., Agerso Y., Emborg H.D., Aarestrup F.M. (2006). First description of an oximino-cephalosporin-resistant, ESBL-carrying *Escherichia coli* isolated from meat sold in Denmark. *J Antimicrob Chemother* 57: 793-794.
 Tham J., Walder M., Melander E., Odenholt I. (2012). Prevalence of extended-spectrum beta-lactamase-producing bacteria in food. *Infect Drug Resist* 5: 143-147.