ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 1, 75-77
www.aaem.pl

Antibiotic resistance of *Escherichia coli* isolated from a poultry slaughterhouse

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Gregova G, Kmetova M, Kmet V, Venglovsky J, Feher A. Antibiotic resistance of *Escherichia coli* isolated from poultry a slaughterhouse. Ann Agric Environ Med. 2012; 19(1): 75-77.

Abstract

The aim of the study was to investigate the antibiotic resistant *E. coli* strains isolated from bioaerosols and surface swabs in a slaughterhouse as a possible source of poultry meat contamination. The highest air coliforms contamination was during shackling, killing and evisceration of poultry. The strains showed resistance to ampicillin (89%), ceftiofur (62%) and cefquinome (22%), while resistance to ampicillin with sulbactam was only 6%. Resistance to streptomycin and gentamicin was detected in 43% *vs.* 14% isolates; to tetracycline 33%; to chloramphenicol and florfenicol in 10% vs. 18% isolates; to cotrimoxazol in 35% isolates; to enrofloxacin in 43 % isolates. The higher MIC of ceftazidime (3.6 mg.l^{-1}) and ceftriaxon (5.2 mg.l^{-1}) revealed the presence of ESBLs in 43% of isolates. From 19 selected phenotypically ESBL positive strains, 16 consisted of CMY-2 genes, while CTX-M genes were not detected by PCR. Maldi tof analysis of selected *E. coli* showed a clear clonal relatedness of environmental strains from various withdrawals.

Keywords

Escherichia coli, antibiotic resistance, ESBL, Maldi tof, poultry slaughterhouse

INTRODUCTION

Microorganisms found in slaughtered poultry, originate from 2 main sources: the environment of the slaughterhouse (live poultry, equipment, staff) and the digestive tract of the birds [1]. A slaughterhouse is usually divided into a clean and a dirty zone, which minimizes contamination of the final products, ensures continuous technical processes, and material flow. The dirty zone includes the area for shackling poultry by the feet and the section for poultry carcass after electrical immobilization. The dead birds are then scaled with water in a closed tunnel. The clean zone consists of the evisceration section, water-chilling, cutting, de-boning and packaging sections. The aim of the present work was to study the coliforms in bioaerosols and antibiotic resistance of *Escherichia coli* from the dirty and clean zones.

MATERIAL AND METHODS

Three withdrawals were made at different times from one Slovakian poultry slaughterhouse. Five samples of bioaerosol and 5 swabs (only for qualitative estimation) were taken from every section of the processing plant; the specimens were specified by various time periods (every 2 weeks) in the winter season as P, B and S. The poultry in each withdrawal came from various farms. A prefix of P, B and S were used for *E. coli* designation, and also used in the Maldi dendrogram.

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Received: 18 July 2011; accepted:04 January 2012

Bioaerosols were collected by means of a sampler MAS-100 Eco. The MAS-100 Eco air monitoring system is a compact sampler intended for use with standard Petri dishes. Petri dishes with Endo agar (ImunaPharm, Slovakia) were placed on top of the dish support of the sampler, and after aspiration of the preset volume of air, they were incubated at 37°C. The plate counts were recalculated per 1 m^3 of air. Surface swabs were resuscitated in buffered peptone water (Oxoid, Basingstoke, UK), and then were subcultured on MacConkey agar (Oxoid).

The bacterial diagnostics and possible clonal relatedness of the studied isolates was determined by Maldi-tof analysis. A sufficient number of stable mass signals of major housekeeping proteins, mainly ribosomal proteins, can be used for bacterial species identification, and for estimation of the similarities between protein spectra of the same species (bacterial fingerprint) [2]. Distance level on the horizontal axis of MSP dendrogram indicates similarity of *E. coli* strains. Species with distance levels under 500 have been described as reliably classified [3]. Bacterial extracts for mass spectrometry measurements were prepared as recommended by the manufacturer of the MS instrument. For Maldi-tof analysis, one colony was spotted onto a ground steel target (Bruker Daltonik GmbH, Leipzig, Germany) and air dried for 15 min. Each sample spot was overlaid with 2 µl of matrix solution (saturated solution of α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile with 2.5% trifluoroacetic acid), and again air dried for 15 min. To identify microorganisms, the raw spectra obtained for each isolate were imported into BioTyper software, version 2.0 (Bruker Daltonik GmbH, Leipzig, Germany), and analysed without any user intervention.

For determination of minimal inhibitory concentration (MIC) according to CLSI guidelines M31-A3 [4], 48 isolates

of *Escherichia coli* were selected. We used ampicillin (AMP), ampicillin and sulbactam (A+IB), ceftazidime (CAZ), ceftazidime with clavulanic acid (CAC), ceftriaxon (CTR), ceftiofur (CFF), cefquinome (CFQ), gentamicin (GEN), streptomycin (STM), neomycin (NEO), nalidixic acid (NAL), enrofloxacin (ENR), chloramphenicol (CMP), florfenicol (FLO), tetracycline (TET) and cotrimoxazol (COT) [5]. Phenotype interpretation of mechanisms of β-lactamases (ESBLs) were readed according the β-lactams (AMP, A+IB, CTR, CAZ, CAC) MIC levels [6]. ESBL genes for CTX-M [7] and CMY-2 [8] were determined by PCR.

RESULTS AND DISCUSSION

The highest air coliforms contamination occurred during shackling, killing and evisceration of the poultry. *E. coli* were resistant only to ampicillin, tetracyclin or enrofloxacin, without ESBLs (extended spectrum betalactamases) production. However, we found *E. coli* with ESBLs and associated with resistance to quinolones (nalidixic acid, ciprofloxacin and enrofloxacin), streptomycin, tetracycline and cotrimoxazol in swabs from many surfaces in all parts of poultry slaughterhouse (Tab. 1).

Table 1. Concentration of airborne coliforms and *E. coli* resistance in surface swabs in a poultry slaughterhouse

Site of sampling	Airborne coliforms (cfu/m³)	*Escherichia coli* resistance
P. withdrawal		
Portioning room	$0.75 \cdot 10^3$	ESBL, TET, STM, CMP, FLO, NAL, ENR, COT
Packaging room	$0.9 \cdot 10^2$	not isolated
Eviscerating room	$2.6 \cdot 10^4$	ESBL, TET, STM, NAL, ENR, CIP, COT
Killing room	$2.5 \cdot 10^4$	ESBL, STM, NAL, COT
Shackling room	$2.07 \cdot 10^4$	ESBL, STM, NAL, ENR, COT
B. withdrawal		
Portioning room	$1.0 \cdot 10^2$	not isolated
Packaging room	$2 \cdot 10^2$	ESBL, STM, GEN, NAL, CIP.ENR, CMP, COT
Eviscerating room	$0.4 \cdot 10^3$	ESBL, STM, NAL, CIP,ENR,FLO, TET, COT
Killing room	$1.8 \cdot 10^4$	ESBL, STM, NAL, CIP,ENR,FLO, TET, COT
Shackling room	$5.9 \cdot 10^4$	ESBL, NAL, CIP, ENR
S. withdrawal		
Portioning room	$0.5 \cdot 10^2$	only ESBLs
Packaging room	$0.5 \cdot 10^2$	not isolated
Eviscerating room	$0.4 \cdot 10^3$	only ESBLs
Killing room	$1.0 \cdot 10^2$	only ESBLs
Shackling room	$1.0 \cdot 10^2$	only ESBLs

During scalding, internal contamination of the carcasses of broilers can occur through the feather follicles [9]. The water-chilling system has been criticized, based on the fact that bacteria can be transferred from chicken to chicken because of contact through water [10]. Poultry may also be contaminated by intestinal bacteria during processing. These microorganisms are easily spread from one carcass to another during defeathering and evisceration [11]. Adequate equipment for cleaning and disinfecting hands and tools must be supplied in workrooms; such equipment must be as close as possible to the workstations, and taps must not be hand-operated; these facilities must have hot and cold running water, cleaning and disinfecting products, and disposable hand towels to cleanse instruments. The temperature of the cutting room and packaging room must be controlled at around 12-15°C [10].

The percentages of betalactams resistance in the 48 *Escherichia coli* isolates recovered from the poultry slaughterhouse showed 89% resistance to ampicillin, 62% resistance to ceftiofur and 22% resistance to cefquinome, while resistance to ampicillin with sulbactam was only 6%. Resistance to streptomycin and gentamicin was detected in 43% *vs.* 14% isolates; to tetracycline 33%; to chloramphenicol and florfenicol in 10% *vs.* 18% isolates; to cotrimoxazol in 35% isolates; to enrofloxacin in 43 % isolates. The higher MIC of ceftazidime (3.6 $mg.l^{-1}$) and ceftriaxon (5.2 $mg.l^{-1}$) revealed the presence of ESBLs in 43% of isolates. From 19 selected phenotypically ESBL positive strains, sixteen consisted of CMY-2 genes, while CTX-M genes were not detected by PCR.

High level resistance to tetracycline (46%) was observed in *E. faecalis* isolated from rectal swabs in a Slovakian poultry setting [12], which is in concurrence with with our *E.coli* observation. The results showed that the source of ESBLs for meat *E. coli* could be environmental microbes from the poultry slaughterhouse. Also in Slovakia, enrofloxacin and ciprofloxacin resistance associated with APEC virulence factors in *E. coli* was detected in poultry faecal strains [13, 14]. Dendrogram resulting from cluster analysis of Maldi tof mass spectra of selected 13 *Escherichia coli* isolates at B, P and S measurements revealed 3 groups of relatedness. The first had only one strain – B621, and 2 others consisted of 7 or 5 *E.coli* strains of all measurements (Fig. 1). Results showed that in the poultry slaughterhouse there was a circulation of similar *Escherichia coli* strains within a longer period.

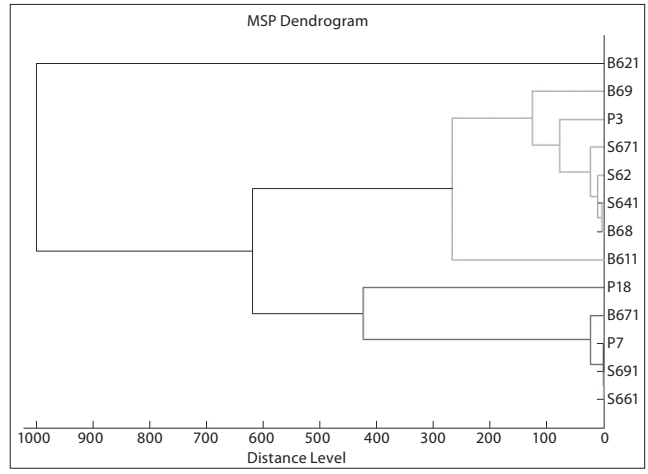

Figure 1. Maldi tof dendrogram of selected environmental (prefix P, B, S from various time periods) *Escherichia coli*

It is widely argued that as meat products are cooked, there is little likelihood that antibiotic-resistant bacteria present in the raw material will colonize in the human gut. This view is challenged by this work, which clearly demonstrates the colonization of humans by antibiotic-resistant *E. coli* in the course of preparing and eating cooked chicken in the home [15].

Acknowledgment

This work was supported VEGA Grants 1/0495/10, 2/0005/11 and APVV-0009-10 from the Slovak Research and Development Agency.

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