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First isolation of ESBL-producing *Salmonella* and emergence of multiresistant *Salmonella* Kentucky in turkey in Poland

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ABSTRACT

The first CTX-M-producing *Salmonella* was described in primary animal production in Poland, due to the antimicrobial resistance monitoring and control program introduced in turkeys. It was associated with the outbreak of multiresistant *Salmonella* Kentucky in non-diseased turkeys, foods and food production environment, but found also in municipal sewage sludge. The emergence along the food chain of clonally related strains resistant to critically important antimicrobial agents, including cephalosporins, quinolones, sulfonamides, aminoglycosides, phenicols, and tetracycline, which are used against foodborne pathogens, poses a serious public health threat.

1. Introduction

Antibiotic resistant bacteria are one of the crucial public health threats. Recently, *Salmonella* resistance to the clinically important classes of quinolones and cephalosporins have been reported in poultry, poultry meat and humans (Bertrand et al., 2006; Boyle et al., 2010; Carattoli, 2008; Cloeckaert et al., 2007; Collard et al., 2007; Fricke et al., 2009; Hasman, Mevius, Veldman, Olesen, & Aarestrup, 2005; Majtan, Majtan, Majtan, Szaboova, & Majtanova, 2006; Rodriguez et al., 2009; Weill et al., 2006). Therefore it was included in antimicrobial resistance monitoring in the EU (EFSA Working Group on Developing Harmonised Schemes for Monitoring Antimicrobial Resistance in Zoonotic Agents, 2008). As of March 2011, cephalosporin resistant *Salmonella* was not noted in animals in Poland.

Salmonella (*S.*) Kentucky was infrequently found in Poland, but since autumn 2009 an increasing number of isolates have been observed. Although not considered a major human foodborne pathogen it was quite often observed in humans from numerous countries (Cardinale, Colbachini, Perrier-Gros-Claude, Gassama, & Aidara-Kane, 2001; European Food Safety Authority & European Centre for Disease Prevention and Control, 2011; Fricke et al., 2009; Levings, Partridge, Djordjevic, & Hall, 2007; Weill et al., 2006; WHO, 2011). Antimicrobial resistant *S.* Kentucky has been reported as a potential risk for increased morbidity and treatment failures (Collard et al., 2007; Weill et al., 2006). We describe the first animal-related *Salmonella* strain resistant to extended-spectrum beta-lactams (ESBL), and characterize *S.* Kentucky isolates from the emerging epidemic in turkey flocks in Poland.

2. Material and methods

2.1. Bacterial strains

Forty five out of 51 *S.* Kentucky strains have been collected between July 2009 and December 2010 (Supplementary data). For the present study 27 strains were selected, which had been isolated in 2010 from: turkeys (N = 15), turkey or unspecified poultry meat (N = 3 and N = 2, respectively), food production hygiene checks (neck skin samples, N = 4), feed (N = 2) and municipal sewage sludge (N = 1). All the strains from *Gallus gallus* and duplicate isolates from the same turkey farm or slaughterhouse were excluded. The only exceptions were 3 strains isolated from turkey farm "A" on May, July and September 2010.

2.2. Microbiological resistance testing and resistance gene identification

Minimum inhibitory concentrations (MICs) were determined with customized Sensititre® plates (EUMVS2, Trek Diagnostic Systems, UK) and interpreted according to epidemiological cut-off values described by the European Committee on Antimicrobial Susceptibility Testing (Supplementary data). Microbiological resistance is presumed in non-wild type (NWT) strains due to the presence of acquired and mutational resistance mechanisms to the drug. Etests® (bioMerieux, Poland) was applied to confirm cephalosporin resistance and differentiate between ESBL and ampC-type phenotypes (Wasyl, Hoszowski,

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Zając, & Skarżyńska, 2010) and bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes were identified by PCR assays (Supplementary data).

2.3. Pulsed Field Gel Electrophoresis (PFGE)

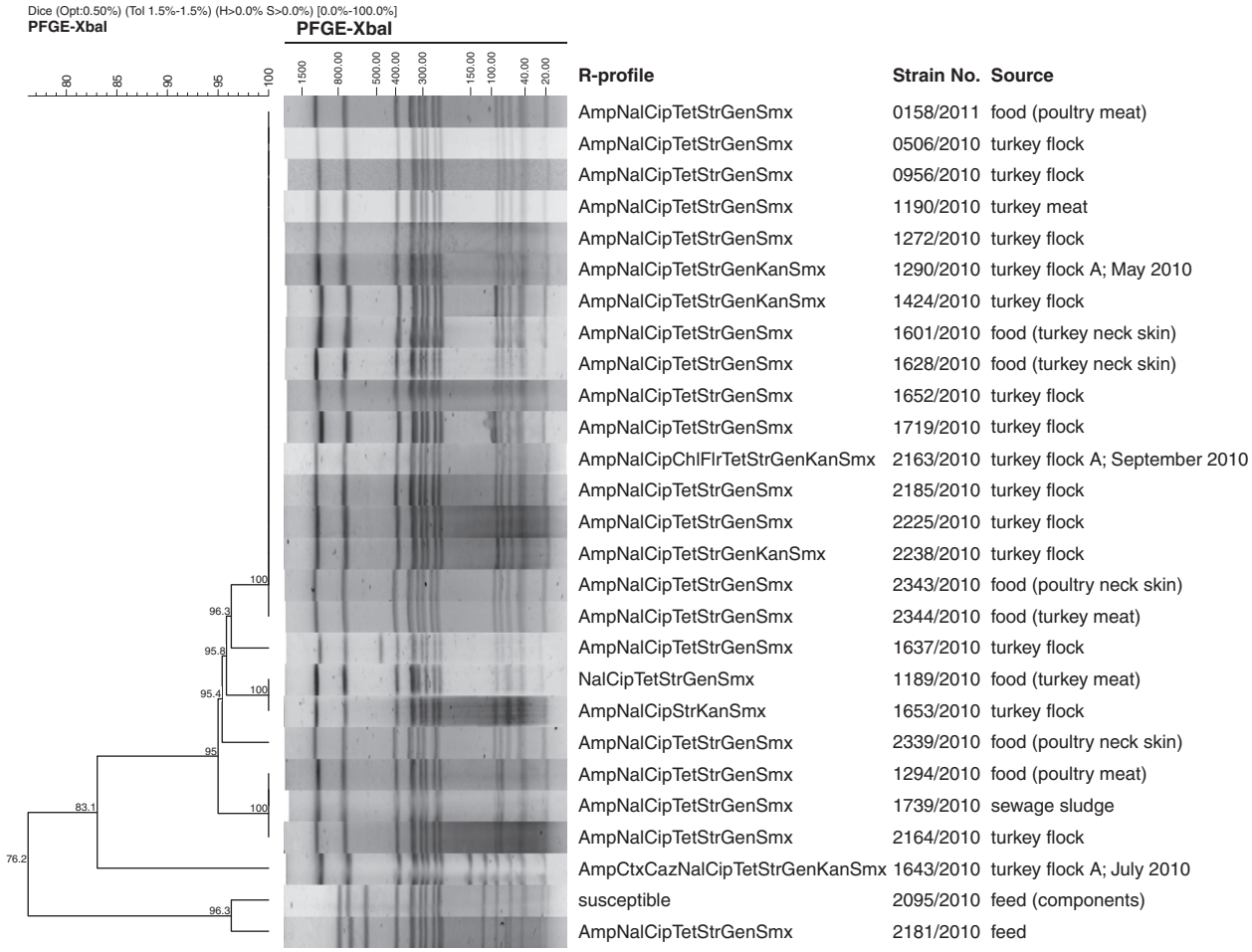
PFGE typing was carried out according to the PulseNet protocol following DNA digestion with XbaI (Ribot et al., 2006). The results were analyzed with BioNumerics (Applied Maths, Belgium) using PulseNet recommended parameters: UPGMA, Dice coefficient, 0.5% optimization, and 1.5% position tolerance. Only bands with a molecular weight higher than 33.3 kb were included in the analysis.

3. Results

Routine antimicrobial resistance monitoring of *Salmonella* isolates revealed that *S.* Kentucky strain #1643/2010 showed microbiological resistance to cefotaxime (MIC>4 mg/L) and ceftazidime (MIC = 16 mg/L). The synergy of clavulanic acid and cefotaxime (MIC_{CTX}>16 mg/L, $MIC_{CTX/clavulanic\ acid}$ = 0.19 mg/L) confirmed the resistance phenotype described as ESBL and the gene responsible for this phenotype was identified as bla_{CTX-M}. The presence of *amp*C-type cephalosporinases was excluded ($MIC_{cefotetan} \leq 0.5$ mg/L; $MIC_{cefotetan/cloxacillin} \leq 0.5$ mg/L). The strain isolated from turkey poults at farm "A" conferred also microbiological resistance to ampicillin, quinolones, tetracycline, aminoglycosides, and sulfamethoxazole (Fig. 1). Two other *S.* Kentucky isolates from environmental samples collected at the same farm on May and September showed similar resistance patterns, but lacked cephalosporin resistance.

The finding of multiresistant, CTX-M-producing strain, which may cause potential threat to public health, drew our attention to the increased number of *S.* Kentucky isolates, indicating an outbreak of the bacteria located at turkey production, and the pathogen spread into food chain (Supplementary data). All outbreak strains were multiresistant to six or more antimicrobials but only one was cephalosporin resistant. The most prevalent profile (AmpNalCipTetStrGenSmx) was observed in 19 strains and a few others additionally showed resistance to kanamycin (N = 3) and phenicols (N = 1), or missed resistance to gentamicin (N = 1) or ampicillin (N = 1) (Fig. 1).

Eight of the obtained XbaI PFGE profiles showed 76.2% similarity and five of them were clustered with 95.0% phylogenetic similarity (Fig. 1). Seventeen strains representing indistinguishable XbaI patterns showed multiple resistance profiles. A few other strains showed up to two bands different to the main profile. They were found in turkey flocks, meat, food processing hygiene checks and municipal sewage sludge. The geographical locations of turkey farms and slaughterhouses were as distant as 600 km and reflected the regions of Poland with intensive



Amp – ampicillin, Ctx – cefotaxime; Caz – ceftazidime, Chl – chloramphenicol, Cip – ciprofloxacin, Flr – florfenicol, Gen – gentamicin, Kan – kanamycin, Nal – nalidixic acid, Str – streptomycin, Smx – sulfametoxazole, Tet – tetracycline

Fig. 1. PFGE and resistance profiles of *S.* Kentucky. Amp — ampicillin, Ctx — cefotaxime; Caz — ceftazidime, Chl — chloramphenicol, Cip — ciprofloxacin, Flr — florfenicol, Gen — gentamicin, Kan — kanamycin, Nal — nalidixic acid, Str — streptomycin, Smx — sulfametoxazole, Tet — tetracycline.