COLIFORM POPULATIONS AND ESBLs IN *ESCHERICHIA COLI* ISOLATED FROM PIG FARM ENVIRONMENT

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1 INTRODUCTION

Agricultural animal production is increasingly regarded a source of airborne pollutants, such as bioaerosols, dust and gases, which are both aggravating and ecologically harmful. The main aerial pollutants in animal houses are derived from animals, feed, litter, excrements and buildings structures.

Although many classical diseases are controlled today, there remain a variety of environmental disorders which can cause considerable losses in performance and lives. Most of these problems occur in pig production and include disorders of the digestive and respiratory tracts, cardiovascular system as well as the skin and skeleton.

Animal workers are exposed to a range of organic and inorganic dusts, endotoxins, fungi and bacteria which have been implicated in health outcomes, concluding respiratory illness, allergies and infections. The health impact of working in the animal industry may also extend beyond the exposed workers. For example, where antibiotics are used in animal farming, workers may be exposed to resistant strains of bacteria, such as gentamicin resistant *E.coli* in poultry housings, providing a mechanism for dissemination into the wider community (Banhazi et al., 2009).

Repeated exposure of bacteria to antimicrobial agents and access of bacteria to increasingly large pools of antimicrobial resistance genes in mixed bacterial populations are the primary driving forces for emerging antimicrobial resistance (Schwartz and Chaslus-Dancla, 2001). Resistance of both commensal and pathogenic bacteria in livestock animals to antimicrobials of clinical importance is now commonplace and is related to their increased use for growth promotion and prophylaxis over the last 50 years (Maynard *et al.*, 2003).

The aim of the study was to investigate antibiotic resistance of microorganisms present in bioaerosols on a pig farm.

2 MATERIAL AND METHODS

In the experiment, samples 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 respective nutrient media (Meat-pepton agar, Endo agar, Sabouraud agar, MacConkey agar) are placed on top of the dish support of the sampler and after aspiration of a preset volume of air, they are incubated at appropriate temperatures (37°C meat-pepton agar, Endo agar for one day and 24°C Sabouraud agar for four days and room temperature). The plate counts were recalculated per 1 m^3 of air.

Susceptibility (MIC) was determined by colorimetric broth microdilution method according to CLSI guidelines using ampicillin, ampicillin and sulbactam, ceftiofur, ceftriaxon, ceftazidime, ceftazidime and clavulanic acid, gentamicin, streptomycin, neomycin, spectinomycin, nalidixic acid, enrofloxacin, ciprofloxacin, chloramphenicol, florfenicol, tetracycline and cotrimoxazol. Resistance mechanisms to beta-lactams e.g. penicillinase (TEM 1,2/SHV1)-high and ESBL were determined by means of interpretative reading of the antibiogram profile and MIC profile. ESBL production was defined as a ≥8-fold reduction in the MIC of ceftazidime in the presence of clavulanic acid with comparison with ceftazidime alone. High level fluoroquinolone resistance (ENR ≥ 16 mg/L) was classified according to Lee et al (2005).

3 RESULTS AND DISCUSSION

Microorganisms present in the air in animal houses may affect negatively the health, growth and productivity of animals.

Bacteria levels are influenced by the density of stocking, the age of the animals, the ventilation system, the microclimate in the animal houses and the amount of dust in the air and deposited on the surfaces. They also

depend on the housing technology (litterless or with litter), system of stocking (continual stocking or the all in –all out system) and feeding (dry or wet feed).

The number of microorganisms in the air in animal houses varies. In most cases it is in the range between 10^3 and 10^6 germs in one cubic metre of air (see Table 1). In farrowing, growing and finishing stage was observed the highest concentration total count of microorganisms as well as coliform and moulds. That relates with increasing temperature, relative humidity and insufficient ventilation. In gestation stage with straw bedded was found after exchange of bedding the concentration of microorganisms decreased mostly by 20 orders. Increasing of microorganisms in weaning due to problems with wit belt manure scraper conveyer.

Relative humidity is probably the most crucial factor controlling bacterial aerosol stability. Airborne bacteria survive better at relative humidity higher or lover than the 50-80% (Ondrašovič et al., 1997). Relative humidity on pig farm varied from 60.2% to 80.9% and temperature from 11.4ºC to 19.6ºC, but concentration of microorganisms was higher than 10^5. Some problems with belt manure scraper conveyor that occurred periodically on the farm could contribute to higher aerial microbial contamination.

Efficient ventilation is one of the principal tenets of intensive animal production, that mean ensure an aerial environment in which can be maintained the animal health and can be sustained their productivity, the employment accomplish his tasks in comfort and without risk to his health and the building and its equipment are protected from corrosion or physical damage (Wathes and Charles, 1994).

The recommended maximum acceptable concentration of microorganisms in the air in animal houses is 250 000 in 1 m^3.

TABLE 1 **Average values and standard deviations of concentrations of several groups of micro-organisms in service piggery stages within the observation period.**

	TCB	TC	Moulds	n
Farrowing stage	$9.3\cdot10^5\pm1.6\cdot10^5$	$9\cdot10^2\pm4.5\cdot10^2$	$3.6\cdot10^4\pm1.3\cdot10^4$	10
Post-weaning stage	$8\cdot10^5\pm3.8\cdot10^5$	$5.6\cdot10^3\pm3.6\cdot10^3$	$6.8\cdot10^4\pm3.9\cdot10^4$	10
Gestation stage	$1.5\cdot10^5\pm8.1\cdot10^4$	$4.5\cdot10^2\pm2.8\cdot10^2$	$2.3\cdot10^4\pm1.4\cdot10^4$	10
Growing stage	$9.6\cdot10^4\pm7.5\cdot10^3$	$5.6\cdot10^3\pm3.2\cdot10^3$	$4.9\cdot10^4\pm2.7\cdot10^4$	10
Finishing stage	$9.8\cdot10^4\pm4.9\cdot10^3$	$2.3\cdot10^3\pm1.04\cdot10^3$	$1.4\cdot10^4\pm5.9\cdot10^3$	10
Vicinity of the farm	$5.6\cdot10^3\pm4.8\cdot10^3$	$6\cdot10\pm2.8\cdot10$	$7.4\cdot10^2\pm7.1\cdot10^2$	10

In this study betalactam and quinolone resistance of *Escherichia coli* isolates was investigated. Phenotypic analysis of MIC concentrations in antibiotic resistant strain revealed the presence of ESBLs and high level of quinolone resistance (Figure 1). Close contact of flies with pigs or their products was associated with colonization of flies with resistant bacteria. Animal husbandry may cause a range of ecological and medical problems. Animal excrements and bioaerosols contain facultative pathogen bacteria of the family *Enterobacteriaceae*.

The CTX-M-type β-lactamases are a rapidly emerging group with a typical extended-spectrum β-lactamase (ESBL)-resistance phenotype. These enzymes, encoded by transferable plasmids, are capable of hydrolyzing broad-spectrum cephalosporins and are inhibited by clavulanic acid, sulbactam and tazobactam. They confer a high level of resistance to cefotaxime but have a low level of activity against ceftazidime. The emergence and dissemination of extended-spectrum cephalosporin-resistant Enterobacteriaceae is increasing and has caused considerable concern.

ESBLs were detected by intepretative reading of antibiotic minimal inhibitory concentrations. CTX-M (group 1, 2 and 9) was screened in phenotype positive *Escherichia coli* strains by PCR CTX-M1 was confirmed with primers according to Carattoli et al. (2008) and by DNA sequencing of PCR product.

The greatest degree of permanent resistance has developed against tetracycline. Depending upon the species of animals, the resistance to various antibiotics differs under field conditions. Antibiotic treatment puts an antibiotic pressure on skin bacteria and changes the nature of the source of airborne microbes (Ondrašovič et al., 1997).

Resistant nonpathogenic *E. coli* and coliform bacteria isolates with specific genes were detected in 23% of milk filters, STEC O157 was detected in 2% of the filters (Cizek et al. 2008).

Intestinal microflora, in the manure can contaminate food chain, water, vegetables, vegetable organic wastes, straw, sawdust, flies and is a potential source of resistant coliform bacteria and introduction of antibiotic residues which might exert a selection pressure.

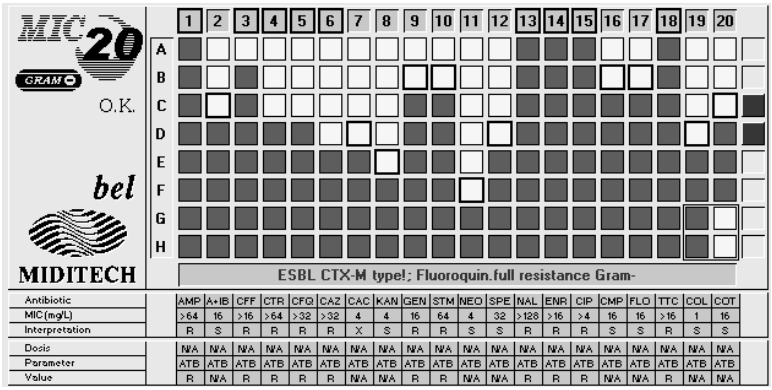

FIGURE 1 **Phenotypic analysis of MIC concentrations in antibiotic resistant strain revealed the presence of ESBL CTX-M type**

In a study on several swine farms in the United States, Jackson *et al.* (2004) determined that tylosin use for growth promotion resulted in erythromycin-resistance in 59% of enterococci isolates, compared to 28% at a farm where tylosin was used for treatment of disease only, and 2% at a farm that did not use tylosin.

National surveillance of Salmonella in swine in the U.S. has revealed resistance to several important antimicrobials including tetracycline (50%), ampicillin (12%), sulfamethoxazole (23%), and streptomycin (23%) (NARMS, 1998). Hoyle *et al.*, (2004) studied ampicillin-resistant *E. coli* in calves in the United Kingdom and determined that ampicillin resistance peaked over 80% within 4 months, steadily declining to less than 10% as the calves aged to 8 months.

The latent period between the introduction of an antimicrobial and the emergence of resistance may vary, once the prevalence of resistance in a population reaches a certain level, reversal of the problem may be extremely difficult (Swartz, 2002).

Although the use of antibiotics as growth promoters is being progressively restricted through EU regulations, they are still used in large quantities in animal rearing for both prophylactic and therapeutic purposes. Kmet et al. (2009) and Kmet and Kmetova (2010) found high levels of multiple resistance in calf and poultry commensal faecal *Escherichia coli* on farms in Slovakia.

E. coli isolates from livestock showed resistance to the largest number of antimicrobials and multidrug resistance was most common in swine fecal samples. Resistance was demonstrated most frequently to tetracycline, cephalothin, sulfisoxazole, and streptomycin (Sayah *et al.*, 2005).

4 CONCLUSIONS

Hygiene of the air and of building surfaces is often less than satisfactory and is potentially a serious limitation to high efficiency of production and good health in those intensive systems of animal husbandry which involve housing at the stage of production cycle. The air and surfaces of buildings may act as reservoirs of primary and opportunistic pathogens. Microorganisms may contribute to the aetiology of environmental diseases, such as calf pneumonia, by the continuous burden which they impose on non-specific host defense mechanisms. Poor air and surface hygiene in livestock buildings is nearly always associated with intensive systems of husbandry and is exacerbated by poor standard of management. Intensive systems usually involve high stocking densities and large flocks or herds, which produce large quantities of wastes on a farm scale. The common aerial pollutants found in livestock buildings are dusts, gases and commensal microorganisms and these are the inevitable by-products of animal existence. They arise from feed, bedding, excreta and animals themselves (Wathes and Charles, 1994).

Resistant microorganisms that exist in bioaerosols in different environments pose hazard to animals and people, particularly through exposure to infective antibiotic resistant pathogens or commensals and related potential mortality and failure of therapy.

The movement of antimicrobial-resistant microorganisms from animal to animal or animal to animal care worker may be facilitated by the crowding of animals into confinements, often with suboptimal hygiene. The co-colonization of animal gastrointestinal tracts by antimicrobial-resistant commensal bacteria and bacterial pathogens may lead to further development of antimicrobial-resistant bacterial zoonoses (Kruse *et al.*, 1999). As much as 75-80% of an antibiotic may pass undigested through an animal, thus its waste may not only harbor high concentrations of antimicrobial-resistant bacteria, but also their resistance genes and raw (undigested) antimicrobial compounds (Campagnolo and Rubin, 1998). This waste is often stored in open air lagoons and/or spread on fields where these compounds, resistant organisms, and antimicrobial-resistance gene reservoirs may move into the environment via aerosolization, infiltration into the groundwater, or runoff into surface water resources.

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