PIG FARMS AND THEIR SURROUNDINGS AS A FACTOR IN THE SPREAD OF ANTIMICROBIAL RESISTANCE

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Abstract: In recent decades, in modern intensive animal husbandry, there has been a worrying trend towards the selection and spread of bacterial strains resistant to antibiotics. This global phenomenon has not only a certain negative effect on therapeutic practice, but also poses a serious risk to the spread of resistance in the food chain and in humans. Various genera from the family *Enterobacteria* like *Salmonella, Shigella, Klebsiella, Yersinia, Citrobacter*, etc. and especially *Escherichia coli* have a special place in this process. The most commonly used antibiotics for treatment of patients with complicated *Salmonella* infections are ceftriaxone and ciprofloxacin, to which the bacteria has already developed antibiotic resistance. For *Escherichia coli*, significantly increased resistance is developed to the third-generation cephalosporins, aminoglycosides, as well as combined resistance to three key antimicrobial groups (fluoroquinolones, third-generation cephalosporins and aminoglycosides). Resistance among *Yersinia* spp. against tetracyclines and fluorochinolones has been reported to the scientific community in numerous studies on human and animal isolates. By using the raw manure as fertilizer, these microorganisms may be transmitted to soil, wastewater or others environmental niches. Further, they can be transmitted via the food chain to humans and animals. Considering that the horizontal gene transfer is one of the most important mechanisms for transmitting of antimicrobial resistance, then after consumption of contaminated food, water, etc., genetically determined antibiotic resistance in animals can easily affect resistance among people, which makes vital drugs ineffective against serious illnesses. For the possible spread of antimicrobial resistance in pigs and related ecological niches - pig farms, manure lagoons, wastewater and soils, large-scale studies are being conducted to clarify their ecological and health significance. In addition, the results obtained will contribute to the development of an effective national strategy for the control of antimicrobial resistance.

Keywords: antimicrobial resistance, pig farms, manure lagoons, wastewater, soils

1. INTRODUCTION

Pork is one of the most widely consumed foods worldwide. Pork production varies from very intensive on farms to the production of one or more pigs in a family's backyard. In both cases, bacterial infections can significantly affect production costs. That's why the routine use of antibiotics has become an integral part of production. Antimicrobials have been used not only to treat clinically ill pigs, but also as part of routine prevention and even growth promotion [1]. In 2000, it was reported that sales of antimicrobials for use in pig farming were estimated at $ 1.7 billion, equivalent to 34% of the global market for animal health antimicrobials [2]. General infections affect weaned pigs to a large extent, while respiratory infections affect slaughter pigs.

Nowadays, the antimicrobial resistance (AMR) is a national and global public health problem caused by the uncontrolled prescribing and use of antibiotics in human and veterinary medicine. For example, in 2013 in China 92,700 tons of antibiotics were used, 52% of which were consumed by animals [3]. In his 2014 review, O'neill reports, that deaths caused by AMR could increase from 700,000 in 2014 to 10 million by 2050 [4]. The resistant bacteria carrying genes for antibiotic resistance (GAR), which can spread from livestock farms to the environment.

In 2016, most of the outbreaks in Europe were caused by bacterial agents (33.9% of all outbreaks), in particular *Salmonella* spp. – 22.3%. The outbreaks caused by these bacteria had a moderate increase compared with 2015 (for *Salmonella* they are 110 more), corresponding to an increase of 11.5%, although as a whole for the period 2010-2016, they decreased [5]. Non-typhoidal *Salmonella* causes approximately 1.2 million illnesses, of which 23,000 were hospitalized, and 450 are deaths each year in the USA. For the treatment of patients with complicated *Salmonella* infections the most commonly used antibiotics are ceftriaxone and ciprofloxacin, to which the bacteria has already developed antibiotic resistance [6]. For *Escherichia coli*, significant increases in infections in the EU and EEA population-weighted mean percentages for third-generation cephalosporin resistance and aminoglycoside resistance, as well as for combined resistance to three key antimicrobial groups (fluoroquinolones, third-generation cephalosporins and aminoglycosides) were observed for the period 2013 –2016 [7]. Up to now, only few European Member States report data from surveillance of *Yersinia* spp. in food, mainly in pig meat and others animal products. Although, there was a statistically significant decreasing trend in 2008–2014 in the in the EU/EEA [8, 9], yersiniosis still remains the third most important zoonosis in the EU [10]. Resistance among *Yersinia*

spp. against tetracyclines and fluorochinolones has been reported to the scientific community in numerous studies on human and animal isolates.

It is crucial to implement the One Health concept to address this important public health issue, including the implementation of good antimicrobial management practices in human and animal health, in order to slow down the development of resistance and prolong useful life of antimicrobials. One Health is the integrated effort of many disciplines working locally, nationally and globally to achieve optimal health for humans, animals and the environment. The focus of this plan is on the actions taken by the responsible state bodies to reduce the risk of new outbreaks.

2. SPREAD OF ANTIMICROBIAL RESISTANCE

The risk of spread of AMR as one of the main threats to health in humans and animals worldwide continues steadily to increase, both in the field of human medicine and in animal husbandry and agriculture. This threat is based on a growing set of evidence provided by research and observation of a growing number of countries around the world [11].

Many countries use swine manure or wastewater for fertilization. But this waste has become a reservoir of resistant bacteria and their GAR. Many antibiotics are not metabolized or degraded in a natural pathway and remain active in the environment for long periods of time [12]. In addition, they can be released into agricultural soils at depth from 20–30 to 40–60 cm via long-term manure application and transfer into groundwater by wastewater from farm and lagoon (Fig. 1). So antibiotic residues and bacteria can persist in soils after application and transfer into groundwater after several years [3]. They exert selective pressure on microorganisms by eliminating the sensitive strains and increasing the chances of survival for those containing GAR [13, 14]. The most common sources of such genetic determinants are hospitals and breeding sites, urban wastewater facilities, manure pits (lagoons), untreated wastewater, various agricultural wastes and others [15].
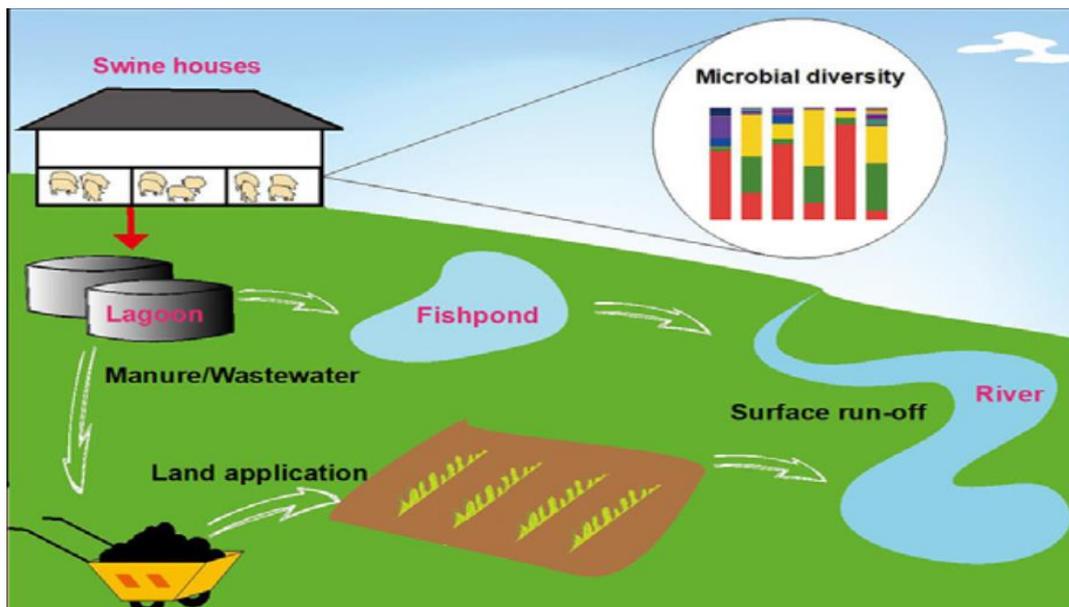

Fig. 1. General presentation of relevance of the developed theme [3]

Resistant bacteria and their GAR can be spread and exchanged in different ways [16]. The molecular basis of antibiotic resistance includes genetic elements such as plasmids, transposons, integrons, genetic islands, etc. that are located in the bacterial genome. If bacteria that carry such elements come into contact with others, they exchange genetic determinants of resistance with each other through horizontal gene transfer [17]. The most common path of distribution along the food chain is through transposons and plasmids between pathogenic and nonpathogenic bacteria in the environment, incl. GAR [15, 18].

3. ANTIMICROBIAL RESISTANCE AMONG THE MAJOR PATHOGENS

According to a report from the European Food Safety Authority (EFSA) and the European Center for Disease Prevention and Control (ECDC), some of the most commonly reported zoonoses in the EU are salmonellosis, yersiniosis and colibacteriosis (especially from shiga-toxin-producing *E. coli* strains, STEC) [19].

Antimicrobial resistance in Salmonella sp.

According to a report of EFSA, the second highest cause of morbidity are bacteria belonging to the genus *Salmonella*. Often *Salmonella* sp. has a low pathogenicity in pigs being more important for zoonotic transmission. However, *S. typhimurium* causes occasional diarrhea, septicemia and often death in grower pigs (6–16 weeks). The incidence increases with Porcine reproductive and respiratory syndrome virus and Porcine circovirus 2 infections. *S. derby* provokes occasional diarrhea in grower pigs and *S. enterica* serovar Choleraesuis – septicemia, diarrhea and death in finishing pigs (12-16 weeks) [1]. It is known that in Bulgaria the most common *Salmonella* spp., which cause serious infectious outbreaks in humans and animals through secondary contaminated fodder from improper storage in pig farms, are *S. enteritidis*, *S. typhimurium*, *S. newport*, *S. javiana* and *S. heidelberg*. In a formal study conducted in Bulgaria (2012) it was revealed the presence of *S. mbandaka* in supplementary feed for pigs, *S. paratyphi* in supplementary feed for sows and *S. kottbus* – in supplementary feed for fattening pigs [20]. The use of antibiotics is not recommended because this might lead to resistance development, as reported in US [21], Taiwan [22] and Japan [23]. More than 2 600 serovars in the world have been identified so far, most of which are pathogenic for humans and animals [24]. Multiple resistance in *Salmonella* isolates is often encoded by genomic island 1, which contains determinants of resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline. It has already been shown that the isolated *Salmonella* from swine feces are often carriers of the GAR including extended-spectrum beta-lactamases (ESBLs) encoded by the genes *bla*TEM, *bla*CTX or *bla*OXA [25]. According to the CAESAR network, resistance in *Salmonella* isolates to third generation cephalosporins (8%) and fluoroquinolones has been detected in the Republic of Serbia [26].

Antimicrobial resistance in Escherichia coli

The bacterial species *E. coli* is a part of the normal intestinal microflora in humans and animals, but it is also one of the most common causes of systemic, gastrointestinal and urinary infections. *E. coli* can be both septicemic and enteric, especially in neonatal piglets. This bacterial species often cause enteric diseases. For example, neonatal and piglet scours appear in the first few days of the piglet's life (1-3 days, respectively 7-14 days) and post-weaning diarrhea – between 5-14 days after weaning, which can lead to dehydration and mortality in severe cases. Antimicrobials can be used effectively against neonatal scours, which can be severe and the piglets can die of septicemia. *E. coli* provoke bacteremia, arthritis and navel infections in post-weaning, and cystitis and nephritis in sows. Studies of *E. coli* isolates from infections in swine have been conducted in different countries on the antibiotic resistance: Belgium, Denmark, England, Finland, France, Latvia, Norway, Poland, Spain, Sweden, Switzerland, Netherlands [27], Canada [28], Germany [29], Japan [30] and Bulgaria [31]. The occurrence of antimicrobial resistance varies greatly between countries and even regions. Thus, the final choice of treatment has to be based on the local situation. The isolates from Belgium, Canada, Denmark, Japan, Latvia, Poland and Spain were more resistant. High resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, trimethoprim and tetracycline has been found. The isolates from Belgium (39 %), Latvia (22%) and Poland (30%), were resistant to fluoroquinolones, compared with these in Canada (0%), Denmark (0%), England (2%), Finland (0%), France (6%), Germany (8%), Norway (0%) and Switzerland (0%) [1, 27-29]. Fluoroquinolone-resistant *E. coli* were also isolated from fecal swine samples in China and Korea. The researchers found plasmid-mediated quinolone-resistance (PMQR) genes in 15.3% isolates and efflux pump activity in 94.9% isolates. The authors reported that the resistance to fluoroquinolones has increased significantly in swine compared to in previous studies in Korea, although fluoroquinolones have been banned as a feed additive since 2009. These authors investigated the *qnr*A and *qnr*B genes, but they did not prove them [32, 33]. Hu et al. (2017) suggested that the predominant plasmid-mediated quinolone-resistance genes detected in human isolates were *qnr*A and *qnr*B, whereas *qnr*S was detected in swine samples [32]. This hypothesis was confirmed by our previous study [31]. The isolates from swine in Belgium (46%), Poland (45%), Spain (20%), Japan (14%), Switzerland (13%), Denmark

(12%) and Germany (12%) were more resistant to gentamicin, compared with these in France (6%), Bulgaria (6%), Finland (0%), Norway (0%) and Sweden (0%). All tested isolates were sensitive to florfenicol [1, 27, 29-31]. It was observed a high percent of resistance in Bulgaria among pathogenic *E. coli* strains isolated from pigs during the period 2010–2015. The resistances to tetracycline antibiotics, streptomycin, spectinomycin, ampicillin, and sulfonamides have increased by twofold and to ciprofloxacin – tenfold compared with study in 2000-2004 [34]. According to a study by the National Diagnostic Research Veterinary Medical Institute (NDRVMI) and University of Forestry in Bulgaria conducted in the period 2012–2014, 75% of *E. coli* isolates were resistant to amoxicillin and erythromycin and 51-75% of them were resistant to ampicillin, oxytetracycline, thiamulin, streptomycin, doxycycline, tylosin, and tilmicosin. It has been found that sensitivity is most strongly established to non-use agents (amikacin, cefquin, and cefotaxime), less commonly used in practice (kanamycin), or new agents in the fluorinated quinolone groups (ciprofloxacin, enrofloxacin, and pefloxacin) and amphenicols [35]. In our previous study, 87.5% of the *E. coli* isolates from swine faeces and lagoons were multidrug resistant. Only 18.75% were resistant to aminoglycosides, 81.25% – to penicillins, 25% – to fluoroquinolones and 6.25% – to macrolides. But the *E. coli* strains were susceptible to monobactams, cephalosporins and carbapenems. We found high resistance to β-lactam and tetracycline antibiotics. We showed increase in resistance to trimethoprim/ sulfamethoxazole from 7.1% in the period 2012–2014 [35] to 43.75% in 2020 [31] and to nalidix acid from 11.1% in the period 2012–2014 [35] to 25% in 2020 [31]. Decreased resistances to doxycycline (from 64.7% in the period 2012–2014 [35] to 37.5% in 2020 [31]), streptomycin (from 63.1% in the period 2012–2014 [35] to 12.5% in 2020 [31]), and erythromycin (from 80% in the period 2012–2014 [35] to 6.25% [31]) have been observed. Dimitrova et al. (2016) also documented that the isolated *E. coli* strains were susceptible to amikacin, cefotaxim, ciprofloxacin and norfloxacin [35]. Urumova (2016) studied and compared the AMR profile in *E. coli* isolates from pigs in the period 2012–2016 from different regions in Bulgaria (Shumen, Ruse, Razgrad, Yambol, and Varna). She found high resistance in growing pigs, compared with that in suckling and fattening pigs, as well as lagoons [34]. It was found that the resistances to ampicillin (68.75%) and amoxicillin/ clavulanic acid (from

2.2% in the period 2012–2016 to 6.25% in 2020 [31]) were doubled and tripled, respectively [34]. The researcher also proved high resistance to streptomycin (69.4%) [34], as reported by previous authors [35]. In the period 2012–2016 was observed a slight resistance to ciprofloxacin (5.2%) [34], which was also confirmed in 2020 (6.25%) [31]. The resistance to tetracycline was almost not changed from 73.3% in the period 2012–2016 [34] to 56.25% in 2020 [31], indicating that the antibiotic continues to be used in swine farms. Only one isolate out of a total of 157 growing animals was found to be resistant to ceftazidime and cefotaxime [34].

Due to the rapid spread of GAR between bacteria, environment and human, treatment of urinary infections in some countries is ineffective in more than 50% of patients. The AMR in EARS-Net countries reaches 10-25%, while over 25% are found in Bulgaria, Cyprus, Italy and Slovakia. Among CAESAR countries (Montenegro, Russia, Northern Macedonia and Turkey) the AMR exceeds 50%, while in Serbia it varies between 25-50% [26]. EARS-Net data show a statistically significant increase in the resistance of *E. coli* isolates in the EU to third generation cephalosporins [36]. Recently, the emergence of *E. coli* carbapenem-resistant has been identified, which is a major challenge and although the proportions of resistance are still low in Europe (around 1% and above), there is a trend of increasing worldwide [26, 36]. The genetic resistance determinants demonstrated in isolates of *E. coli* include ESBLs encoded by various plasmid genes (*bla*SHV, *bla*CMY-2, *bla*TEM etc.), as well as a number of GAR for resistance to quinolones (*qnr*), trimethoprim (*dhf*), aminoglycosides (*aac*(3)), etc. [37]. As high levels of *E. coli* resistance have been reported in farmed animals in Europe, incl. carbapenemase-expressing isolates and plasmid-mediated colistin resistance [36]. It was conducted a study of widespread distribution of genetic determinants of resistant *E. coli* strains isolated from the resident intestinal microflora of healthy pigs. The results showed the prevalence of *aad*A1 genes for determining streptomycin/ spectinomycin resistance, *tet*(A) – tetracycline resistance and *str*A/*str*B – streptomycin resistance. There is a limit information about the presence of the genes *sul*1 and *sul*2, which determine the bacterial resistances to sulfamethoxazole, *bla*TEM – to aminopenicillins and cephalosporins of first generation, *tet*(B) – to tetracyclines and *int*l gene, which is responsible for the synthesis of the integrase enzyme from class 1 integrons. The lowest distribution is for the *aac*C2

gene, which is responsible for the resistance to gentamicin, kanamycin, tobramycin and netilmicin [34]. In 2020 was confirmed the presence of the β-lactamase genes *bla*TEM/*bla*SHV in one isolate out of a total of 8 from lagoon and *amp*C in three isolates out of a total of 8 from fattening swine faeces and in four isolates from lagoons. In the period 2012–2016 in Bulgaria was found high resistance to tetracycline in growing pigs and fattening swine, which is a worrying fact as coliforms resistant to this antibiotic may be ingested during consumption. This also applies to the antibiotics ampicillin and amoxicillin, which continue to be used in veterinary practice. Probably, less commonly applied are streptomycin, erythromycin, and doxycycline [31, 34].

Antimicrobial resistance in Yersinia enterocolitica

The pathogenic bio/serotypes of *Y. enterocolitica* strains are the third most common causative agents of food zoonosis in the EU in the last twenty years. A number of studies have already provided data on the epidemiology of *Y. enterocolitica* [19] and the circulation of GAR among strains isolated from patients, including antibiotics used as growth promoters in pigs [38-40] [22-24]. A study conducted in Switzerland showed that isolates of *Y. enterocolitica* (4/O:3) from tonsils and faeces of slaughter pigs showed the same profile of genes in virulence and antibiotic resistance as well as isolates from clinical cases of yersiniosis in humans [41]. Similar findings have been reported in Italy, where the percentage of resistant strains is significantly higher [42]. This is evidence for transmission of genetic resistance determinants in the food chain and confirmed the etiological role of biotype 4 in human yersiniosis. Studies on the prevalence of resistant strains of *Yersinia*, conducted in India showed a resistance to tetracyclines, aminoglycosides and macrolides [43]. Multi-resistant *Y. enterocolitica* strains isolated from slaughtered pigs and pork have also been detected in Malaysia with a 90% rate [44].

4. DISEASE CONTROL IN SWINE PRODUCTION

However, it is difficult to obtain accurate estimates on the prevalence of various bacterial diseases. The obtained data from diagnostic laboratories are often from samples of difficult clinical cases. The "right" environment is very important for pigs and disease prevention. It is important to understand that the use of antibiotics in swine farming does not mean disease control. Unfortunately, this is a common practice in many swine farms. The challenge, therefore, is to correct the main problems of governance. For example, when the temperature is constant high and there is no draft, then diarrhea after weaning proceeds slightly. There are two approaches – avoiding the use of infectious agents and avoiding clinical disease. The first can be achieved by starting a healthy herd or by depopulating and repopulating a clean stock. Another way to disease control is to avoid buying pigs from farms with sick animals, as well as to ensure strict biosecurity when entering the farm. The second approach is more difficult. It is achieved through herd management, pig farming and the environment, and immunity. Pigs that are privately owned by families suffer much less from infectious diseases. On farms, mixing of pigs of different ages and / or with pigs coming from other farms should be avoided. Raising piglets in groups with their sows also reduces the transmission of the pathogen in the swine farm. Last but not least, piglets need to be weaned when their immune system has become mature enough to cope with intestinal diseases on its own. The small number of pigs in the barn leads to a low rate of respiratory diseases, lack of stress, low mortality and higher growth [1].

5. CHOICE OF THERAPY

The choice of therapy depends primarily on the type of disease. There are very few independent studies that compare different antimicrobial drugs for the same disease. The antimicrobial resistance test is used to determine whether a particular antimicrobial agent should be used to treat a disease [1]. The test is usually based on a standard microbiological method to determine whether an isolate should be considered resistant or sensitive to a particular antibiotic according to the clinical breakpoints in EUCAST [31]. Antimicrobial agents with high clinical efficacy and to which a pathogen is sensitive are usually selected for treatment. It should also be borne in mind that resistance can be transmitted from pigs to humans, and therefore the consequences for human health must be keep in mind. Fifteen years ago, the World Health Organization developed a list of critical antibiotics for human health and recommended that the use of these agents in animal prevention be limited as much as possible [45]. Most isolates are sensitive to cephalosporins and fluoroquinolones, but these antimicrobial classes are considered critical to human health and their use in food production should be limited or avoided as much as possible. Continuous monitoring at national, regional and farm level is needed to assist the veterinarian in choosing the most optimal treatment [1].

6. CONCLUSION

The intensive breeding of pigs for meat production may be accompanied by the carrying pathogens and commensal microorganisms resistant to antimicrobial agents in the intestinal tract of these animals. By using the raw manure as fertilizer, these microorganisms may be transmitted to soil, wastewater or others environmental niches. Further, they can be transmitted via the food chain to humans and animals. Considering that the horizontal gene transfer is one of the most important mechanisms for transmitting of AMR, then after consumption of contaminated food, water, etc., genetically determined antibiotic resistance in animals can easily affect resistance among people, which makes vital drugs ineffective against serious illnesses. The fact is also alarming for established over the last 10 years significantly higher proportions of resistance in the case of *Salmonella* spp., *E. coli* and *Yersinia* spp. for which is proven that they are resistant to commonly used antibiotics. Therefore, in addition to measures to reduce the use of antibiotics in animal husbandry, veterinary and medical clinical practice, the prevalence of AMR and important GAR as a risk factor for human and animal health should be monitored and regulated.

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