COST is supported by
the EU Framework Programme
Horizon 2020

COST Action FA1401PiGutNet

Open document on the risks associated with long-term antibiotic exposure to the variability of the gut microbiota

Industrial livestock production in its present form relies heavily on the widespread use of antimicrobials to improve animal health, welfare and productivity. Antimicrobials are used on livestock farms for a number of reasons: (i) as therapeutics; (ii) more commonly as metaphylactics, meaning that the presence of clinical illness in one animal triggers drug treatment of the whole herd or flock; (iii) prophylactics; and (iv) growth promotion. A ban on the use of growth promoters was implemented throughout the EU in 2006. However, this has not led to any consistent decrease in antibiotic consumption. Typically, the growth promoter ban has prompted compensatory increases in metaphylactic and prophylactic use. The result is that in Europe, the volume of livestock usage of antibiotics continues to rival that of medical usage and in the USA (which recently introduced a voluntary ban on growth promoters), agricultural usage exceeds medical usage [1]. However, there has been some localized successes: for example, a more than 50% reduction in the usage of antibiotics (notably macrolides) in pigs was achieved from 1992 to 2008 in Denmark without any loss in productivity [2].

The discussion of AMR is often focus on human health outcomes. However, a broader consideration of the impacts on animal and environmental health is essential. Prescott discussed the complex epidemiology of AMR and stated that 'resistance anywhere is resistance everywhere' [4]. AMR is described as an ecological problem and as 'a highly multifaceted topic at the interface of human, animal and plant health, food hygiene and environmental science' by Butaye et al [6].

Antimicrobial usage and the resultant selective pressure have been recognized as the main drivers for AMR [7,8,9], leading to therapeutic failures, to medicine and veterinary medicine as well.

Rushton stated that antimicrobials should be seen as a common good [10]. In light of this, human behaviour and attitudes around antibiotic prescribing and use should be considered both in human and veterinary medicine. Decisions around antimicrobial usage in livestock should consider the trade-offs that occur between restoring animal health and productivity, provision of animal welfare and impacts on livelihoods, hunger and poverty alleviation versus the risk of driving resistance.

Moreover in pig farming where intensive livestock production is usually inline with antimicrobials consumption a lot has to change in order to tackle AMR. Changes must focus in three main pillars:

- In general enhancement of pig health and welfare.
- Innovative solutions for alternatives to antibiotics.
- Changing in attitudes and behaviors.

Animal living conditions, management practices and exposure to pathogens influence strongly the degree of antibiotics use. The better the husbandry and rearing conditions, the better the animal health status the less need to antibiotics use. High levels of internal and external biosecurity measures, (e.g. all in all out production systems, feed and water hygiene, cleaning and disinfection procedures), combined with changes in management practices (e.g. Lactation length, small groups of animals, stress decrease during critical periods for the piglet life like weaning, environmental enrichment and higher status of welfare) can decrease the need for antibiotics.

Moreover new specific and innovative solutions must be developed and promoted in order to replace antibiotics. For example the use of vaccines and auto vaccines can promote the general health status of pigs. Implementation of new feeding strategies, with the use of pre- and probiotics, minerals, acidifiers can act like a counterweight to antibiotics use. Research on gut immunology, gut microbiota can favor gut health and maturation especially in newborn, improving immunological competence and control of intestinal infectious disease. Animal genetics and breeding strategies can provide significant knowledge on genetic markers for disease resistance and development of animal with natural resistance in a variety of pathogens.

Of great importance are changes in human behaviors. It will be very difficult to achieve antibiotics reduction, and rational use in livestock production without the participation of veterinarians, agricultural consultants and famers. Moreover a broad One Health approach to the evidence-gathering surveillance, data analysis, intervention design and evaluation is proposed.

The use of antimicrobial agents selects for both antimicrobial-resistant bacteria and antimicrobial resistance determinants. Moreover antibiotics can influence and alter the intestinal microbiota.The gut microbiota is a vital component of a healthy animal. The gastrointestinal tract of the pig contains a large and diverse number of microorganisms. The swine intestinal microbiota is not homogeneous. The swine gut is composed by few bacterial phyla, like Bacteroidetes and Firmicutes [11]. At the genus level, Prevotella, Lactobacillus, Treponema, Roseburia, and Streptococcus are among the most dominant [13]. In the swine

ileum, the microbiota is dominated almost entirely by Firmicutes at the phylum level and by Anaerobacter, Turicibacter, and Escherichia at the genus level [15].

The age of the pigs is an important factor, as nursing piglets have a very different gut microbiota from post weaned pigs [11,16]. For example, the phylum Proteobacteria is more abundant prior to weaning [11,17]. The differences in the gut microbiota of pre- versus post-weaned piglets are driven largely by diet change from the easily digestible sow's milk to grain-based feed that occurs at weaning. The immune system maturation, over time, stabilizes the swine gut microbiota resulting in a more resistant to dietary alterations [11,14]. The texture of the feed and size [19], as well as the sanitary conditions of the housing environment, can also alter the gut microbiota [28].

The effect of antimicrobials on the emergence of antimicrobial-resistant bacterial strains has focused on pathogenic organisms. However, antimicrobials do not target only pathogenic bacteria. The commensal microbiota is also a target of the antimicrobials.

Janczyk et al. [20] observed a decrease in both diversity and richness in the ileal microbiota of 39-days old piglets that had been administered a single dose of amoxicillin intramuscularly at birth. Holman et al. [13] described that pigs that were fed tylosin-supplemented feed exhibited a rapid increase in tylosin-resistant fecal anaerobes. The concentration of the macrolide resistance gene erm(B) was similarly affected, with a 10-fold increase observed in tylosin supplemented pigs. Despite a progressive decrease in tylosin concentration in the feed and a 2-week withdrawal period prior to shipping, neither tylosin-resistant anaerobes nor the concentration of erm(B) was significantly affected. Even more, the total number of anaerobes was not affected by either antibiotic, only the resistant proportion was altered. Conversely, chlortetracycline, which was administered at a lower dosage, had no measurable effect on either chlortetracycline resistance in total anaerobes or

on the abundance of selected tet genes [12]. Rettedal [21] in piglets fed chlortetracycline for 2 weeks following weaning, noticed a significant change in the ileal microbiota largely associated with a decrease in Lactobacillus johnsonii and Turicibacter and with an increase in Lactobacillus amylovorus. Thymann et al. [22] using RFLP, described that weaned pigs treated with intramuscularly amoxicillin, and in feed ZnO administration, presented lower microbial diversity in the small intestine comparing with untreated pigs.

On the other hand Bosi et al. [23] found that amoxicillin, tilmicosin and doxycycline did not affect the jejunal microbiota of 3-week-old pigs. Total enterobacteriace and lactobacillace were also not affected by any of the three antibiotics. Kalmokoff et al. [24] reported no impact on the swine fecal microbiota in

commercial pigs fed either tylosin or virginiamycin over a 15-week period. Poole et al. [27] to investigate the effect of 50 mg of chlortetracycline included in feed, on the fecal microbiota of post weaned swine over a 28-day period. Authors recorded that chlortetracycline did not have any impact on bacterial diversity or on community structure diversity. Similarly, Kim et al. [14] documented no difference at the bacterial phylum or class levels in 10 pigs continuously fed either tylosin or a control, antibiotic-free diet, for 12 weeks starting at 10 weeks of age.

It is obvious, that although a significant number of studies have been conducted on the swine intestinal microbiota following antimicrobial administration, no clear pattern has emerged in terms of the alteration of specific bacterial genera or species. For example, the relative abundance of the lactic acid bacterial genera Lactobacillus and Streptococcus, as well as Coprococcus, has been reported to be both increased and reduced following in-feed antibiotic administration [25, 11, 15]. The same has been documented for the phylum Bacteroidetes [11, 15, 26].

Nonetheless, the resilience of the swine gut microbiota to long-term changes due to the administration of antimicrobials is apparent. Older pigs also appear to exhibit fewer changes in their gut microbiota compared with younger swine in response to in-feed antibiotics.

PiGutNet identifies these points as essential to design new tools for improving the health status of pigs by reducing the risk of gut dysbiosis and the spread of AMR genes, in the framework of One Health Approach.

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