Antimicrobial resistance in Danish pigs: A cross sectional study of the association between antimicrobial resistance and geography, exposure to antimicrobials, and trade

Antimicrobial resistance is a worldwide problem of paramount importance for both humans and animals. To combat the emergence of antimicrobial resistance, the problem must be targeted in all major reservoirs as it is assumed that a high level of AMR genes in environmental reservoirs can increase the risk of human pathogens becoming resistant. Pigs might constitute an important reservoir. Therefore, it is important to manage antimicrobial resistance in pigs. Before effectiveactions can be initiated, it is crucial to know which factors are associated with the levels of antimicrobial resistance in pigs. The Danish pig farm is an ideal place to study AMR, as the pigs are all kept together in a confined space and managed in a similar manner. Furthermore, movements of pigs between farms and the purchase of antimicrobials for use at the farm are registered in national databases, thus facilitating the collection of information on relevant factors. The aim of this PhD project was to study the relationship between the levels of antimicrobial resistance genes and geographical factors in Danish pig farms: the geographical location of the farm, the exposure to antimicrobials, and the trade patterns. Data collection was necessary in order to fulfil the aim of the project, and early in the project it became evident that a thorough consideration of how the data should be collected was needed. This resulted in three different projects, each contributing to the planning process. Throughout the entire PhD project, the focus was on seven antimicrobial resistance genes - ermB, ermF, sull, sullt, tet(M), tet(O), and tet(W). The levels of these genes were determined using a quantitative real-time polymerase chain reaction (qPCR). The erm genes confer resistance against macrolides, the sul genes confer resistance against sulfonamides, and the tet genes confer resistance against tetracyclines. It was necessary to determine the minimum number of individual faecal samples that should be pooled together to represent the average level of antimicrobial resistance genes in the farm. It was estimated that a sample pooled from individual samples from five pigs was optimal. The pooling method was optimised, and was found to have strong agreement with previously used pooling methods. Samples were collected from 687 Danish pig farms in February and March 2015. However, 6 farms did not fulfil the criteria for the target population and were excluded. The samples were collected at five abattoirs located on Zealand and in Jutland. The sample material was faeces, and it was collected by squeezing a small amount out of the rectum at the slaughter line. After collection the samples were sent to the laboratory. The samples were pooled into one aliquot per farm and DNA was extracted from the faecal material. The antimicrobial resistance gene levels were then measured using quantitative real-time PCR. As the samples were collected at the abattoir it was not possible to know in advance which farms were available for sampling. Consequently, it was not possible to estimate whether the sampledfarms were representative of the target and study populations prior to sampling. However, an evaluation of the representativeness of sampled farms (in terms of farm size and geographical location) was carried out post-sampling. It was found that the sampled farms were representative of the target population. Furthermore, there was a significant difference in farm size between the oversampled area in the northern part of Jutland and an undersampled area in the western part of Jutland and an oversampled area in the northern part of Jutland. A simulation study showed that the sampling procedure would inevitably result in a bias towards larger farms and with some non-randomness in the spatial distribution. One of the aims of this PhD project was to estimate the quantitative relationship between the antimicrobial resistance gene levels and antimicrobial exposure. Previous studies have indicated that antimicrobial exposure in early periods of a pig’s life can influence the antimicrobial resistance genes levels found later in life. In order to quantify the relationship, an estimate of the antimicrobial exposure throughout the entire lifetime of a slaughter pig was required. An algorithm to estimate the LEA was therefore developed. Previous studies have shown that livestock farms in close proximity share common pathogens. Furthermore, there have been indications that phenotypic antimicrobial resistance can be spatially clustered. Therefore, the spatial pattern of the antimicrobial resistance gene levels was assessed, and both areas with high levels and areas with low levels of some of the genes were found. However, it was concluded that the geographical location of the farm had only a minor effect on the antimicrobial resistance gene levels. Antimicrobial exposure is the most important risk factor for the development of antimicrobial resistance. However, previous studies of the relationship between antimicrobial resistance and antimicrobial exposure have focused on phenotypic antimicrobial resistance in a small number of farms. In this PhD project, the focus was on genotypic antimicrobial resistance. The quantitative relationship between the levels of seven antimicrobial resistance genes and the lifetime exposure of 11 different antimicrobial classes was estimated. It was found that some antimicrobial classes had a positive correlation with the levels of some antimicrobial resistance genes, but a negative correlation with other antimicrobial resistance genes. In conclusion, it was found that even though exposure to antimicrobials was associated with the antimicrobial resistance gene levels, it could only explain 10% - 42% of the variation in the gene levels. The microflora of a pig is established soon after birth. The source of the microflora is bacteria in the environment and from the sow. It has been shown that antimicrobial exposure of the sow will influence the antimicrobial resistance levels in the piglet. Therefore, the correlation between the levels of antimicrobial resistance genes in finisher farms and that of the sow farms with which they had trade connections was assessed. A significant correlation was found for most of the antimicrobial resistance genes with correlation coefficients ranging from 0.06 to 0.47. Of the three factors that were studied in this PhD project, only the antimicrobial exposure and the level of antimicrobial resistance in the sow farm with a trade connection were found to have a considerable effect on the antimicrobial resistance genes. However, it became apparent during the project that many other factors could affect the levels of antimicrobial resistance genes. This could lay the foundation for future studies.