



# The faecal resistome and microbiome of farmers and slaughterhouse workers: A metagenome-wide cross-sectional study

Liese Van Gompel<sup>1</sup> | Roosmarijn Luiken<sup>1</sup> | Rasmus Hansen<sup>2</sup> | Patrick Munk<sup>3</sup> | Martijn Bouwknecht<sup>4</sup> | Lourens Heres<sup>4</sup> | Gerdit Greve<sup>1</sup> | Peter Scherpenisse<sup>1</sup> | Betty Jongerius-Gortemaker<sup>1</sup> | Monique Tersteeg-Zijderfeld<sup>1</sup> | Silvia García-Cobos<sup>5</sup> | Wietske Dohmen<sup>1</sup> | Alejandro Dorado-García<sup>1</sup> | Jaap Wagenaar<sup>6,7</sup> | Bert Urlings<sup>4</sup> | Frank Aarestrup<sup>3</sup> | Dik Mevius<sup>6,7</sup> | Dick Heederik<sup>1</sup> | Heike Schmitt<sup>1,8</sup> | Alex Bossers<sup>1,7</sup> | Lidwien Smit<sup>1</sup>

1 Institute for Risk Assessment Sciences, Utrecht University, NL; 2 Intomics, DK; 3 National Food Institute, Danish Technical University, DK; 4 Vion Food Group, NL; 5 University Medical Centre Groningen, University of Groningen, NL; 6 Department of Infectious Diseases and Immunology, Utrecht University, NL; 7 Wageningen Bioveterinary Research, NL; 8 Centre for Infectious Disease Control, National Institute for Public Health and the Environment, NL

## Introduction

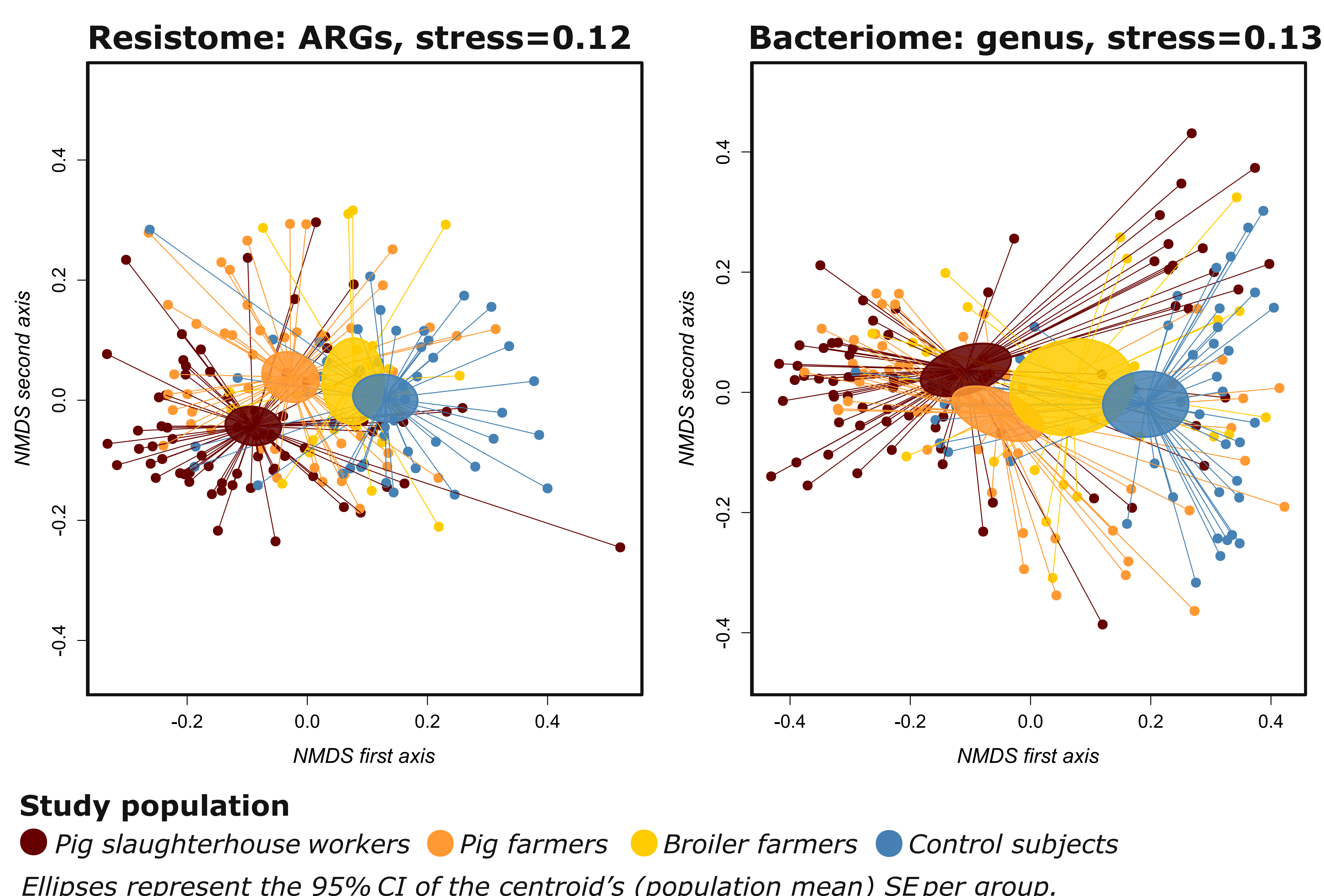
By studying the entire human faecal resistome and associated bacteriome, the diversity and abundance of faecal antimicrobial resistance genes (ARGs) can be comprehensively characterized. Prior culture-based studies have shown associations between occupational exposure to livestock and carriage of specific antimicrobial resistant bacteria. Using shotgun metagenomics, the present study investigated the abundance and diversity of 194 faecal resistomes and bacteriomes from humans occupationally exposed to ARGs in livestock and a control population in the Netherlands. In addition, we sought to identify determinants for the resistome and bacteriome composition of these populations.

## Results

Pig slaughterhouse workers and pig farmers had a higher total ARG abundance compared to broiler farmers and control subjects (Figure 1). Tetracycline,  $\beta$ -lactam and macrolide resistance gene clusters dominated the resistome of all studied groups. No significant resistome alpha diversity differences (species richness, Shannon diversity index) were found among the four populations. However, the resistome beta diversity showed a separation of the mean resistome composition of pig and pork exposed workers from broiler farmers and controls, independent of their antimicrobial use (Figure 2).

We demonstrated differences in resistome and bacteriome composition between slaughter line positions, pig versus broiler exposed workers, as well as differences between farmers and employees versus family members (Table 1, PERMANOVA). In addition, we found a significant correlation between the bacteriome and resistome (Procrustes analysis) of the studied populations. Furthermore, an in-depth analysis of the pig and pork exposed workers compared to the controls, revealed significantly higher levels of 30 ARGs in pig and pork exposed workers compared to the control subjects (DESeq2 analysis).

**Figure 2: The distributions of the resistome & bacteriome population means associated with pig & pork workers were distinct from those of (broiler farmers) & controls.**



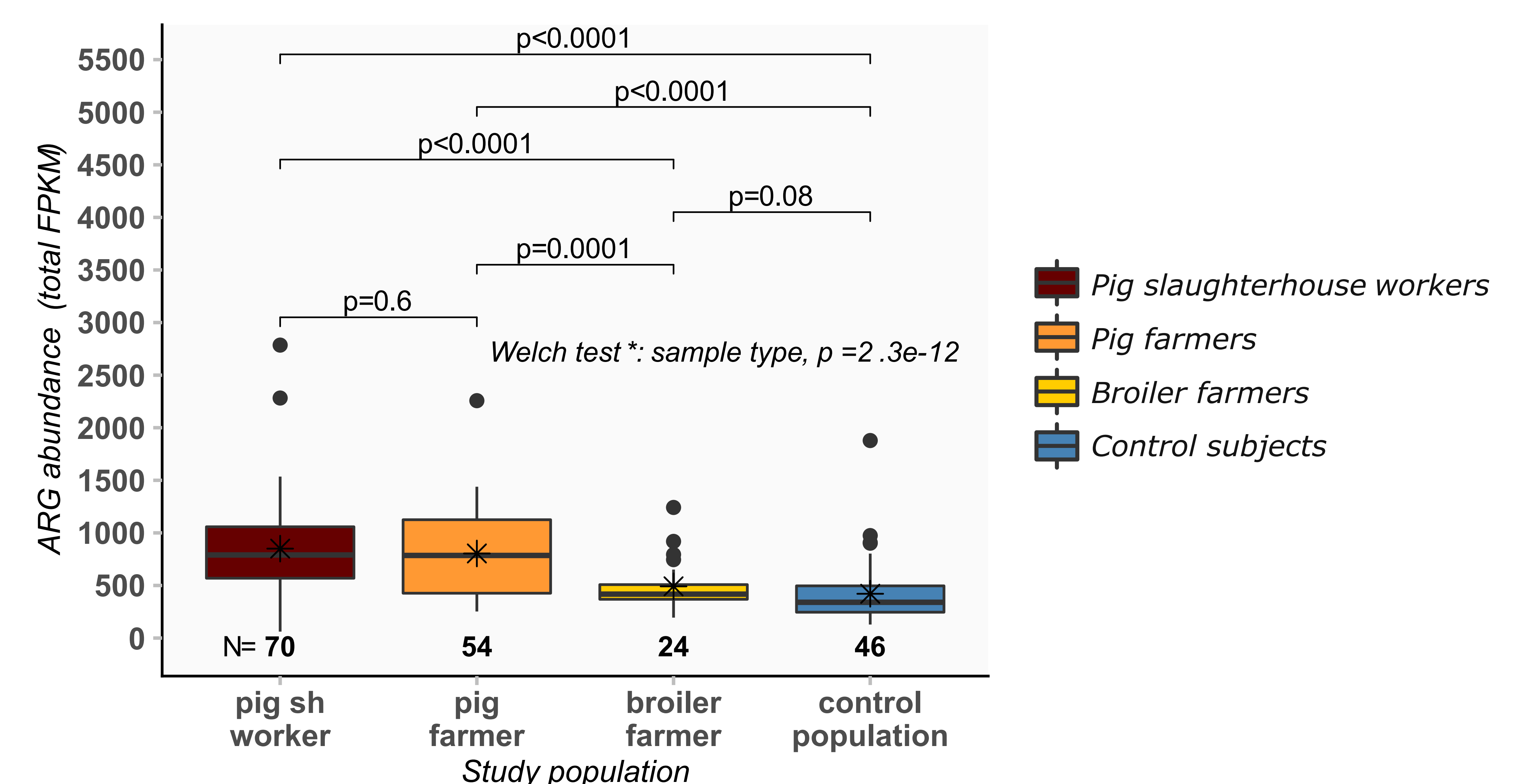
## Conclusion & Future plans

We found an increased ARG carriage in persons working in the Dutch pork production chain compared to broiler farmers and controls. Additionally, we showed significant differences in resistome and bacteriome composition of pig and pork exposed workers compared to a control group, as well as within-population (farms, slaughterhouse) compositional differences. Our results suggest direct or indirect livestock contact as a determinant for human faecal ARG carriage. Future work will focus on the integration of additional farm (animal, dust) and pig slaughterhouse (carcass) resistomes and bacteriomes into the models.

## Methods

Faecal samples and questionnaires (e.g. regarding personal characteristics, antibiotic use & animal contact) were collected from persons working or living on pig and broiler farms, working in a pig slaughterhouse (N=148) and from the general population (Lifelines cohort, N=46). Total faecal DNA was extracted and sequenced using shotgun metagenomics (Illumina HiSeq4000). ARGs (resistome) and bacterial genomes (bacteriome) were classified by mapping reads to the ResFinder and NCBI bacterial reference genome databases. Alpha-diversity indices and Bray-Curtis (BC) dissimilarities were calculated and multivariate analyses were performed (NMDS, PERMANOVA, SIMPER, DESeq2 analysis).

**Figure 1: Pig farmers & slaughterhouse workers carry significantly higher antimicrobial resistance gene (ARG) abundances in their faeces compared to broiler farmers & control subjects.**



**Table 1: Significant resistome & bacteriome compositional differences were identified between & within populations independent of antimicrobial use.**

Study population	Categories used in the PERMANOVA analysis	Resistome: Explained variation	Bacteriome: Explained variation
<b>Differences between populations</b>			
<b>Full population (4 groups)</b>	(1) Pig slaughterhouse workers, (2) Pig & (3) Broiler farms, (4) Controls	12.3% (p=0.001)	14.9% (p=0.001)
<b>Full population excl. low risk categories (low animal contact) (4 groups)</b>	(1) Pig slaughterhouse workers, excl. workers from deboning, (2) Pig & (3) Broiler farmers & employees, excl. family members, (4) Controls	19.1% (p=0.001)	26.1% (p=n.a.*)
<b>Differences within populations</b>			
<b>Abattoir workers (3 groups)</b>	Workers from: (1) Lairage to dehairing, (2) Evisceration to Cooling, (3) Deboning	7.3% (p=n.a.*)	5.4% (p=0.09)
<b>Farms (2 groups)</b>	(1) Pig farms, (2) Broiler farms	5.4% (p=0.004)	4.7% (p=0.008)
<b>Farms (2 groups)</b>	(1) Pig and broiler farmers & employees, (2) Pig and broiler farmer's family members	5.6% (p=0.001)	14.3% (p=n.a.*)

\*The assumption of homogeneity of variances was not met. However, a test at a higher resistome/bacteriome clustering level was found significant.