In silico Mapping of Microbial Communities and Stress Responses in a Porcine Slaughterhouse and Pork Products through its Production Chain

Guillermo Campos Calero[1], Natacha Caballero Gómez[1], Leyre Lavilla Lerma[1], Nabil Benomar[1], Charles W. Knapp[2], Hikmate Abriouel[1*]

[1]Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Universidad de Jaén, 23071-Jaén, Spain.
[2]Centre for Water, Environment, Sustainability & Public Health; Department of Civil and Environmental Engineering; University of Strathclyde, Glasgow, Scotland, United Kingdom.

[*]**Correspondence:** Dr. Hikmate Abriouel, Área de Microbiología. Departamento de Ciencias de la Salud. Facultad de Ciencias Experimentales. Edif. B3. Universidad de Jaén. Campus Las Lagunillas s/n. 23071-Jaén, Spain. Tel.: 34-953-212003; fax: 34-953-212943.
hikmate@ujaen.es

Abstract

The use of shotgun metagenomic sequencing to understand ecological-level spread of microbes and their genes has provided new insights for the prevention, surveillance and control of microbial contaminants in the slaughterhouse environment. Here, microbial samples were collected from products and surrounding areas though a porcine slaughter process; shotgun metagenomic DNA-sequencing of these samples revealed a high community diversity within the porcine slaughterhouse and pork products, in zones originating from animal arrival through to the sale zones. Bacteria were more prevalent in the first zones, such as arrival- and anesthesia-zones, and viruses were prevalent in the scorching-and-whip zone, animal products and sale zone. Data revealed the dominance of Firmicutes and Proteobacteria phyla followed by Actinobacteria, with a clear shift in the relative abundance of lactic acid bacteria (mainly *Lactobacillus* sp.) from early slaughtering steps to proteobacteria and then to viruses—suggesting site-specific community compositions occur in the slaughterhouse. *Porcine-type-C oncovirus* was the main virus found in slaughterhouse, which causes malignant diseases in animals and humans. As such, to guarantee food safety in a slaughterhouse, a better decipher of ecology and adaptation strategies of microbes becomes crucial. Analysis of functional genes further revealed high loads of diverse genes associated with stress, especially in early zones (animal and environmental surfaces of arrival zone with 57710 and 40806 genes, respectively); SOS responsive genes represented the most prevalent, possibly associated with genomic changes responsible of biofilm formation, stringent response, heat shock, antimicrobial production and antibiotic response. The presence of several antibiotic resistance genes suggests horizontal gene transfer, thus increasing the likelihood for resistance selection in human pathogens. These findings are of great concern, with the suggestion to focus control measures and establish good disinfection strategies to avoid gene spread and microbial contaminants (bacteria and virus) from the animal surface into the food chain and environment.

Keywords:
slaughterhouse; metagenomics; *in-silico* analysis; bacteria; virome; stress genes.

1. Introduction

Slaughterhouses represent major reservoirs of bacteria, fungi, yeasts, viruses, prions and parasites pathogenic to humans and animals (Bahnson et al., 2005; Heredia and García, 2018; Krog et al., 2019; Viegas et al., 2016), and special interest has been given to antimicrobial-resistant (AMR) pathogenic bacteria and their genes, which have been increasingly spreading through the food chain, creating medical and veterinary challenges (Verraes et al., 2013; Voss et al., 2005). Gene mobilization has evolved from the decades of selective pressures caused by antibiotics used as growth promoters, prophylactic and therapy agents and now transcend ecosystems (Davies and Davies, 2010). Among the genes being exchanged include those responsible for cellular defense, stress tolerance, organism survival and persistence (Maisonneuve and Gerdes, 2014; Norman et al., 2009). Thus, the emergence of new bacterial strains with enhanced resistome and robustness represent a greater threat in food safety and public health (Schmithausen et al., 2018). The higher genetic exchange promotes microbial and functional diversity, which becomes prevalent in food-processing environments. Unfortunately, microbial activity is an inherent feature of food preparations, and the environmental conditions and nutrient availability in a slaughterhouse shape the microbial diversity and thus the metabolic pathways, by which bacteria survive. The detection of their metabolites (primary and secondary) could become used as indicators to ensure food safety.

Metagenomic and bioinformatic tools can provide a greater sense of food safety (Abriouel et al., 2017; Andjelković et al., 2017)—providing deeper insights into the defense mechanisms used by bacteria to withstand stress (and increased resistance) in a slaughterhouse. Moreover, better information on microbial diversity and gene flow remains a critical aspect of food processing systems related to food safety and human health. Previous studies have reported the high diversities of resistomes and virulomes in swine farms and faeces (Munk et al., 2018; Noyes et al., 2017; Zhu et al., 2013). Campos Calero et al. (2018) recently reported high microbial loadings, and their associated high diversity and richness of antimicrobial resistance genes (ARGs) and virulence genes (VGs) throughout a pig slaughterhouse in Jaén (Spain)—both related to two main sources of contamination: bacterial contamination on animal surfaces and the evisceration process. As such, in this study, we compared metagenomic gene sequences associated with bacterial stress response, biofilm formation, and competitive defense (e.g., as a consequence of pathogen spread) in different process areas, animal surfaces, and meat products throughout a pig slaughterhouse in Jaén (Spain). It remains crucial to understand

and determine the occurring species, their abundances and their resistance/defense mechanisms with the aim to reduce their spread and the impact on the environment and health. Furthermore, microbiome mapping was also done with the aim to identify potential indicator organisms in each slaughterhouse processing area and/or meat product necessary for surveillance and control of microbial contaminants.

2. Materials and Methods

2.1. Samples

The animal/environmental samples used in the present study were collected from a local pig slaughterhouse (Jaén, Spain) representative of those in the region, described previously by Campos Calero et al. (2018). Selected zones were sampled as follows: "animal arrival" (MA, MS), anesthesia (AA, AS), "scorching and whip" (FA, FS), evisceration process (kidney R1 and R2; lean MGR1 and MGR2) and sale (EA, ES). ("xA" in sample nomenclature denote animal/meat origin; whereas, "xS" represent surfaces in the slaughterhouse).

2.2. Bioinformatic analyses

2.2.1. Metagenomic DNA and construction of metagenomic libraries

Genomic DNA were previously extracted and purified by Campo Calero et al. (2018) from the different animal and environmental samples used in this study. Construction of the metagenomic libraries was done using the Illumina Nextera XT DNA Library Prep Kit (Illumina, Inc., San Diego, CA, United States) according to the manufacturer's instructions. The resulting reads were assembled using SPAdes program version 3.13.0 (Bankevich et al., 2012). Assembly and annotation were done at Lifesequencing S.L. (Valencia, Spain). The mean coverage for the *k*-mer 77 with the best N50 and L50 values was evaluated using QUAST program version 5.0.0 (Gurevich et al., 2013).

2.2.2. Identification

2.2.3. Gene prediction and functional annotation

All contigs were used for coding-DNA-sequence (CDS) prediction via Prodigal program version 2.6.3 (Hyatt et al., 2010). The predicted CDS were annotated using Eggnot-mapper program version 2.0.0 (Huerta-Cepas et al., 2017) against several databases: Gene Ontology (GO), Clusters of Orthologous Groups of proteins (COG),

Kyoto Encyclopaedia of Genes and Genomes (KEGG) and KEGG BRITE. To identity the genes involved in stress pathways, the annotated sequences were screened for genes encoding for peptides induced during biofilm formation, heat shock, SOS response, stringent response, and production of antimicrobials and antibiotic resistance.

3. Results

3.1. Microbiome profile. Each process in the porcine slaughterhouse and pork products could be represented by distinct microbial community structures**,** based on16S-rRNA phylo-taxonomics. Most bacteria were characterised in the animal-arrival (MA and MS) and the anesthesia (AA and AS) zones with relative abundances of 74-97%; and viruses were found in the rest of area such as scorching-and-whip (FA and FS), evisceration process (kidney R1 and R2; lean MGR1 and MGR2) and sale (EA and ES) zones (Fig. 1A). However, archaea were only detected at very low proportions in the animal-arrival (MA and MS; 0.66%) and anesthesia (AA and AS; 0.26%) zones (Fig. 1A).

In terms of phyla, bacteria were represented mainly by *Firmicutes* ranging from 46-55% and 84-91% in the anesthesia (AA and AS) and the animal-arrival (MA and MS) zones, respectively (Fig. 1B); followed by *Proteobacteria* in the anesthesia (AA and AS), scorching and whip (FS) zones and also kidney (R1) product having relative abundances ranging from 16-21% (Fig. 1B). The remaining phyla were under-represented (0.006-7% of relative abundance), including: *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, *Spirochaetes*, *Deinococcus-Thermus* and *Tenericutes* (Fig. 1B). However, archaea were only represented by *Euryarchaeota* (0.26-0.66%) in the animal-arrival (MA and MS) and the anesthesia (AA and AS) zones (Fig. 1B).

Genus-level dentification further demonstrates greater diversity among the several zones (Fig. 1C and D). *Lactobacillus* was highly detected in the animal-arrival (MA and MS; 61-62%) and the anesthesia (AA and AS; 22-31%) zones (Fig. 1C); however, their proportions declined throughout the remaining processing zones, ranging from 0.01 to 0.4% (Fig. 1D). Among *Lactobacillus* species, *L. reuteri* (11-27%), *L. amylovorus* (12-23%), *L. johnsonii* (1.6-13%) and *L. ruminis* (0.2-4%) were more frequently detected in the animal-arrival (MA and MS) than the anesthesia (AA and AS) zones (Fig. 1E). Besides *Lactobacillus* spp., *Acinetobacter* spp. and *Aerococcus* spp. (15-17% relative abundance) were also present in the anesthesia zones (AA and AS; Fig. 1C)—mainly represented by *Acinetobacter* sp. and *Aerococcus viridans*, respectively (Fig. 1E). However, the low proportion of bacteria recovered from the rest of zones corresponded mainly to *Acinetobacter johnsonii* (9.7%)*, Brevundimonas diminuta* (3.86%) and

Sphingopyxis sp. (1.45%) in FS zone; *Pseudomonas* sp. (9.87%), *Pseudomonas fragi* (1.88%) and *Acinetobacter johnsonii* (1.78%) in R1 product; and *Lactobacillus* phage Sha1 (1.86%) in MGR2 product (Fig. 1F).

Virus, comprised only of *Gammaretrovirus* genus, represented a major group in scorching-and-whip (FA and FS), evisceration process (kidney R1 and R2; lean MGR1 and MGR2) and sale (EA and ES) zones (Fig. 1D), with *Porcine type-C oncovirus* being the most abundant species (74-99%) (Fig. 1F).

Regarding the archaea detected in the animal-arrival (MA and MS) and the anesthesia (AA and AS) zones, *Methanobrevibacter* spp. (0.26-0.65%) and *Methanosphaera* spp. (0-0.01%) were present (Fig. 1C), and corresponded mostly to *Methanobrevibacter* sp. and *Methanosphaera stadtmanae*, respectively (Fig. 1E).

3.2. Screening and quantification of defense-response stress genes

The functional screening of the complete libraries with regards to stress genes, encoding proteins involved in antimicrobial resistance and various defense strategies, was done by metagenome annotation analysis. The results revealed the presence of genes for antibiotic response, heat shock, SOS response, stringent response, biofilm formation and antimicrobial production. The relative abundances of genes involved in defense found in all metagenomes were most prevalent in samples from arrival zone (MA and MS) with 57710 and 40806 genes, respectively, followed by anesthesia zone AS (35433 genes) and AA (18762 genes) (Table 1). However, samples with lower relative abundance of genes were animal products, such as MGR1 (22 genes) and R2 (24 genes), and animal and environmental surfaces in the sale zones ES (34 genes) and EA (75 genes) (Table 1). In regards to specific genes involved in several responses, different abundance profiles of functional-gene clusters were detected in order as follows: SOS response > biofilm formation > stringent response > heat shock > antimicrobial production > antibiotic response (Fig. 2A, Table 1). The relationship between gene distribution and sample origin clearly indicated that SOS response genes were highly associated with animal surface in the arrival zone (MA), followed by environmental surface in arrival (MS) and anesthesia (AS) zones (Fig. 2B). Stringent-response genes were mainly represented in MA, MS and AS animal/environmental surfaces (Fig. 2B). Biofilm formation, heat shock and antimicrobial production genes were primarily detected in MA followed by MS and AS (Fig. 2B).

3.2.1. SOS response proteins in animal and environmental surfaces. Analysis of all metagenomes indicated a high prevalence of genes involved in SOS response, encompassing a total of eight groups of encoding genes (Supplementary Materials). DNA repair and recombination proteins and homologous recombination proteins were predominant in animal and environmental surfaces in the arrival and anesthesia zones (Table 2). Other gene groups coding for SOS response were those for DNA replication and repair protein RecF, DNA recombination/repair protein RecA, Repressor DNA-binding, DNA-damage-inducible protein D, enoyl-CoA hydratase and protein ImuA (Table 2). However, genes encoding DNA-damage-inducible protein 1 were absent in all metagenomes (Table 2). Furthermore, the relative abundance of genes coding for SOS response was lower in animal surface than in environmental surface, except in the arrival zone ("MS"; Fig. 3, Table 2). Their abundances declined during meat processing in the slaughterhouse especially in animal products and sale zone, with only DNA repair and recombination proteins, homologous recombination proteins and Repressor DNA-binding being detected (Fig. 3, Table 2).

3.2.2. Stringent response in animal and environmental surfaces. Nine groups of genes encoded for stringent responses (Supplementary Materials). Two-component regulatory systems were found in all metagenomes and were most prevalent in animal and environmental surfaces of arrival and anesthesia zones; other genes included (in decreasing prevalence) beta-galactosidase, RNA polymerase sigma-70 factor, ECF subfamily, RNA polymerase primary sigma factor and RELA proto-oncogene (Fig. 3, Table 3). However, FMN reductase (NADPH), staphopain B, guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase and stress response kinase A were least abundant and absent in most metagenome samples (Table 3). On the other hand, DNA-directed RNA polymerase subunit D was not detected in any metagenomes (Table 3). Similarly, as for SOS response, the relative gene abundances declined through the processes, and guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase being absent and in animal products and sale zone (Fig. 3, Table 3).

3.2.3. Heat shock response proteins in animal and environmental surfaces. Fifteen groups of genes encoding for heat shock proteins were found in all metagenomes, including: *rpoH, ko03020, POL5, groES, groEL, dnaK, K04043, dnaJ, K09526, K03283, ibpA, nnrD, clpB, hsp70, hsp60, hrcA, grpE, ctsR, yabO* and *hsp20*, among others (Supplementary Materials). Genes coding for RNA polymerase were the most prevalent

in animal and environmental surfaces of arrival and anesthesia zones followed by tRNA pseudouridine32 synthase / 23S rRNA pseudouridine746 synthase, molecular chaperone DnaK, ATP-dependent Clp protease ATP-binding subunit ClpB, chaperonin GroEL and DnaJ homolog subfamily B member 11 (Table 4). Other genes coding for ADP-dependent NAD(P)H-hydrate dehydratase, HSP20 family protein, molecular chaperone GrpE, heat-inducible transcriptional repressor, Class I heat-shock protein chaperonin GroES, transcriptional regulator of stress and heat shock response, RNA polymerase sigma-32 factor, molecular chaperone IbpA, and heat shock protein family A were less abundant (Fig. 3, Table 4). However, heat-shock 70kDa protein 1/2/6/8, DnaJ homolog subfamily C member 6 and DNA polymerase phi were not found in any metagenomes (Table 4). As mentioned for other stress responsive genes, animal and environmental surfaces of arrival and anesthesia zones were the most prevalent zones in terms of relative abundance (Fig. 3, Table 4).

3.2.4. Biofilm formation response in animal and environmental surfaces. Genes coding for biofilm formation were found in all metagenomes, and they were more prevalent on animal and environmental surfaces of the arrival, anesthesia, and scorching and whip zones (Table 5). The detected genes such as *accR, acfD, acm2, acpA, acsbg2, acsf, acta, adrA, adrB, aguD, algA, amiA, amiC, amiD, aml1, amsD, amsF, amyE, appA, appB, appC, appD, appF, apr, araB, araD, arbF*, among others (Supplementary Materials) clustered into four gene groups. These genes (ordered in terms of relative abundances) encode for quorum sensing, *Escherichia coli* biofilm formation, *Vibrio cholerae* biofilm formation and *Pseudomonas aeruginosa* biofilm formation (Fig. 3, Table 5). Furthermore, some animal products showed moderate relative abundances of genes such as MGR2 and R1; however, the other animal products and sale zone exhibited lower relative gene abundances (Fig. 3, Table 5).

3.2.5. Antibiotic response proteins in animal and environmental surfaces. A total of six gene groups were found; RNA polymerase nonessential primary-like sigma factors, holin-like protein LrgB, and non-homologous end-joining pathways were the most abundant antibiotic-responsive proteins, followed by protein Veg and *Methylococcus capsulatus* Bath (Fig. 3, Table 6). However, Membrane-associated protein TcaA was less abundant and DNA-directed DNA polymerase IV was absent in all metagenomes (Table 6). Among the detected genes, *rpoS, irgB, tcaA, veg, mca* and *pol4* were also found (Supplementary Materials).

3.2.6. Antimicrobial production response proteins in animal and environmental surfaces. Among the analyzed metagenomes, there were six antimicrobial-production encoding gene groups. Streptomycin biosynthesis was the most abundant (Table 7); other gene groups, ordered in terms of relative abundance, were as follows: biosynthesis of enediyne antibiotics > cephalosporin-C deacetylase > biosynthesis of vancomycin group antibiotics > penicillin amidase > isopenicillin-N N-acyltransferase like protein (Fig. 3, Table 7). Interestingly, animal products except MGR2 and R1 had all genes, and sale zone exhibited either few or no genes for antimicrobial-production proteins (Fig. 3, Table 7). Among the detected encoding genes, *cah* and *ial* were also detected (Supplementary Materials).

4. Discussion

Understanding the microbial ecology within a slaughterhouse environment remains crucial to provide new insights for the prevention and control of microbial contamination. Metagenomic approaches shed light on the microbial diversity and functional genes that cause serious consequences on food safety and quality; further they can help elucidate gene spread in the environment (Alves et al., 2018; Durso et al., 2011; Singh et al., 2018). In this study, metagenomic analyses revealed different microbial community structures in several slaughterhouse zones, from animal arrival to sales. Bacteria were more prevalent in the first zones such as arrival or anesthesia zones on both the animal and environmental surfaces. However, the relative abundances of viruses were higher in scorching-and-whip zone, animal products and sale zone.

Dominant bacterial communities in a porcine slaughterhouse on both from animal and environmental surfaces highly depend on the area from which samples were taken and are influenced by conditions in the processing plant and the hygienic measures applied. The dominance of Firmicutes and Proteobacteria phyla followed by Actinobacteria aligned with the data by Bridier et al. (2019), which previously reported the dominance of Proteobacteria followed by Bacteroidetes, Firmicutes and Actinobacteria in a pig slaughterhouse environment. Few metagenomic studies have been carried out on slaughterhouse environmental surfaces (Bridier et al., 2019; Campos Calero et al., 2018; Hall et al., 2013); nevertheless most focused on gut microbiomes (Crespo-Piazuelo et al., 2019; Kim et al., 2015; Quan et al., 2018). We hypothesise that the distribution of microbial populations on environmental surfaces can indicate contamination sources and microbial evolvement throughout processing.

During the first slaughtering steps, i.e., animal arrival and anesthesia zones, lactic acid bacteria (LAB) such as *Lactobacillus* sp. were the most dominant bacteria, represented by *L. reuteri*, *L. amylovorus*, *L. johnsonii* and *L. ruminis* followed by *Aerococcus viridans.* These LAB may have originated from many sources, as they are abundant in highly nutritive environments, including decomposing plant material, vegetables and also animal cavities (such as the mouth, genitals, and the intestinal and respiratory tracts); furthermore, their high resistance to environmental conditions promote their survival (Konig and Fröhlich, 2009). However, Gram-negative proteobacteria were represented by *Acinetobacter* sp., *Ac. johnsonii, Brevundimonas diminuta, Sphingopyxis* sp., *Pseudomonas* sp. and *P. fragi*, and they were recovered from anesthesia zone, scorching and whip zone, and animal products.

As reported previously by Campos Calero et al. (2018), culture-dependent methods showed high mesophilic loads such as pseudomonads, staphylococci, *E. coli* and LAB in all unit processes on both animal (and products) and process surfaces (MA, MS, FA, FS) with viability counts ranging from 3.5–7.71 $\log_{10}$ CFU/ml. However, the metagenomic analysis with total community DNA provides a better measure of the relative abundance of these bacteria in each sampling area than culture-based methods, as many microorganisms are not detected by these methods such as viruses. On the other hand, the results by Bridier et al. (2019) indicated that bacterial communities in the pig slaughterhouse were dominated by the Moraxellaceae family, particularly four genera: *Acinetobacter, Moraxella, Psychrobacter* and *Enhydrobacter*. As literature lacks metagenomic assessments of microbial diversity and population dynamics in pig slaughterhouse, the data presented here uniquely demonstrates a shift in the relative abundance of LAB in early steps of slaughtering, being superseded by proteobacteria and then viruses suggesting a process-specific composition within the slaughterhouse.

Concerning viruses, the data indicated that porcine endogenous retroviruses (PERVs), which exist in the genome of pigs, were present from the beginning of the porcine slaughterhouse and became most dominant in the scorching-and-whip area until the sale zone, and also animal products. The most prevalent PERVs were represented by *Gammaretroviruse* genus, which was spread from the animals once slaughtered. Retroviral genomes become integrated into the genome of infected germ cells and thus they are detected in organs and tissues, which can be used for xenotransplantation (Acharya et al., 2019). This genus included mainly *Porcine-type-C oncovirus* species and cause malignant disease in animals and humans as shown *in vitro* by Acharya et al. (2019); similarly Kim et al. (2016) reported that viral zoonosis occurred under particular

host conditions, such as immunosuppressive treatment and transplantation with host-adapted virus-producing cells, and thus transmission of PERV was created from different recipient cells *in vivo*. However, the *in-vivo* studies by Denner (2018) showed that PERV transmission has not been observed in any of the many preclinical and clinical xenotransplantation trials performed so far, and not in any of the many experimental PERV-infection experiments. As such, further consideration of *in-vivo* PERV transmission is required. The presence of porcine viromes in slaughterhouse, including animal and environmental surfaces, represents great relevance not only for clinical safety in xenotransplantation but also in food safety due to viral zoonosis transmission.

We must better decipher the ecology and adaptation strategies of microorganisms to guarantee food safety in a slaughterhouse. Elucidating the diversity and function of genes involved in stress response in animal and surface environments of a porcine slaughterhouse becomes crucial to understand community fate and resilience, and this is best accomplished via metagenomic analysis. The high microbial load in the first slaughterhouse zones such as animal-arrival and anesthesia was directly associated with highly prevalent and diverse repertoire of stress genes, including SOS-response genes being the most abundant. The significance of SOS response in bacterial fitness was largely reported in the context of a complex physiological environment suggesting that SOS response maybe involved in the subsequent stress responses such as antibiotic and other antimicrobial resistance (Samuels et al., 2019). In a slaughterhouse, changing environmental conditions, the density animals and the presence of competing microbes may induce and activate the SOS response; this thus suggests that bacteria become subjected to high levels of stress including DNA damage. Samuels et al. (2019) reported that SOS response played a vital role in colonization of the murine gut; the presence of commensal competing bacteria contributes to stress, and the SOS-response promotes effective pathogen colonization. Similarly, the first slaughterhouse zones are characterized by a high microbial loads and thus high colonization and high genomic changes as a result of SOS response may occur inducing other potential defense responses.

A network of eight gene groups involved in SOS response consisted mainly of DNA repair and recombination proteins, and homologous recombination proteins, which contribute to bacterial adaptation to external stressors, including antibiotic resistance, (Recacha et al., 2017) and bacterial pathogenesis and virulence (Li et al., 2010; Samuels et al., 2019). Furthermore, other stress responses such as biofilm formation, stringent response, heat shock, antimicrobial production and antibiotic response correlated with

SOS response; all had high relative abundances in the arrival and anesthesia zones. As such, either SOS-response induced subsequent stress responses, or *vice versa*; this is widely reported in the literature as part of the induction process for biofilm formation (e.g., Gotoh et al., 2010; Goneau et al., 2015), which is further mediated by four gene-groups coding for quorum sensing, *Escherichia coli* biofilm formation, *Vibrio cholerae* biofilm formation and *Pseudomonas aeruginosa* biofilm formation. These gene groupings were found mainly in arrival and anesthesia zones, followed by environmental scorching and whip zone and some animal products (R1 and MGR2).

Furthermore, genomic changes may occur due to the activation of error-prone DNA polymerases and antibiotic-resistance development, which are found in zones of the slaughterhouse. In this sense, there are the propensity of higher mutation rate and acquisition of new genes, which become of great concern since gene spread in food chain and in the environment are responsible of acute, difficult-to-cure infections (Beaber et el., 2004). SOS response activation can induce horizontal gene transfer; for example Crane et al. (2018) reports that zinc block of the SOS-response system also inhibited the horizontal transfer of antibiotic resistance genes in enteric bacteria. Similarly, stringent response may induce the SOS response, or *vice versa*; both responses represent main pathways leading to the generation of persister bacteria and multidrug-resistant bacteria (Lebeaux et al., 2014; Strugeon et al., 2016). In this respect, among the most abundant antibiotic response genes, the RNA polymerase nonessential primary-like sigma factor has been shown to mediate various cellular responses linked to stress conditions (Gruber & Gross, 2003), followed by holin-like protein LrgB and non-homologous end-joining. Moreover, heat-shock induced SOS response and biofilm formation (Roncarati & Scarlato, 2017), thus a complex network regulating stress genes has been established.

Regarding antimicrobial production, genes involved in streptomycin biosynthesis were the most abundant determinants despite the absence of Ab-producing *Streptomyces griseus*, which suggest horizontal gene transfer of these genes to other bacteria in this environment. Also, cephalosporin-, vancomycin- and penicillin-encoding genes were detected in the slaughterhouse zones, which raise great concern since they may represent origins of antibiotic resistance, thus increasing the likelihood for selection in human pathogens, especially if they are located on genetic mobile elements.

5. Conclusions

The diversity and richness of stress genes were shown especially in the early zones are of great concern and indicate that exhaustive control measures are required to reduce the

risk of pathogen spread and their genes throughout slaughterhouse and thus to pork products and environment. Within zones for the arrival of animals and anesthesia, SOS responsive genes were the most prevalent, and could be inducing genomic changes responsible of biofilm formation, stringent response, heat shock, antimicrobial production and antibiotic response. Throughout meat chain production, the high relative abundance of virome was mainly associated with *porcine-type-C oncovirus*, thus representing another public-health related risk. As such, focused disinfection measures in the first steps in slaughtering may reduce risks to food safety and consumer health by minimising gene spread and microbial contamination (bacterial and viral) from animal surfaces into the food chain and environment.

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Figure legend

Figure 1. Taxonomic composition of microbial communities on animal and environmental surfaces of a meat-production chain within a porcine slaughterhouse. Bar plots display the relative abundance of domain **(A)**, phylum **(B)**, genus **(C and D)** and species **(E and F)**. MA, animal surface from arrival zone; MS, environmental surface from arrival zone; AA, animal surface from anesthesia zone; AS, environmental surface from anesthesia; FA, animal surface from scorching and whip zone; FS, environmental surface from scorching and whip zone; samples from evisceration process (kidney R1 and R2; lean MGR1 and MGR2); EA, animal surface from sale zone; ES, environmental surface from sale zone.

Figure 2. Distribution and relative abundance of stress genes detected in metagenomic samples from different slaughterhouse zone/product surfaces [MA, animal surface from arrival zone; MS, environmental surface from arrival zone; AA, animal surface from anesthesia zone; AS, environmental surface from anesthesia; FA, animal surface from scorching and whip zone; FS, environmental surface from scorching and whip zone; samples from evisceration process (kidney R1 and R2; lean MGR1 and MGR2); EA, animal surface from sale zone; ES, environmental surface from sale zone]. **(A)** relative abundance of stress genes throughout meat chain production. **(B)** Distributions of stress genes and their abundances in metagenomic results from different slaughterhouse zone/product surfaces (visualized *via* Circos). The length of the bars

on the outer-ring represents the relative abundance of stress genes in each metagenome sample. Each stress gene was represented by a specific ribbon color, and the width of each ribbon demonstrates the abundance of each gene.

Figure 3. Distribution and relative abundance of specific stress genes detected within metagenomic samples of different slaughterhouse zone/product surfaces [MA, animal surface from arrival zone; MS, environmental surface from arrival zone; AA, animal surface from anesthesia zone; AS, environmental surface from anesthesia; FA, animal surface from scorching and whip zone; FS, environmental surface from scorching and whip zone; samples from evisceration process (kidney R1 and R2; lean MGR1 and MGR2); EA, animal surface from sale zone; ES, environmental surface from sale zone].