Resistome and virulome diversity of foodborne pathogens isolated from artisanal food production chain of animal origin in the Mediterranean region

Frédérique Pasquali,[1] Lucia Gambi,[1] Alessandra De Cesare,[2] Cecilia Crippa,[1] Vasco Cadavez,[3,4] Ursula Gonzales-Barron,[3,4] Antonio Valero,[5] Fouad Achemchem,[6] Alex Lucchi,[1] Antonio Parisi,[1,7] Gerardo Manfreda[1]

[1]Department of Agricultural and Food Sciences, Alma Mater Studiorum Università di Bologna, Italy; [2]Department of Veterinary Medical Sciences, Alma Mater Studiorum Università di Bologna, Italy; [3]Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; [4]Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; [5]Department of Food Science and Technology, UIC ENZOEM, Agrifood Campus of International Excellence (ceiA3), Universidad de Córdoba, Spain; [6]LASIME Laboratory, Agadir Superior School of Technology, Ibn Zohr University, Agadir, Morocco; [7]Experimental Zooprophylactic Institute of Puglia e della Basilicata, Bari, Italy

Abstract

The aim of the present study was to investigate the resistome and virulome diversity of 43 isolates of *Listeria monocytogenes*, *Salmonella enterica* and *S. aureus* collected from artisanal fermented meat and dairy products and their production environments in Portugal, Spain, Italy and Morocco. After DNA extraction, genomes were sequenced, and *de novo* assembled. Genetic relationships among genomes were investigated by SNP calling and in silico 7-loci MLST. Genomes of the same species belonged to different ST-types demonstrating the circulation of different clones in in the same artisanal production plant. One specific clone included genomes of *S.* Paratyphi B belonging to ST43 and repeatedly isolated for more than a year in an artisanal sausage production plant. No genomes but three (belonging to *Salmonella enterica*), were predicted as multiresistant to different antimicrobials classes. Regarding virulence, genomes of *L. monocytogenes* belonging to ST1, ST3 and ST489, as well as genomes of *S.enterica enterica* (ST43, ST33, ST314, ST3667, ST1818, ST198) and ST121 *S. aureus* were predicted as virulent and hypervirulent. The occurrence of virulent and hypervirulent *L. monocytogenes, Salmonella enterica* and *S. aureus* strains in artisanal fermented meat and dairy productions as well as in their finished products suggests the need for a specific focus on prevention and control measures able to reduce the risk of these biological hazards in artisanal food productions.

Introduction

Recent years have seen an increased consumer demand for artisanal foods and beverages. These products are generally obtained from small-scale local productions and are perceived as healthier and more genuine by consumers, increasing their attractiveness and popularity (Capozzi *et al*., 2020; Frizzo *et al*., 2020). At the same time, these small-scale productions are often less standardized than industrial ones with higher involvement of product handling by staff personnel and a challenging control of production parameters. Beside this, several small-scale industries do not have a standardized process and developed Hazard Analysis and Critical Control Points plan in place. In these conditions, the management and control of biological hazards might be particularly challenging (Ditlevsen *et al*., 2020; Tamang *et al*., 2020; Halagarda *et al* 2022).

According to EFSA and ECDC (2021a), the consumption of contaminated food in 2020 caused 3,086 cases of foodborne outbreaks, 20,017 cases of illness, 1675 hospitalizations and 34 deaths in 27 member states.

Pathogenic bacteria in foods of animal origin may exhibit antibiotic resistance patterns that can be transmitted through foods. This phenomenon is of great concern, especially since antibiotic-resistant pathogenic bacteria are increasingly found in foods, including those of animal origin (Gourama, 2020; Alsayeqh *et al*., 2021).

According to the World Health Organization, foodborne antibiotic-resistant microorganisms represent one of the top ten threats to public health and food safety (WHO, 2021). The main problem is the coexistence of virulence and antibiotic resistance genetic determinants, which can lead bacteria to survive the antimicrobial treatment and cause illness through ingestion of contaminated food (EFSA and ECDC, 2021b).

Correspondence: Lucia Gambi, Department of Agricultural and Food Sciences, Alma Mater Studiorum – University of Bologna, Via del Florio 2, 40064 Ozzano dell'Emilia (BO), Italy.
Tel.: +39.051.2097900.
E-mail: luciagambi@gmail.com

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Major foodborne pathogens of public health significance include *Listeria monocytogenes, Salmonella enterica* and *Staphylococcus aureus* (Abebe *et al*., 2020). *L. monocytogenes* is the causative agent of listeriosis. In 2020, 16 *L. monocytogenes* foodborne outbreaks associated to 120 cases 83 hospitalizations and 17 deaths were reported in seven EU countries (EFSA and ECDC, 2021a). The most common implicated food vehicles were 'fish and fish products', 'other or mixed meat and products thereof' and 'cheese'.

Salmonellosis in 2020 in Europe, was the second most commonly reported foodborne infection in humans (after campylobacteriosis and was associated to a high number of foodborne outbreaks. *Salmonella* was associated to 694 foodborne outbreaks, 3,686 cases of illness, 812 hospitalizations and 7 deaths. In Italy, *S.* Enteritidis was responsible for a single outbreak linked to cheese, and that caused 86 cases, eight hospitalizations and one death (EFSA and ECDC, 2021a). As for previous years, the two food vehicles most involved in strong-evidence foodborne salmonellosis outbreaks were 'eggs and egg products' and 'pig meat and products thereof'.

Forty-three food poisoning outbreaks caused by *S. aureus* toxins with 402 cases and 32 hospitalizations, were reported by six European states. Different kinds of cheese (i.e., soft cheese, raw milk cheese) seem to be one of the major contributors to foodborne outbreaks (EFSA and ECDC, 2021a). According to EFSA and ECDC report (2021a) *Salmonella and S. aureus* toxins were also identified in two outbreaks associated with the consumption of contaminated dairy products.

As of today, studies related to antimicrobial resistance (AMR) in food animals have largely focused on commercial-scale production systems and less on artisanal and traditional (small-scale) production (Garham *et al*., 2017).

Within the H2020 PRIMA European funded project ARTISANEFOOD (http://www.ipb.pt/artisanefood/), partners from Portugal, Spain, Italy, and Morocco identified local, artisanal fermented food products of dairy and meat origins. In order to gain a snapshot of the sanitary and hygienic status of these productions, samples were collected from raw materials, intermediate and finished products as well as the environment (Pasquali *et al*., 2022). Since few data on antimicrobial resistance and virulence properties of isolates from artisanal productions are available (Graham *et al*., 2017) forty-three isolates collected in the ARTISANEFOOD project belonging to *L. monocytogenes*, *Salmonella enterica* and *S. aureus* were investigated for their resistome and virulome, in order to elucidate their diversity and potential dissemination within small-scale production industries.

Materials and methods

Sampling

Isolates were collected as previously described (Pasquali *et al*., 2022). Briefly, the sampling took place in four different Mediterranean countries: Italy, Portugal, Spain and Morocco. In each country local meat and dairy artisanal productions were selected. For each production samples of raw materials, semi-finished and finished fermented products, as well as from food contact surfaces were sampled along with the production environment. In each artisanal production, two to five replicates of each of 12 to 15 samples were collected in four to six batches from March 2019 to December 2021 for a total of approx. 2,800 samples. ISO standard methods as well as PCR and biochemical tests were applied for the isolation and identification of *L. monocytogenes*, *Salmonella enterica* and *S. aureus* as previously described (Pasquali *et al*., 2022). Retrieved bacterial pathogens are reported in Table 1.

Sequencing and bioinformatic analyses

DNA was extracted using MagAttract HMW DNA Kit (Quiagen, Hilden, Germany). Libraries were built using the Nextera DNA sample Prep Kit (Illumina, Milan, Italy). Whole genome was sequenced by Illumina MiSeq platform (Milan, Italy). Reads of 250 bp on average, were submitted to RefSeq Masher Matches v. 0.1.2 for species confirmation (https://github.com/phac-nml/refseq_masher). Reads that didn't match the previous identifications were removed from the following analysis. *De novo* assemblies were built by Unicycler v. 0.5.0, which includes Spades v. 3.14.0 (https://github.com/rrwick/Unicycler), and quality checked by QUAST v. 5.2.0 (https://github.com/ablab/quast). The phylogenetic analysis of the genomes was performed by *in silico* MLST v. 2.22.0 (https://github.com/tseemann/mlst) and SNPpy v. 4.6.0 (https://github.com/tseemann/snippy). Based on the core SNP alignment, a high-resolution phylogeny tree was built including the conserved nucleotide variant sites shared by all genomes. Genomes ArFCLM01, ArFASE04 and NCBI Nucleotide Accession ASM1342v1 were used as *L. monocytogenes*, *Salmonella enterica*, *S. aureus* as reference genomes respectively. In order to identify true phylogenetically informative SNPs, the reference genome should be closely related to the genome of the studied isolates (Besser *et al*., 2018). For this reason, genomes ArFCLM01, ArFASE04 were selected as belonging to the most represented ST-Types among *L. monocytogenes* and *S. enterica* newly sequenced genomes of the present study. This approach was not applicable to *S. aureus* genomes, due to the high diversity of ST-types, country and food origins. Therefore, public genome ASM1342v1 was chosen as reference for *S. aureus* newly sequenced genomes. PhyML v. 3.1 was used to analyze the SNP differences between isolates based on maximum likelihood algorithm and phylogenetic trees were visualized with iTOL v. 6 (Letunic and Bork, 2021). A pairwise SNP distance matrix was generated using snp-dist v. 0.6.3 (https://github.com/tseemann/snp-dists). Analyses of the resistome and virulome of all genomes were performed using ABRicate v1.0.1 (https://github.com/tseemann/abricate). Sequencing data are available at NCBI Database under BioProject Accession number PRJNA876122.

Results and Discussion

The genetic relationships as well as the resistome and virulome were analyzed in 43 isolates belonging to *L. monocytogenes, S. enterica*, and *S. aureus* collected in fermented meat and dairy based artisanal foods produced in the Mediterranean area.

Listeria monocytogenes

Ten isolates of *L. monocytogenes* belonged to Spanish Iberian raw-cured sausage ("salchichón") production (raw meat, environment and final product) and four samples collected from Italian salami production environment (Table 1).

Statistics of *de novo* assemblies showed the quality of sequenced genomes with number of contigs ranging from 25 to 32, N50 ranging from 262311 to 533374, and largest contig from 505969 to 1222483.

According to the phylogenetic tree, *L. monocytogenes* genomes clustered according to the country of origin and ST-Type (Figure 1A). The fourteen genomes were gathered in 5 clades: clade 1 included four Spanish ST8 genomes (between 599 and 739 SNPs difference); clade 2 included only one Spanish ST451 genome. Clade 3 gathered Spanish ST1 genomes (between 65 and 73 SNPs difference) and clade 4 Spanish ST3 genomes (5 SNPs difference). Clade 5 included the Italian's ST489 genomes (between 51 and 79 SNPs difference).

Based on the phylogenetic tree clustering and the SNP distance values, data suggest that different clones of *L. monocytogenes* were circulating in the Spanish raw-cured sausage plant from July to October 2020. On the contrary, in the Italian salami plant, a single clone of *L. monocytogenes* was observed specifically isolated from water drainage swab samples in September 2020. The same Italian clone was not observed in following samples, neither in the environment nor in raw materials and finished products. Regarding ST-types, ST1 and ST8 are frequently identified in clinical settings, suggesting the concern for the

Table 1. Bacterial pathogens sequenced in this study.

Sample code	Sample	Country	Isolation matrix*	Isolation date	Species	ST-type	Antimicrobial resistance associated genes
ArFCLM01	LM1	Spain	sausage – FP	20/07/2020	*L.monocytogenes*	ST3	*fosX*
ArFCLM02	LM2	Spain	sausage – FP	20/07/2020	*L.monocytogenes*	ST3	*fosX*
ArFCLM03	LM3	Spain	sausage -RM	15/09/2020	*L.monocytogenes*	ST1	*fosX*
ArFCLM04	LM5	Spain	sausage-E	15/09/2020	*L.monocytogenes*	ST8	*fosX*
ArFCLM05	LM6	Spain	sausage-E	30/09/2020	*L.monocytogenes*	ST451	*fosX*
ArFCLM06	LM7	Spain	sausage - RM	20/10/2020	*L.monocytogenes*	ST1	*fosX*
ArFCLM07	LM8	Spain	sausage – FP	20/10/2020	*L.monocytogenes*	ST8	*fosX*
ArFCLM08	LM9	Spain	sausage – FP	20/10/2020	*L.monocytogenes*	ST1	*fosX*
ArFCLM09	LM13	Spain	sausage – E	20/10/2020	*L.monocytogenes*	ST8	*fosX*
ArFCLM10	LM14	Spain	sausage – E	20/10/2020	*L.monocytogenes*	ST8	*fosX*
ArFFLM01	2SWD2A	Italy	salami – E	29/09/2020	*L.monocytogenes*	ST489	*fosX*
ArFFLM02	2SWD2B	Italy	salami – E	29/09/2020	*L.monocytogenes*	ST489	*fosX*
ArFFLM03	2SWD5A	Italy	salami - E	29/09/2020	*L.monocytogenes*	ST489	*fosX*
ArFFLM04	2SWD5B	Italy	salami - E	29/09/2020	*L.monocytogenes*	ST489	*fosX*
ArFASE02	S1-1A	Portugal	sausage – FP	28/01/2021	S. Paratyphi B	ST43	*aac(6')-Iaa*
ArFASE03	S1-1B	Portugal	sausage- E	10/11/2019	S. Paratyphi B	ST43	*aac(6')-Iaa*
ArFASE04	S1-2A	Portugal	sausage – FP	28/01/2021	*S.* Paratyphi B	ST43	*aac(6')-Iaa*
ArFASE05	S2-1A	Portugal	sausage – E	10/11/2019	*S.* Paratyphi B	ST43	*aac(6')-Iaa*
ArFASE06	S2-2C	Portugal	sausage – E	10/11/2019	*S.* Paratyphi B	ST43	*aac(6')-Iaa*
ArFASE07	S2-3B	Portugal	sausage – E	10/11/2019	*S.* Paratyphi B	ST43	*aac(6')-Iaa*
ArFASE11	S4-3C	Portugal	sausage – E	08/12/2019	*S.* Paratyphi B	ST43	*aac(6')-Iaa*
ArFMSE01	SALM1	Morocco	sausage – FP	22/10/2019	*S.* Hadar	ST33	*aac(6')-Iaa, aph(3'')-Ib, aph(6)-Id, blaTEM-1B, dfrA12, sul1, tet(A)*
ArFMSE02	SALM10	Morocco	sausage – FP	14/01/2020	*S.* Kentucky	ST314	*aac(6')-Iaa*
ArFMSE03	SALM2	Morocco	sausage – FP	22/10/2019	*S.* Montevideo	ST3667	*aac(6')-Iaa*
ArFMSE04	SALM24	Morocco	sausage – FP	13/10/2020	*S.* Hadar	ST33	*aac(6')-Iaa, aph(3'')-Ib, aph(6)-Id, blaTEM-1B, dfrA12, sul1, tet(A)*
ArFMSE05	SALM25	Morocco	sausage – FP	13/10/2020	*S.* Albany	ST1818	*aac(6')-Iaa*
ArFMSE06	SALM3	Morocco	sausage – FP	22/10/2019	*S.* Seftemberg	ST198	*aac(6')-Iaa, aadA2, aph(3'')-Ib, aph(6)-Id, blaTEM-1B, dfrA12, sul1, tet(A)*
ArFCSA01	SA18	Spain	sausage – RM	29/06/2020	*S. aureus*	ST15	*blaZ, tet(K)*
ArFCSA02	SA19	Spain	sausage - RM	29/06/2020	*S. aureus*	ST15	*blaZ, tet(K)*
ArFCSA03	SA20	Spain	sausage - RM	29/06/2020	*S. aureus*	-	*blaZ, tet(K)*
ArFCSA04	SA25	Spain	sausage-FP	15/09/2020	*S. aureus*	ST7	-
ArFCSA05	SA33	Spain	sausage-FP	20/10/2020	*S. aureus*	ST7	-
ArFCSA06	SA34	Spain	sausage-FP	20/10/2020	*S. aureus*	ST7	-
ArFCSA07	SA48	Spain	sausage - RM	31/08/2020	*S. aureus*	ST8	-
ArFCSA08	SA49	Spain	sausage - RM	31/08/2020	*S. aureus*	ST8	-
ArFDSA01	SA101	Spain	cheese - FP	22/09/2020	*S. aureus*	ST121	*blaZ, str*
ArFDSA03	SA103	Spain	cheese - FP	22/09/2020	*S. aureus*	ST121	*blaZ, str*
ArFDSA04	SA105	Spain	cheese – RM	18/02/2020	*S. aureus*	ST398	*blaZ, tet(M)*
ArFESA01	L2 CP1582	Italy	cheese – FP	05/06/2020	*S. aureus*	-	-
ArFESA03	L6 CP11285	Italy	cheese – FP	15/03/2021	*S. aureus*	ST8	*blaZ*
ArFESA04	L5 CP122	Italy	cheese – FP	29/01/2021	*S. aureus*	-	-
ArFFSA03	6SBR4	Italy	salami - SFP	09/12/2020	*S. aureus*	ST5	*blaZ*
ArFMSA01	ST.AU.3	Morocco	sausage - FP	22/10/2019	*S. aureus*	ST15	*blaZ, tet(K)*

FP: finished product, SFP: semifinished product, RM: raw material (minced meat or milk), E: production environment.

potential transfer of these isolates to humans through food consumption.

Regarding antimicrobial resistance prediction, few AMR associated genes were detected. Particularly, all genomes carried exclusively the *fosX* gene associated to fosfomycin resistance (Table 1).

Regarding virulence prediction, all fourteen *L. monocytogenes* genomes but one (ArFCLM05, ST451) showed a full length *inlA* gene (Figure 2A). Listeria Pathogenic Islands 1 (LIPI 1) was detected in all genomes, however, ST1 genomes lacked *actA* gene generally located in LIPI 1 along with *prfA, actA, hly, mpl, plcA, plcB*, and *iap* genes. In addition, ST1, ST3 and ST489 genomes included LIPI 3 (*llsA, llsG, llsH, llsX, llsB, llsY, llsD, llsP*), which has been associated, together with LIPI 1, to increased virulence and specifically to invasiveness (*llsX* gene of LIPI 3) (Vilchis-Rangel *et al*., 2019).

Salmonella enterica

Seven *Salmonella enterica* isolates were collected from Portuguese alheira sausage production (final product and environment), and in six Moroccan merguez sausages (Table 1). Portuguese isolates belonged to *S. enterica subsp. enterica* serovar Paratyphi B and Moroccan isolates to *S. enterica subsp. enterica* serovars Hadar (2 isolates), Kentucky, Montevideo, Albany and Senftemberg.

Statistics of *de novo* assemblies showed quality of sequenced genomes with number of contigs ranging from 23 to 56, N50 ranging from 231038 to 614135, and largest contig from 521807 to 1308384. Based on SNP calling, a phylogenetic tree of all *S. enterica* genomes was inferred (Figure 1B). Genomes clustered according to the serovar and ST-Type. The thirteen genomes were gathered in 7 clades: clade 1 including the seven Portuguese *S.* Paratyphi B ST43 genomes (between 0 and 1 SNPs difference), clade 2 with the two Moroccan *S.* Hadar genomes of ST33 (411 SNPs difference), clades 3, 4, 5 and 6 each including one Moroccan isolate: *S.* Kentucky (ST314), *S.* Montevideo (ST3667), *S.* Albany (ST1818), *S.* Senftemberg (ST198), rispectively. Based on the phylogenetic tree clustering, data suggest that one clone of *S.* Paratyphi B was circulating among the Portuguese sausage from November 2019 to January 2021 with SNP differences from 0 to 1 (Figure 1B). On the contrary, in the Moroccan sausage plant, different *Salmonella* serovars were observed. Regarding ST-types, ST43 of *S.* Paratyphi B of the Portuguese genomes has been already described in human infections in Europe and South America (Castellanos *et al*., 2020). *S.* Hadar ST33 has been already described in animals and food of animal origin (Carrol *et al*., 2021).

Regarding the antimicrobial resistance prediction all *Salmonella* isolates carried the *aac(6')-Iaa* gene conferring aminoglycoside resistance (Table 1). Both genomes of *S.* Hadar additionally carried *aph(3")-Ib*, *aph(6)-Id* (both associated to aminoglycoside resistance) and *tet*(A) (tetracycline resistance) (Table 1). Along with *S.* Hadar, *S.* Senftemberg showed a multiresistant predicted profile with additional genes *aadA2* (aminoglycoside resistance), *blaTEM1-B* (beta-lactam resistance), *dfrA12* (trimethoprim resistance), *sul1* (sulphonamide resistance) and *tet*(A) (Table 1).

Regarding virulence prediction, *Salmonella* genomes carried from 100 to 107 virulence genes (Figure 2B). Genes of virulence plasmid pSV were not detected. However, virulence genes of *Salmonella* pathogenicity islands SP1 (*orgABC, prgHIJK, sipABCD, sicAP, spaOPQRS, invABCEFGHIJ),* *SP2 (ssaGHIJKLMNOPQRSTUV, sscAB,*

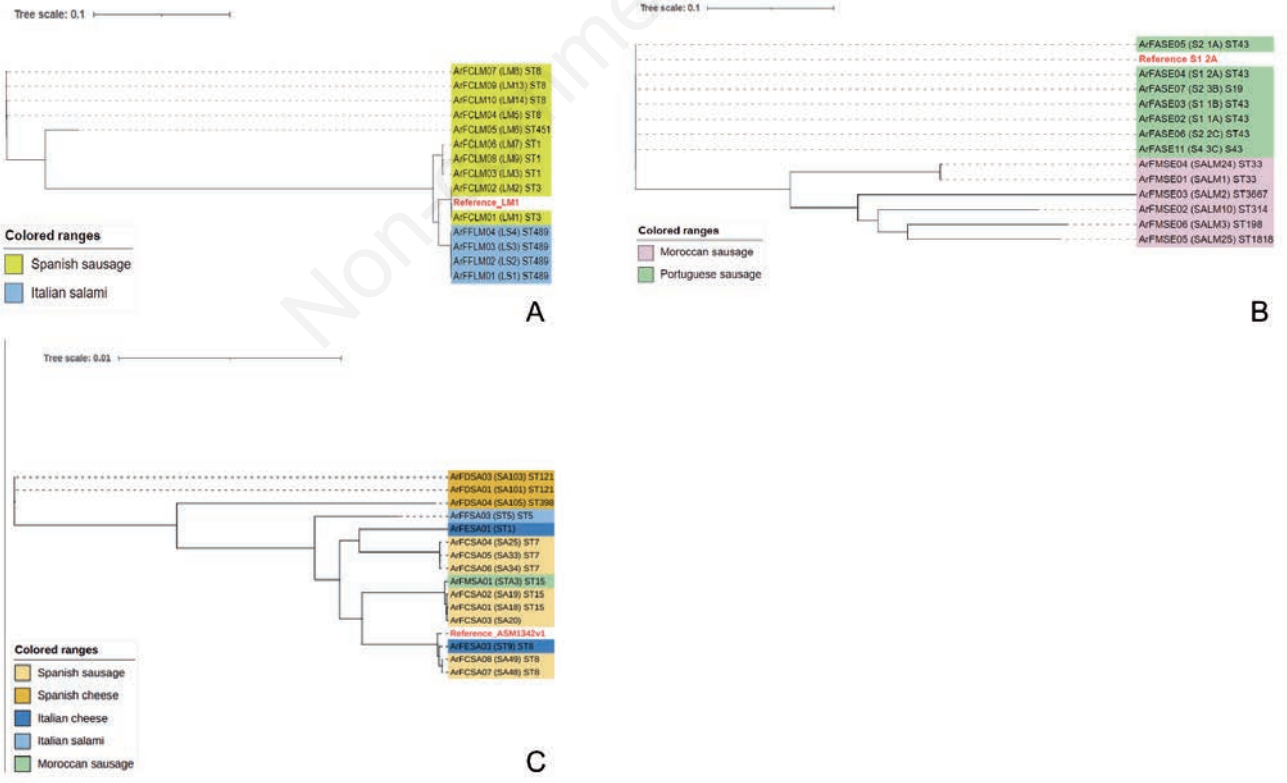

Figure 1. Core SNPs-based phylogenetic trees of A) *L. monocytogenes,* B) *Salmonella spp*. and C) *S. aureus* genomes.

sseABCDEFGIJK1K2L, ssaCDE,) and SP3 (*misL, mgtBC*) were detected along with *sopE2* gene described in the literature as virulence marker of UK and Italian *S.* Typhimurium monophasic variant clades (Marcus *et al*., 2000; Palma *et al*., 2018). *S.* Paratyphi B was the serovar with the highest number of virulence genes (107) and harbored *grvA, ratB, shdA, sod C1, sseI/srfH* genes not found in genomes of other serovars. Among these genes, *grvA* and *sodC1* have been described as part of Gifsy-2 phage contributing to the virulence of *S.* Typhimurium (Ho *et al*., 2001).

Staphylococcus aureus

A total of fifteen *S. aureus* isolates were subjected to genomic analysis: three from Spanish "*salchichón*", eight from Spanish cheese, three from Italian cheese, one from Italian salami and one from Moroccan merguez sausage (Table 1).

Statistics of *de novo* assemblies showed the quality of sequenced genomes with number of contigs ranging from 15 to 39, N50 ranging from 230321 to 815868, and largest contig from 372180 to 1017875. Based on SNP calling, a phylogenetic tree of all *S. aureus* genomes was inferred. As for *L. monocytogenes* and *Salmonella enterica*, also *S. aureus* genomes clustered according to ST-Type and country (Figure 1C). The fifteen genomes were gathered in 7 clades. Clade 1 included the two *S. aureus* ST121 genomes originating from Spanish cheese and collected the same day in September 2020 (40 SNPs of difference). Clades 2, 3 and 4 gathered only one genome each belonging to ST398 (Spanish cheese), ST5 (Italian salami) and ST1 (Italian soft cheese) respectively. Clade 5 included three *S. aureus* ST7 strains collected from Spanish sausage in the time frame of one month from September to October 2020 (between 10 to 37 SNPs difference). Clade 6 gathered three strains belonging to ST15 and one *S. aureus* strain to which the ST-Type was not attributed. In this clade one strain (ArFMSA01) originated from Moroccan merguez sausage, whereas the other three (ArFCSA01, ArFCSA02, ArFCSA03) were all from Spanish meat used for sausage production collected on the same day in September 2020 (28 to 36 SNPs of difference). Finally, clade 7 gathered three ST8 *S. aureus*, one from Italian soft cheese and two from Spanish raw meat for "*salchichón*" production. The two strains from Spanish meat (ArFCSA07, ArFCSA08) were isolated the same day in August 2020 and showed only one SNP of difference. Based on the phylogenetic tree clustering and the SNP distance matrix, data suggest that different clones of *S. aureus* were co-existing among Spanish sausage production as well as Italian soft cheese (Figure 1C). Regarding ST-types, ST121 is a *S. aureus* globally disseminated hypervirulent clone (Rao *et al*., 2015). ST5 and ST8 have been associated to hospital acquired Methicillin Resistant *S. aureus* (HA-MRSA) and ST398 was found both in humans and pig/pork meat (Deurenberg *et al*., 2007; van Belkum *et al*., 2008). ST5, ST8, ST15, ST121 have also been described in humans, food and wildlife (Lv *et al*., 2021; Heaton *et al*., 2020; Ghebremedhin *et al*., 2009).

Regarding antimicrobial resistance prediction in *S. aureus*, isolates ArFDSA01, ArFDSA03, ArFDSA04, ArFCSA01, ArFCSA02, ArFCSA03, ArFFSA03, ArFESA03, ArFMSA01 carried *blaZ* gene associated to beta-lactamase production and beta-lactam resistance (Table 1). Although generally located close to *blaZ* gene, no *mec* genes were identified predicting all *S. aureus* strains as methicillin susceptible (Hiramatsu *et al*., 2013). ArFDSA01 and ArFDSA03 additionally carried *str* gene associated to streptomycin resistance.

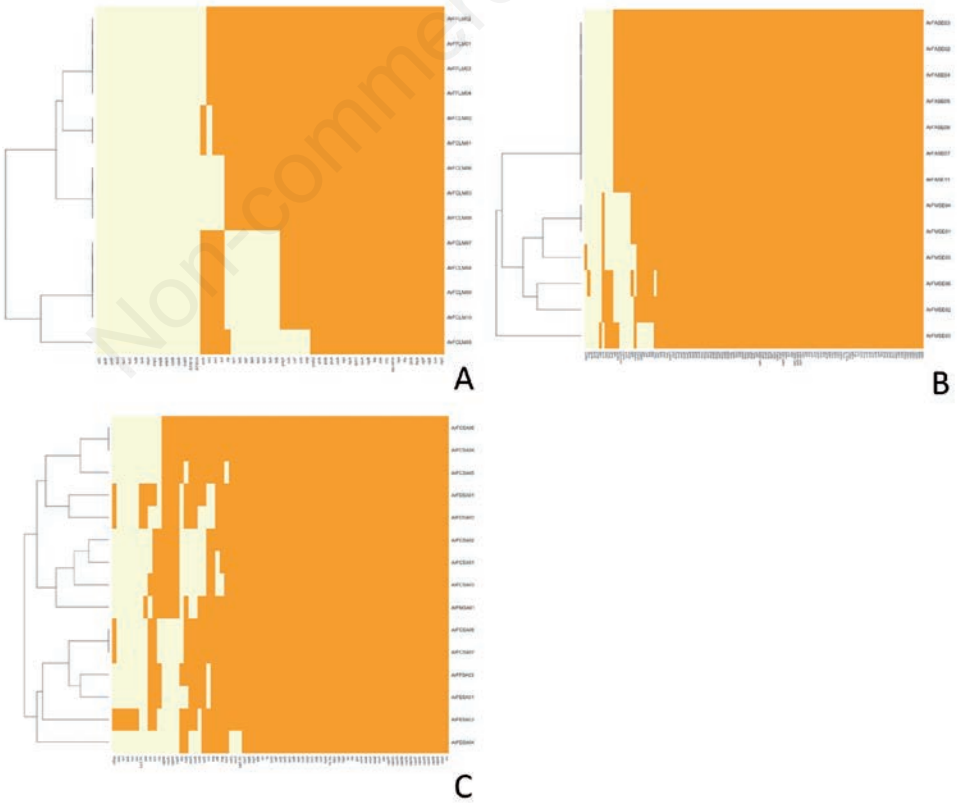

Figure 2. Heatmaps of virulome of A) *L. monocytogenes*, B) *Salmonella spp.*, C) *S. aureus* genomes (yellow: absence (<80% of sequence identity), orange: presence (>80% of sequence identity).

ArFDSA04 additionally carried *tet(M)* and ArFCSA01, ArFCSA02, ArFCSA03 and ArFMSA01additionally carried *tet(K)* gene both associated to tetracycline resistance. No resistance associated genes were detected in genomes ArFCSA04, ArFCSA05, ArFCSA06, ArFCSA07, ArFCSA08, ArFESA01. None of the isolates was predicted as multiresistant.

Regarding virulence prediction, all fifteen *S. aureus* genomes carried from 54 to 67 virulence genes (Figure 2C). ST121 genomes, described as associated to hypervirulent strains, carried the characteristic *lukS–lukF* genes coding for proteins LukS–PV and LukF–PV responsible of the assembly of PVL a bicomponent pore-forming cytotoxin closely related to the development of *S. aureus* infection (Hu *et al*., 2015). The other thirteen genomes but one (ArFDSA04) carried the *lukF* gene but not the *lukS* gene. Additionally, haemolysin related genes were found in all genomes (*hlb, hld, hlgA,hlgBx, hlgC*). Enterotoxin related gene *seb* was found exclusively in ST121 (ArFDSA01, ArFDSA03) and one ST15 (ArFMSA01) genome (Rao *et al*., 2015).

Conclusions

L.monocytogenes, Salmonella enterica and *S. aureus* were isolated from meat-based and dairy artisanal food productions of the Mediterranean area. These foodborne pathogens might persistently circulate among the plants and contaminate the final product. Whole genome sequencing based analyses were effective in building a high-resolution phylogeny among the genomes as well as a full characterization of their resistome and virulome. Regarding antimicrobial resistance, most isolates were predicted as resistant to β-lactams or aminoglycosides. Only three isolates (belonging to *Salmonella enterica*) were predicted as mutiresistant to antimicrobials of different classes. Regarding virulence, isolates of *L. monocytogenes* (belonging to ST1, ST3 and ST489), as well as isolates of *Salmonella enterica* (ST43, ST33, ST314, ST3667, ST1818, ST198) and *S. aureus* (ST121) were predicted as virulent and hypervirulent suggesting the need of specific attention on control measures able to reduce the risk of these biological hazards in artisanal food productions.

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