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Occurrence and genomic characterization of antimicrobial-resistant and potential pathogenic *Escherichia coli* from Italian artisanal food productions of animal origin

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Abstract
Escherichia coli can harbor a broad repertoire of virulence and antimicrobial resistance (AMR) genes, which can be exchanged across the human gastrointestinal microflora, thus posing a public health risk. In this study, six batches of artisanal soft cheese and a 6-month ripened fermented dried sausage were investigated to assess the occurrence, phylogeny, and genomic traits (AMR, virulence, and mobilome) of *E. coli*. Thirty and three strains isolated from salami and cheese food chains, respectively, were confirmed as *E. coli* by whole genome sequencing. The accumulation of single nucleotide polymorphism differences within small clusters of strains encompassing batches or processing stages, combined with high serotype and phylogroup diversity, suggested the occurrence of different contamination phenomena among the facilities. A total of eight isolates harbored plasmid-mediated resistance genes, including one cheese strain that carried an IncQ1 plasmid carrying AMR determinants to macrolides [*mph*(B)], sulfonamides (*sul1*, *sul2*), trimethoprim *(dfrA1),* and aminoglycosides [*aph*(3")-Ib and *aph*(6)-Id]. A pool of virulence-associated genes in the class of adhesion, colonization, iron uptake, and toxins, putative *ColV*-positive iron uptake systems *sit*, *iro,* or *iuc* (eight salami and two cheese), plasmid-encoded hemolysin operon *hly*ABCD (one salami), and potential atypical enteropathogenic *E. coli* (three salami environment) were reported. Overall, our findings underscore the importance of routine surveillance of *E. coli* in the artisanal food chain to prevent the dissemination of AMR and virulence.

Introduction
Escherichia coli represents one of the most investigated gram-negative bacteria that typically inhabit the human and warm-blooded animal gastrointestinal microflora (Jiang *et al*., 2014). Nonetheless, this species also encompasses opportunistic pathogens implicated in a variety of intestinal and extra-intestinal illnesses. These traits are linked to the acquisition of particular virulence features associated with an enhanced ability for niche adaptation as well as a wide range of diseases. Virulence traits are often carried by genetic elements, including plasmids, transposons, and pathogenicity islands that can be mobilized to other strains, generating unique and novel virulence patterns (Kaper *et al*., 2004). Pathogenic *E. coli* can colonize different environments, such as soil and water, generally as a consequence of fecal contamination (McAuley *et al*. 2014). Moreover, specific pathotypes, above all Shiga toxin-producing *E. coli* (STEC), have also been implicated in many foodborne outbreaks, which continue to be a major cause of human illness worldwide. On the farm-to-fork continuum, environmental, animal, or human sources can all contribute to bacterial contamination at any stage (Yang *et al*., 2017). Owing to the pathogen's persistence, many factors such as the use of contaminated raw materials, inadequate hygienic conditions during food handling, and post-processing cross-contamination from equipment and the environment may take place (Jiang *et al*., 2014; Yang *et al.,* 2016). Besides pathogenic strains, commensal *E. coli* may represent a significant reservoir of antimicrobial resistance (AMR) in the food chain (Kaspar, 2006). The AMR burden is expanding in *E. coli*, which ranks third among the list of the 12 antibiotic-resistant 'priority pathogens' indicated by the WHO (Denamur *et al*., 2021). Moreover, its capacity to acquire multidrug resistance genes makes it a useful bioindicator for AMR tracking (Ramos *et al*., 2020). Accordingly, the presence of multidrug-resistant (MDR) strains carrying many plasmid-encoded AMR genes suggests a potential public health risk due to the dissemination of antimicrobial-resistant isolates and resistance genes through the food chain. For instance, it has previously been observed that ready-to-eat foods, such as meat-based preparations, may have the potential to harbor *E. coli* and promote the transfer of AMR genes across the human gastrointestinal microbiota (Lima *et al*., 2017). To our knowledge, few studies have investigated the occurrence of AMR and virulence features in *E. coli* populations isolated from dairy and meat-based artisanal food productions in Italy, which represent an intrinsic branch of local food culture and heritage. This study was set out to explore the occurrence, phylogenetic linkage, AMR and virulence profiles of *E. coli* isolates from Italian cheese and salami artisanal productions.

Materials and Methods

Sampling overview

The dairy and pork meat-based artisanal products tested were represented by a soft cheese made of pasteurized cow milk, added by salt, calf rennet, and starter cultures, and a 6-month ripened fermented dried sausage, commonly named "salami", from an Italian swine autochthonous breed with no addition of starter cultures or nitrites/nitrates. Sampling was performed as previously described (Pasquali *et al*., 2022; Pasquali *et al*., 2023). Briefly, 6 batches of each production were sampled at the 2 artisanal facilities, located in Northern Italy, between January 2020 and May 2021, by collecting raw materials (cheese: raw and pasteurized milk, calf rennet; salami: minced meat mixture), intermediate products (cheese: cheese stored within warm and maturation room; salami: salami stored for 1 week inside drying area as well as for 3, 10 and 18 weeks inside ripening area), final products (cheese: cheese packed on day 0 of production along with stored cheese at day 4, 8, 11 and 15 at 2°C, 8°C and 2°C for 5 days followed by 8°C for 10 days; salami: 28 weeks ripened salami), and environmental swabs from processing areas (cheese: wall, manhole and gloves of workers inside warm, maturation and packaging area; salami: wall, manhole, processing surfaces and filler stuffer machine inside stuffing, drying and ripening area). 5 replicates were collected for each sample unit per batch, resulting in a total of 1170 tested samples (750 and 420 in the cheese and salami food chains, respectively).

Escherichia coli isolation, identification, and whole genome sequencing

E. coli isolation from cheese and salami samples was performed by enriching one aliquot of 25 g of each food sample unit (raw materials, intermediate and final products) or environmental swabs in buffered peptone water (Thermo Scientific™,Milan, Italy) at 37°C overnight. Following, a 10-µL loopful of the overnight-enriched culture was streaked onto MacConkey agar (Thermo Scientific™, Milan, Italy) and incubated at 37°C for 24 hours. A total of 5 colonies displaying the typical *E. coli* morphology, such as a pink color surrounded by a dark pink area of precipitated bile salts, dry and round-shaped, were selected from each plate, harvested, purified, and submitted to species-level identification by biochemical test (RapID™ ONE System, Thermo Scientific™, Milan, Italy). Strains identified as *E. coli* were then submitted to DNA extraction with the Chelex method (Kimura *et al*., 1999) for molecular identification by polymerase chain reaction (PCR) (Clermont *et al*., 2000). The DNA of the strains confirmed as *E. coli*, by using both the biochemical and PCR methods, was extracted using the MagAttract HMW DNA Kit (Qiagen, Hilden, Germany) and then submitted to library preparation using the Nextera XT DNA Library Preparation Kit (Illumina, Milan, Italy). The whole genome sequencing was in paired end (250 bp) using the Illumina MiSeq platform (Milan, Italy).

Bioinformatics workflow for strains' molecular typing

Raw reads were *de novo* assembled using Unicycler v0.5.0 (https://github.com/rrwick/Unicycler), and the quality of the assembled contigs was evaluated with contig_info v2.01 (https://gitlab.pasteur.fr/GIPhy/contig_info). ReferenceSeeker v1.8.0 (https://github.com/oschwengers/referenceseeker) was used to confirm strains' taxonomy by matching genomic sequences with closely related reference genomes hosted in the RefSeq pre-built database (https://www.ncbi.nlm.nih.gov/refseq). *E. coli* acknowledged O:H serotypes and phylotypes were respectively assessed with SeroTypeFinder v2.0.1 (https://cge.food.dtu.dk/services/SerotypeFinder/) and ezclermont v0.7.0 (https://github.com/nickp60/EzClermont). Contigs were screened for antimicrobial resistance and virulence genes by ABRicate v1.0.1 (https://github.com/tseemann/abricate) using the Resfinder and Ecoli_VF databases. The location of both antimicrobial resistance and virulence genes was predicted by plasmid reconstruction and typing using MOB-recon (https://github.com/phac-nml/mob-suite). Phylogenetic inference was calculated by a single nucleotide polymorphism (SNP)-calling approach using the Snippy v. 4.6.0 tool (https://github.com/tseemann/snippy). Reads were mapped against the

reference genome *E. coli* O157:H7 str. Sakai (NCBI RefSeq assembly no. GCF_000008865.2) to identify nucleotide variants, and an alignment of core SNPs has been further produced with snippy-core. The latter was used to build a maximum likelihood phylogenetic tree by PhyML v. 3.1, which has been visualized with iTOL (https://itol.embl.de/). Finally, a pairwise SNP distance matrix was generated using snp-dist v. 0.6.3 (https://github.com/tseemann/snp-dists). The paired-end reads included in this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB71359 (https://www.ebi.ac.uk/ena/browser/view/PRJEB71359).

Antibiotic susceptibility assay

Confirmed *E. coli* strains have been submitted to the standard broth microdilution phenotypic assay for minimum inhibitory concentration (MIC) evaluation (ISO, 2019). The Sensititre™ EUVSEC ready-to-use plates (Thermo Scientific, Milan, Italy) have been used to assign MIC values for 14 antibiotics (sulfamethoxazole, trimethoprim, ciprofloxacin, tetracycline, meropenem, azithromycin, nalidixic acid, cefotaxime, chloramphenicol, tigecycline, ceftazidime, colistin, ampicillin, and gentamicin). Antimicrobial sensitivity or resistance patterns' have been defined following the European Committee on Antimicrobial Susceptibility Testing clinical breakpoints for all antimicrobials except tetracycline, azithromycin, and nalidixic acid, for which the Clinical and Laboratory Standard Institute breakpoints have been chosen.

Results

A total of 34 *E. coli* strains were identified by biochemical and PCR methods over the 1170 tested samples, resulting in an occurrence rate of 3% (*Supplementary Table 1*). The highest proportion of *E. coli* isolates was detected in the salami production (31 out of 34; 91%) because *E. coli* was detected in all the batches. Except for batch 1, *E. coli* isolation started with raw material (meat mixture) and persisted for all batches until the final product (SBR28). Moreover, positive food samples (raw material, semi-finished, and finished products) from batches 2, 3, and 4 were collected at all sampling sites. Besides the high recovery from food matrices, 6 strains were identified from the environment in all batches except the first and second. Interestingly, environmental strains were mainly isolated on the surfaces of the table and filler stuffer machine within the stuffing room (processing of the meat mixture) in batches where the meat mixture was also tested as positive (batches 3, 4, 5, and 6), showing the meat and table surface cross-contamination. On the other hand, a few strains (n=3) were isolated from cheese, 2 from batch 3 (semi-processed cheese stored in the warm room and last day of storage at refrigeration temperature), and one from the finished product of batch 6. None of the isolated *E. coli* colonized the cheese processing environment. After whole genome sequencing (WGS), all strain species identities were corroborated except for one salami strain isolated in ripening week 3 in batch 4, which was classified as *Enterobacter hormaechei*. Excluding this strain, the 33 confirmed *E. coli* were characterized by good assembly quality, with n° of contigs ranging from 78 to 394, genome size from 4.7 to 5.2 Mb, GC% from 50 to 51%, and N50 from 91064 to 553490 (*Supplementary Table 1*).

Phylogenetic linkages showed that strains harboring the same phylogroups (A, B1, B2, C, D, E, F, and G) clustered nearby on the core-SNPs tree (Figure 1) and gathered together in high-distance clades. Among them, several smaller clusters encompassing 2 to 4 isolates were displayed according to serotype prediction, carrying lower genetic distance (from 526 to 2271 SNP variants) and reflecting a high serotype diversity. In fact, a total of 15 different O and H types were designated over the 33 *E. coli* strains, resulting in 19 O:H combinations. For 6 strains only H types were indicated since conventional serotype information was not available for O antigens (Figure 1). Concerning genetic relationships based on SNPs, *E. coli* strains isolated from the same salami batch resulted in being genetically distant, with few exceptions for small clusters among batch 1 (1SBD5, 1SBR3 and 1SBR181 from 784 to 903 SNPs), batch 3 (3MB1 and 3SBD4 with 608 SNPs), batch 6 (6SWM3 and 6SBR282 with 660 SNPs) and batch 2 (2MB1 and 2SBR4 with 526 SNPs). The latter showed the

smallest genetic distance harbored across the entire dataset, suggesting the absence of an *E. coli* clonal population colonizing the salami artisanal facility. The absence of a persistent clone throughout processing stages and environments was confirmed by the wide SNP distance between the initial and following manufacturing processes and the final products (batch 1>66 kSNPs, batch 2>23 kSNPs, batch 3>71 kSNPs, batch 4>48 kSNPs, and batch 5>85 kSNPs). Similarly, high genetic distance was observed within the 3 *E. coli* genomes of cheese production (from 560 to 50971 SNP differences).

A total of 20 antimicrobial resistance genes (ARGs) have been found (Figure 1), carrying resistances to seven different antibiotic classes, such as aminoglycosides [*aac*(3)-Via, *ant*(3")-Ia, *aph*(3")-Ib, *aph*(6)-Id], β-lactams (*bla*CFE-1, *bla*CMY-50, *bla*CMY-101, *bla*CMY-113, *bla*CMY-155, *bla*TEM-1B), trimethoprim (*dfr*A1, *dfr*A5), macrolides [*mph*(B)], quinolones (*qnr*B19), sulfonamides (*sul*1, *sul*2) and tetracyclines [*tet*(A), *tet*(B), *tet*(C)]. All genomes carried β-lactamases, among which *bla*CFE-1 was the most represented (27 out of 33 strains). Moreover, the multidrug resistance (MDR) gene *mdf*(A) was also commonly identified in all genomes. The other identified AMR genes were carried by a total of 8 strains: 3 from cheese and 5 from salami. The highest resistance rate was reported by the 6CP4 genome (cheese final product, batch 6), carrying ARGs associated with all seven antibiotic classes. Mobilome analyses (*Supplementary Table 2*) highlighted that 9 of these genes were distributed between 3 distinct plasmids, among which *mph*(B), *sul*1, *sul*2, *dfr*A1, *aph*(3")-Ib and *aph*(6)-Id were all carried by a conjugative IncQ1 plasmid. High resistances were also observed for the remaining cheese strains (batch 2), which harbored genes encoding resistances for all classes except macrolide and quinolone. Moving to salami, the most resistant strains resulted in 2SBR183 (18 weeks of ripening, batch 2) and 6STM2 (table of the mixture processing area, batch 6), associated with 4 antimicrobial classes, followed by 3SM1 (filles stuffer machine inside the mixture processing area, batch 3), which reported 3 classes of genotypic resistance. Except for *mdf*(A), β-lactamases *bla*CFE and *bla*CMY, *dfr*A5, and the 3SM1 *tet*(B) gene, the residual AMR pattern appeared as horizontally acquired, harbored by IncQ1, IncFIA, IncFIB, or IncFIC plasmids (*Supplementary Table 2*). Besides resistome profiles, the antibiotic susceptibility assay highlighted a few AMR phenotypes to 7 antibiotics (Figure 1) and exhibited an overall positive correlation with the presence of ARGs, concerning tetracycline [n=8, *tet*(A), *tet*(B) or *tet*(C)], sulfamethoxazole (n=5; *sul*1 and/or *sul*2), ampicillin (n=5; *bla*TEM-1B), trimethoprim (n=3, *dfr*A1 or *dfr*A5), ciprofloxacin and nalidixic acid (n=1, *qnr*B19). The only discordance between genotype and phenotype was observed for gentamycin. In particular, among the 4 resistant strains, only 2 carried the corresponding *aac*(3)-Via gene. Further analyses should be performed to investigate the molecular basis of this phenotypic resistance.

The virulence profiling assessed 142-247 genes among the 33 strains and encompassed different classes, including the type II, III, V, and VI secretion systems (T2SS, T3SS, T5SS, and T6SS), adhesion-invasion or colonization factors (*e.g.*, fimbriae and flagella), as well as iron uptake systems (*e.g.*, enterobactin, aerobactin, yersiniabactin, and salmochelin) (Figure 2). All genomes carried adhesion and invasion (*csg* and *ibe*), colonization (fimbriae *fim*, flagella *flg* and *fli*), and iron uptake systems (enterobactin *ent* and *fep*) virulence genetic traits. A high reporting rate was also observed for hemolysin E (*hly*E, 91%), serum resistance complements protein (*trat* 53%), Stg (*stg*ABCD, 48%), and ycbQRST fimbriae (91%). Moreover, relevant abundances were predicted for many iron uptake system operons, including salmochelin (*iro*BCDEN, 36%), aerobactin (*iuc*ABCD and *iut*A, 36%), ferrous iron/manganese transport system (*sit*ABCD, 36%), and yersiniabactin (*ybt*AEPQSTUX, 24%).

Although none of *E. coli* genomes reported shiga toxins 1 (stx1) and 2 (stx2) virulence factors typical of STEC pathotypes, 5 salami strains harbored one STEC virulence markers, encoding for α-hemolysin *hly*A (2SBD5 and 6MB5) or intimin *eae* (4STM3, 5STM5, and 6STM2). These latter 3 *eae* positive strains, isolated from salami environmental production (table surface of the stuffing room), were positive for all genes (*eae*, *tir*, *espA*, *espD*, *espB*) of the pathogenicity island Locus of Enterocyte Effacement (LEE) where *eae* is located (Hernandes *et al*., 2009). Moreover, they were negative for shiga-toxin producing associated genes *stx1* and *stx2* as well as for the *ehxA* gene

(plasmid-mediated biomarker of enterohemorrhagic *E. coli* that codes for hemolysin) and the *btp* gene [plasmid-mediated biomarker of typical enteropathogenic *E. coli* (EPEC) coding for bundle forming pili]. This genetic profile suggests these 3 strains belong to atypical EPEC. Although typical EPEC has been associated with diarrheal symptoms in children, the pathogenicity of atypical EPEC is controversial since these bacteria were isolated both from healthy and diseased people (Afset *et al*., 2006; Slinger *et al*., 2017;). However, 2 biomarkers were described as associated with a-EPEC pathogenicity: the *nleB* gene, coding for a protein involved in biofilm formation, and the *efa1/lifA* gene, coding for the lymphocyte inhibitor factor A. Both genes are located in modules 2 and 3, respectively, of the O island OI-122 (Afset *et al*., 2006; Bugarel *et al*., 2011; Slinger *et al*., 2017). The 3 atypical-EPEC strains harbored module 2 of OI-122 exclusively and were positive for the *nleB* gene but not for the *efa1/lifA* gene.
Unlike AMR prediction, the highest virulence repertoire was linked to salami production, led by the 6MB5 strain (raw material, batch 6) with 247 virulence genes. Mobilome typing revealed a total of 20 strains carrying plasmid-encoded virulence traits (*Supplementary Table 2*). Virulence plasmids were spread in both cheese (n=2) and salami (n=9) isolates and acted as vehicles of iron uptake systems, such as *sit* and/or salmochelin *iro* and aerobactin *iuc* (n=9 salami and 2 cheese), T2SS *etp* gene (N=2 salami), or *hly*ABCD hemolysin operon (n=1 salami).

Discussion

The present study aimed to assess the occurrence and reconstruct the phylogeny of *E. coli* populations colonizing different batches of 2 Italian artisanal food facilities (cheese and salami) and to predict the burden of AMR and virulence genes among the isolated strains.
Phenotypic and PCR analyses identified a total of 34 strains disseminated among the tested facilities, showing a predominance from the salami artisanal chain compared to cheese (31 in comparison to only 3 isolates). *E. coli* was isolated in all 6 salami batches, and for most of them, *E. coli* was isolated throughout the processing stages, from raw material to final product. Moreover, 6 strains have been isolated from environmental sites, especially in the processing area of the meat mixture.
Although fermented dry sausages are commonly designated as microbiologically stable ready-to-eat meat products because of their low water activity and pH, foodborne pathogens, including *E. coli* STEC, have been demonstrated to survive production processes and colonize these products through contaminated raw meat, additives, processing equipment, or as a result of post-processing contamination (Dalzini *et al*., 2015). Besides outbreaks of *E. coli* O157:H7 linked to pork meat salami reported worldwide (MacDonald *et al*., 2004; Conedera *et al*., 2007), commensal *E. coli* were also identified in Italian pork meat salami (Rega *et al*., 2021) and fermented pork meat products (Rega *et al*., 2023). Additionally, *E. coli* has also been assessed in cheese made from pasteurized milk (Trmčić *et al*., 2016).
WGS of isolated strains allowed us to check and confirm the species identity by excluding from the dataset one misidentified strain as *E. hormaechei*, supporting WGS as the best practice for facilitating more precise species identification (Besser *et al*., 2018). The accumulation of high SNP differences within small clusters of strains encompassing the same batches or processing stages, in agreement with the high serotype and phylogroup diversity, suggests the occurrence of different contamination phenomena in the facility. Since the porcine reservoir is commonly known to be a source of *E. coli* (Reid *et al*., 2017), livestock farming may have been a possible source of contamination. However, we were not able to trace back to the farm origin to verify this statement. However, we cannot rule out the possibility of additional sources, such as the workers involved in product manufacturing.
Given the increasing worldwide public health concern represented by antimicrobial-resistant *E. coli* (Dhawde *et al*., 2018), we sought to identify AMR genes, along with their localization and phenotypes among isolated strains. Other than intrinsic resistances to β-lactamases, with *bla*CFE-1 as predominant and MDR *mdf*(A) gene, 8 strains additionally carried β-lactamases (*bla*TEM-1B), aminoglycoside [*aac*(3)-Via, *ant*(3")-Ia, *aph*(3")-Ib, *aph*(6)-Id], trimethoprim (*dfr*A1), macrolide [*mph*(B)], quinolone (*qnr*B19), sulfonamide (*sul*1, *sul*2) and/or tetracycline [*tet*(A), *tet*(B), *tet*(C)]

acquired genes, encoded by different conjugative or mobilizable IncQ1, IncFIA, IncFIB, or IncFIC plasmids. Phenotypic resistances (ampicillin, gentamicin, trimethoprim, ciprofloxacin, nalidixic acid, sulfamethoxazole, and tetracycline) had high consistency with resistome profiles. Resistances to sulfamethoxazole and gentamicin have been reported in the pork food chain (Rega *et al*., 2023), as well as tetracycline and ampicillin resistances in pig farms (Bryan *et al*., 2004; Boripun *et al*., 2023). Moreover, all confirmed antibiotic resistances have been assessed in cheese samples in Lebanon and Egypt (Ombarak *et al*., 2018; Hussein *et al*., 2023). The cheese isolate with highest AMR repertoire (6CP4) carried a MDR IncQ1 plasmid mediating resistance to macrolides [*mph*(B)], sulfonamides (*sul*1, *sul*2), trimethoprim (*dfrA1*), and aminoglycosides [*aph*(3")-Ib and *aph*(6)-Id]. IncQ plasmid, carrying resistances to the same classes [*aph*(3″)-Ib, *aph*(6)-Id, *dfr*A17, *mph*(A) *sul*1, *sul*2] has been identified in human extraintestinal pathogenic *Escherichia coli* (ExPEC) (Rehman *et al*., 2022). IncQ plasmid has been described as mobilizable plasmids maintained in many bacterial species, thus contributing to AMR gene dissemination (Oliva *et al*., 2017). Carriage of identified AMR plasmids suggests that these genes may spread to other pathogens and commensal inhabitants in the human gastrointestinal tract, making it more challenging to choose and apply suitable therapeutic treatments. Given the wide range of *E. coli* virulence factors with specific functions and activities (*e.g.*, adhesion, invasion, attachment, iron acquisition, motility, and toxin activity), usually encoded by mobile genetic elements, we aimed to examine the distribution of these markers in our sequences.

A broad repertoire was detected (from 142 to 247 genes), including many of the above-mentioned virulence determinants, with the highest number carried by one salami raw material strain. A possible reason for this is that phylogroup B2 *E. coli*, belonging to this strain, has long been known to have a larger number of virulence genes compared to other phylogroups (Sannes *et al*., 2004). Virulence factors common to all strains (*csg*, *fim*, *ibe*) or most of them (*hly*E, *iuc*) have been associated with colonization, fitness, effectors, and toxin pathogenicity mechanisms used by different pathotypes of enteric pathogenic *E. coli* to cause intestinal and extraintestinal diseases in humans, although their pathogenic efficacy warrants more investigations (Pakbin *et al*., 2021).

A total of 3 *E. coli* strains isolated from the salami production environment, harbored the LEE locus as well as module 2 of O island OI-122, suggesting these strains as belonging to atypical enteropathogenic *E. coli* (a-EPEC). A-EPEC strains with partial OI-122 have already been isolated from diarrheal patients, suggesting that the pathogenicity of these 3 strains cannot be ruled out (Xu *et al*., 2017). A total of 11 strains reported plasmid-mediated iron uptake systems (*sit*, *iro* or *iuc*), among which 10 can be considered putative *ColV*-positive according to the screening methodology described by Liu *et al*. (2018). ColV plasmids are also commonly found in ExPEC isolates from various sources and are significantly associated with animal husbandry, such as pigs (Wyrsch *et al*., 2022). Considering that the *ColV* gene repertoire may enable strains that acquire them to promote enhanced virulence, urinary tract infection, and sepsis (Reid *et al*., 2022), this may suggest our strains as possible contributors to the mobilization of virulence features between food animals, the environment, and humans, thus posing serious public health risks. Analogous outcomes may arise for plasmid-encoded hemolysin operon *hly*ABCD, promoting *hly*A hemolysin secretion, which is a critical virulence factor acknowledged in ExPEC isolates (Beutin, 1991; Bauer and Welch, 1996).

Conclusions

This study provided genomic insight into the occurrence and abundance of antimicrobial and virulence-associated genes of *E. coli* from salami and cheese artisanal food chains, suggesting the possible dissemination of many acquired markers. Overall, our findings highlighted the need to enforce hygiene measures in food handling processes, notably within the artisanal context, to prevent contamination with antimicrobial-resistant and potentially pathogenic bacteria.

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Online supplementary material:

Supplementary Table 1. Metadata (food product, batch and isolation site), species detection and assembly quality of 34 strains collected from artisanal productions. Preliminary identification carried out with biochemical and polymerase chain reaction methods as well as whole genome sequencing-based species identity are both reported.

Supplementary Table 2. Identification of plasmid-encoded antimicrobial resistance and virulence genes combining ABRicate and MOB_suite results. Plasmid typing, such as replicon (rep) types, relaxase type, mobility and size, as well as reconstruction of putative plasmids (plasmid ID), are also highlighted.

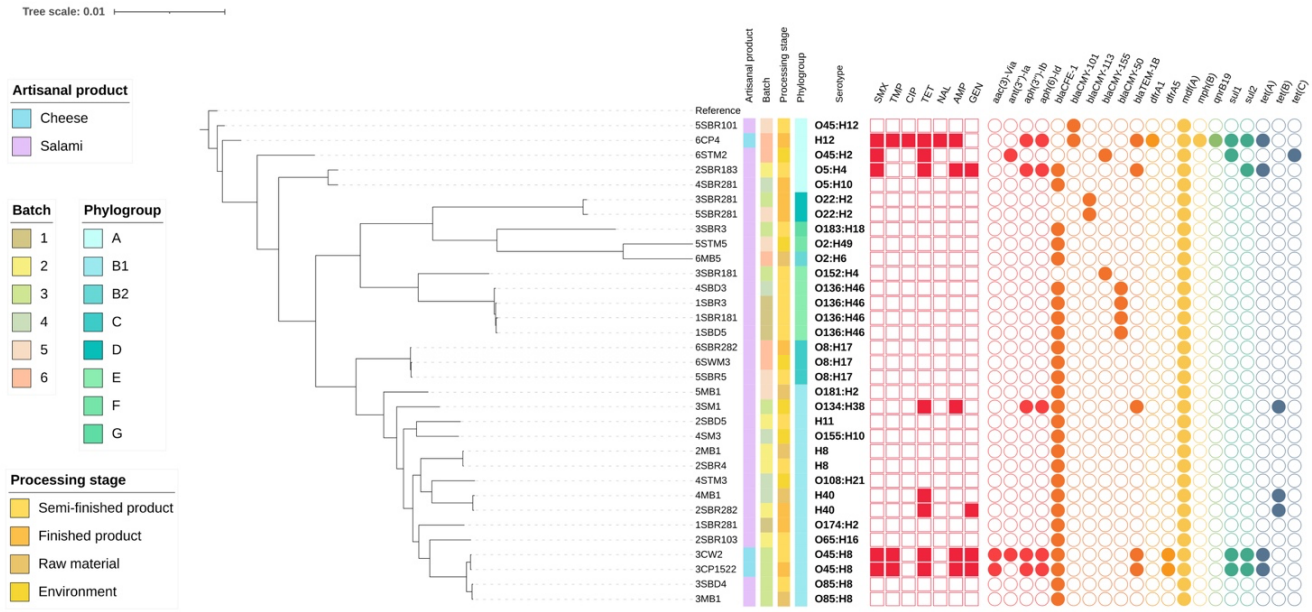

Figure 1. Single nucleotide polymorphism-based maximum likelihood phylogenetic tree of 33 confirmed *E. coli* annotated with artisanal products, batch, processing stages, phylogroups and serotypes. Antimicrobial susceptibility testing (red squares) and detection of AMR genes (colored circles according to the antimicrobial class) are also displayed in the tree, where filled shape indicates presence whereas absence is marked with only shape outline. The tree is rooted in the reference genome *Escherichia coli* O157:H7 str. Sakai (NCBI RefSeq assembly no. GCF_000008865.2). SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; TET, tetracycline; NAL, nalidixic acid; AMP, ampicillin; GEN, gentamicin.

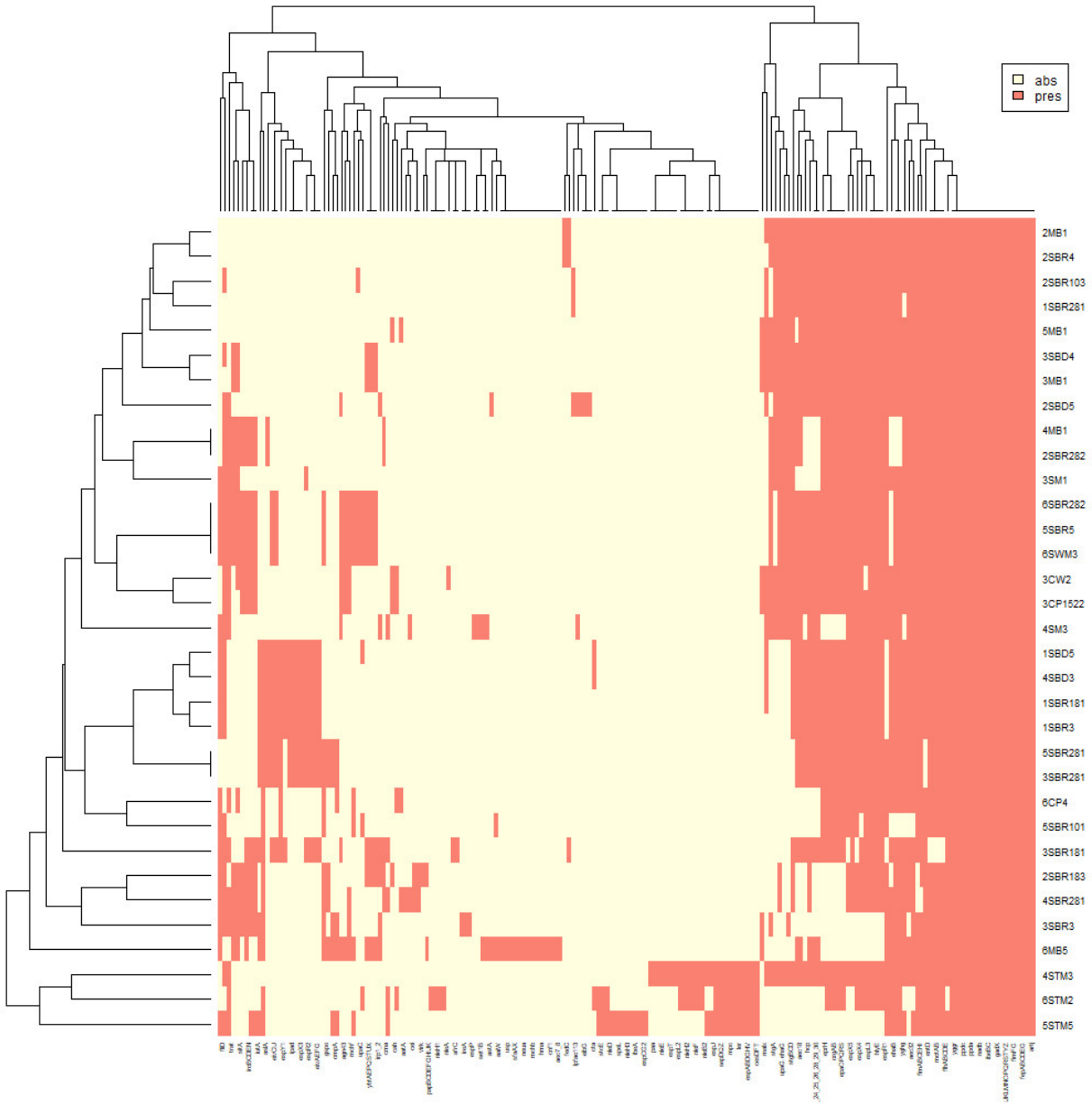

Figure 2. Heatmaps of virulence-associated genes of 33 *Escherichia coli* strains. Presence of genes is indicated with red color, whereas yellow display gene absence.