



MICROBIOLOGICAL ANALYSIS OF OFFICIAL DAIRY PRODUCT SAMPLES AND CHARACTERISATION OF THE GENOTYPIC AND PHENOTYPIC PROFILES OF ANTIMICROBIAL RESISTANCE AND VIRULENCE IN BACTERIAL ISOLATES

Pietro Di Taranto¹, Fiorenza Petruzzi¹, Angelica Bianco¹, Laura Del Sambro¹, Viviana Manzulli¹, Giovanni Castelli¹, Simona Faleo¹, Antonella Didonna¹, Annita Maria Damato¹, Carmine Pedarra¹, Giovanni Normanno², Antonio Parisi¹

¹Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy; ²Dipartimento di scienze agrarie, alimenti, risorse naturali e ingegneria, Università degli Studi di Foggia, Italy.

Between 2024 and 2025, 345 official dairy product samples were collected by Local veterinary Authorities of Foggia and analysed at Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata. Specifically, 119 samples were tested for Salmonella detection (ISO 6579-1:2017/Amd 1:2020); 172 for Listeria monocytogenes (Lm) detection (ISO 11290-1:2017); 119 for Lm enumeration (ISO 11290-2:2017); 133 for Shiga toxin-producing Escherichia coli (STEC) detection (ISO/TS 13136:2012); 188 for β -glucuronidase-positive E. coli enumeration (ISO 16649-2:2001); 189 for coagulase-positive staphylococci (CoPS) enumeration (ISO 6888-2:2023); 59 for staphylococcal enterotoxins detection (ISO 19020:2017) and 54 for aerobic microorganisms (AM) at 30°C enumeration (ISO 4833-1:2022). Presumptive colonies of Salmonella and Lm were confirmed by MALDI-TOF MS. Confirmed Salmonella isolates were serotyped (ISO/TR 6579-3:2014) and tested for antimicrobial susceptibility by broth microdilution (Sensititre™ EUVSEC), following Commission Implementing Decision (EU) 2020/1729. Minimum inhibitory concentrations were interpreted according to EUCAST epidemiological cut-off values. CoPS were subjected to PCR for the detection of staphylococcal enterotoxin genes (sea to see and ser) (AFSSA EU RL Protocol). DNA from the bacterial strains was purified using the MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific). Sequencing libraries were prepared according to the Illumina protocol and sequenced on an Illumina MiSeq platform. The obtained reads were assembled de novo using SPAdes. Bioinformatic analysis were performed using AMRFinderPlus for the identification of antimicrobial resistance

(AMR) genes and virulence factors. Overall, 31 samples (9.0%, 31/345) were found to be non-compliant. Non-compliances involved the enumeration of AM at 30°C (18.5%, 10/54), E. coli (3.7%, 7/188), CoPS (2.1%, 4/189) and the detection of Salmonella (1.7%, 2/119), STEC (0.8%, 1/133) and Lm (4.1%, 7/172). Both Salmonella isolates were identified as S. Apeyeme. One isolate was susceptible to all antimicrobials tested, whereas the other showed a multidrug-resistant profile, with resistance to azithromycin, tigecycline, colistin and tetracycline. AMR was confirmed by the detection of mph(A), tet(X3), mcr-1 and tet(A) genes. Both Salmonella strains harbored virulence-associated genes (invA, avrA, pipA, sseK2, iroB, iroC, lpfB, icmF, sciR, sinH) implicated in host cell invasion, intracellular survival, iron acquisition and intestinal colonisation. The STEC isolate was identified as serotype O55:H12 belonging to sequence type (ST) 101 and harbored the stx1 virulence gene. AMR genes detected included (blaTEM-1, sul2, aph(3'')-Ib) conferring resistance to β -lactams, sulfonamides and streptomycin. Lm strains belonged to Lineage I and were assigned to ST1 (3/7, 42.8%), ST6 (2/7, 28.6%) and ST200 (2/7, 28.6%). All Lm strains carried the virulence genes (inlA, inlB, IntA, inlC, inlP) associated with host-cell invasion, immune modulation, intracellular spread and placental colonisation. Among CoPS, the sea gene was detected in one isolate. This study highlights the importance of microbiological surveillance and genomic characterisation of foodborne pathogens to support risk assessment and strengthen food safety measures along the dairy production chain. The detection of pathogens carrying relevant AMR and virulence determinants underscores their potential threat to public health.