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Main topic : Antimicrobial resistance: A worldwide concern

Occurrence of extended-spectrum b-lactamase (ESBL) producing and/or colistin resistant Enterobacteriaceae in ready to eat foods in Apulia and Basilicata (Italy)

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Introduction - Extended-spectrum b-lactamase (ESBL) producing and/or colistin resistant Enterobacteriaceae have been largely isolated in food producing animals, mostly bovines, pigs and poultry (1). The aim of the present study was to investigate the presence of ESBL producing and/or CR Enterobacteriaceae in ready to eat foods collected in two Italian Regions, Apulia and Basilicata.

Methods - A total of 293 samples, including 202 dairy products, 64 ice-creams and 27 sausages were analyzed. Five g or ml of each sample were incubated in 45 ml of BPW at 37°C for 24 h; then 1 ml of broth was enriched in 10 ml of EE Broth 1X (ThermoFisher Scientific) at 37 °C for 24 h. Thereafter 10 ml of enrichment was streaked on CHROMAGAR ESBL and CHROMAGAR COL-APSE plates (CHROMagar), incubated at 37°C for 24 h. Typical colonies were selected from both the medium, subcultured on Blood Agar and identified by MALDI-TOF mass spectrometry. Phenotypic confirmation was performed using combined-disk test (2) and MIC-Strip colistin test (MERLIN-A Bruker Company) on Enterobacteriaceae isolated from CHROMAGAR ESBL and CHROMAGAR COL-APSE respectively. Both ESBL producing and colistin resistant Enterobacteriaceae were genotyped using Whole Genome Sequencing (WGS) (3).

Results – Among the 293 samples, 10 (3%) were positive for ESBL producing and 13 (4%) for colistin resistant Enterobacteriaceae respectively. Of the 10 ESBL producing positive samples, 4% (9/202) were represented by dairy products and 2% (1/64) by ice-cream. Of the 13 colistin resistant positive samples, 6% (4/64) were represented by ice-cream, 4% (8/202) by dairy products and 4% (1/27) by sausages. Among the 10 ESBL producing Enterobacteriaceae isolated, 7 were identified as *Enterobacter cloacae* (6 from dairy products and 1 from ice-cream), 1 as *Enterobacter kobei*, 1 as *Escherichia coli* and 1 as *Citrobacter braakii* (all from dairy products). Of the 13 colistin resistant Enterobacteriaceae, 4 were identified as *Enterobacter cloacae* (2 from dairy products and 2 from ice-cream), 3 as *Enterobacter hermannii* (2 from dairy products and 1 from sausages), 2 as *Klebsiella pneumoniae* (both from ice-cream), 2 as *Enterobacter asburiae*, 1 as *Enterobacter bugandensis*, 1 as *Citrobacter freundii* (all from dairy products) (Table 1).

WGS analysis was preliminary performed on 3 ESBL producing and on 2 colistin resistant strains. None of the 3 ESBL producing strains (all *Enterobacter cloacae*, 2 from mozzarella and 1 from caciocotta cheese) was found positive for ESBL encoding genes. Among the 2 colistin resistant strains, *Enterobacter hermannii* from sausages and *Enterobacter asburiae* from mozzarella, were found positive for *mcr8* and *mcr9* genes respectively. All the 5 tested strains resulted positive for the main virulence factors typical of Enterobacteriaceae (*fepA*, *entA*, *galU*, *gmhA*, *lpA*, *kdsA* / *lpxAC* / *rfaD*, *ompA*, *fimABCDEFGHI*).

Conclusions - This study clearly demonstrates the possible dissemination of ESBL producing and colistin resistant Enterobacteriaceae in food. This finding represents a potential hazard to human health, especially given the fact that the genes encoding these resistance determinants are often associated with motile genetic elements that can be transferred to both commensal and pathogenic bacteria.