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Monitoring of health and hygiene parameters in the Carabinieri's collective catering facilities

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Abstract
Hygiene and health parameters were monitored at several Carabinieri canteens in central and southern Italy to assess the management aspects related to collective catering services. Between February 2024 and March 2025, 89 food matrices normally served in 23 canteens located in six Italian regions were subjected to microbiological assessment. Food sample aliquots (100-150 g) were sterilely collected and tested for total mesophilic count (TMC) and, using qualitative and quantitative detection methods, pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157, and *Salmonella* spp. Suspicious colonies were identified using the Vitek 2 system (bioMérieux, Paris, France) and confirmed by performing end-point polymerase chain reaction assays. Statistical analysis calculated the following parameters: mean, standard deviations (SD), Shapiro-Wilk test, and the two-tailed *t*-test ($p<0.05$). The mean TMC value was 2.65 log CFU/g (SD=1.21); more specifically, the highest [3.22 log CFU/g (SD=1.36)] was observed in Puglia, while the lowest [1.74 log CFU/g (SD= 0.63)] was observed in Molise. The *t*-test showed statistically significant differences in TMC values among the following regions: Abruzzo-Puglia ($p=0.038$), Campania-Marche ($p<0.001$), and Lazio-Campania ($p=0.04$). The three tested pathogens (*L. monocytogenes*, *E. coli* O157, and *Salmonella* spp.) were never observed. This work and the data obtained provide a picture of the good hygiene and health status of the Carabinieri canteens in central and southern Italy; however, the anthropogenic bacterial detection highlights the importance of the correct application of good hygiene practices to ensure the protection of the health of the military community.

Introduction
Since 2014, the Italian military contingents exclusively regulated the collective canteens restricted in the context of foreign operating theaters, as described by the General Inspectorate of Military Health (Defense General Staff - General Inspectorate of Military Health, 2014).
However, a consistent legal evolution was introduced in the Carabinieri Corps Headquarter in 2021, by the adoption of Publication No. C-19 entitled "*Compendium of logistic provisions of the Carabinieri Force*" (Carabinieri Corps Headquarter, 2024). More specifically, this act identifies requirements for temporary service canteens and operational trainings; it includes both kitchen and distribution shelters characterized by a submitted certified activity start notice (also known as the acronym SCIA). It implies the necessity of specific procedures, included in the manual, about the correct application of the so-called good hygienic practices, reported in the European Regulation No. 852/2004 (European Parliament and Council of the European Union, 2004), performed by the employed military personnel, as described by Pulze *et al*. (2023). Based on the hazard analysis and critical control point concepts, military canteens must apply the high hygienic standards in different productive flows in order to administer safe food to the consumers. These conditions highlight the critical necessity of a correct and flexible application of the above-mentioned concepts, in different military contexts, which can vary from the routine activities in barracks to the emergency ones (Falconer Hall *et al*., 2022).
For this purpose, this study represents the first Italian investigation about hygienic and health parameters, which involved 23 Carabinieri collective canteens distributed in six regions (southern-central Italy). The performed screenings involved 89 foodstuffs, collected between February 2024 and March 2025, which were normally administered to the military employees.
These analyses are necessary and essential to verify the correct hygienic levels of foods and to verify the behavioral standards performed by the military personnel in the collective canteens.

Materials and Methods
Sample collection, processing and microbiological analyses
A total of 23 Carabinieri collective canteens, distributed among six south-central Italian regions (Abruzzo, Campania, Lazio, Marche, Molise and Puglia), as graphically illustrated by Figure 1, were involved in this study between February 2024 and March 2025.

For this purpose, 89 food matrices were collected among the routinely served foodstuffs (both animal origin and vegetable ones) to the military forces, and the sampling procedures included aliquots of 100-150 g, which were sterilely performed. After each sampling, specimens were transported under refrigerated conditions and processed for microbiological screenings at the laboratory level. A schematic and detailed representation of the analyzed foods is also presented in Figure 1.

From each conferred food matrix, three aliquots of 25 g were aseptically collected with mono-usage scalpels (Monopec Scalpels, Thermo Fisher Scientific™, Waltham, MA, USA). They were introduced into sterile stomacher bags (BagMixer®, Interscience, Puycapel, Cantal, France) that contained different diluents based on the different microbiological analytes to be detected. The considered ones were total mesophilic count (TMC), *Listeria monocytogenes*, *Escherichia coli* O157, and *Salmonella* spp.

For the TMC, an aliquot of 25g of food was introduced in 225 mL of maximum recovery diluent (Oxoid Ltd., Basingstoke, Hampshire, UK) and stomached for 90 seconds at room temperature. After that, ten-fold dilutions were plated using the plate count agar (Oxoid Standard Plate Count Agar, Oxoid Ltd., Basingstoke, Hampshire, UK) and the quantitative results were reported as log CFU/g (ISO 4833-1:2013/AMD-1:2022).

The third 25 g aliquot was used to discover *L. monocytogenes* and was introduced in 225 mL of supplemented Half Fraser broth (Half Fraser Broth, ThermoFisher Scientific, Waltham, MA, USA), stomached for 90 seconds, and incubated at 37°C for 24 hours. This last step was followed by the plating onto ALOA agar (Oxoid Ltd., Basingstoke, Hampshire, UK), as reported in the ISO 11290-1/2:2017 (ISO, 2017b).

The *E. coli* detection method was performed following the ISO 16654:2017 (ISO, 2017a) by using an aliquot of 25 g diluted in 225 mL of buffered peptone water (BPW) (Oxoid Ltd., Basingstoke, Hampshire, UK), stomached for 90 seconds and incubated at 37°C for 18-24 hours. The last step was the plating onto McConkey agar (Oxoid Ltd., Basingstoke, Hampshire, UK).

Concerning *Salmonella* spp. detection, the collected food aliquot (25.0 g) was diluted in 225 mL of BPW (Oxoid Ltd., Basingstoke, Hampshire, UK), stomached for 120 seconds, and incubated at 37°C for 18-24 hours. Then, 0.1 mL of the sample was transferred into 10 mL of Rappaport-Vassiliadis diluent (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 42°C for 24 hours. Finally, a loopful was plated onto xylose lysine doexychlolate agar (Oxoid Ltd., Basingstoke, Hampshire, UK), as described by the ISO 6579-1/2:2020 (ISO, 2020).

Based on the obtained colonies and their respective morphologies, all suspected ones were identified using the biochemical automated method VITEK® 2 system (bioMérieux, Paris, France) in agreement with manufacturer instructions. More in detail, the identification process for gram-positive and negative bacterial strains was performed using specific cards named VITEK® ID-GP and VITEK® ID-GN (VITEK® 2 system, bioMérieux, Paris, France). It implied the suspension of three pure colonies into 3.0 mL of sodium chloride solution (0.45%) with a final turbidity of 0.5 McFarland. The analytical workflow ends with the cards' loading in the system which was able to produce results by 8 hours from the start. The possible positive samples required the biomolecular confirmations using end-point uniplex and/or multiplex polymerase chain reaction assays.

Statistical analysis

The IBM® SPSS 20.0 statistics software version (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses. It firstly implied a descriptive statistical analysis with the Shapiro-Wilk test to determine the normal distribution of the obtained microbiological data expressed as CFU/g. For each value, mean (μ) and standard deviation (SD) were also calculated. The two-tailed *t*-test was applied to compare the quantitative microbiological results (considered as dependent variables) with the different screened Carabinieri canteens distributed among six Italian regions. The statistical significance was considered if the p-value was <0.05. Finally, the confidence intervals (CI 95%) was calculated, when applicable, for all percentage values.

Results

From a general perspective, the 23 screened Carabinieri military canteens demonstrated high hygienic levels based on the obtained TMC medium value 2.65 log CFU/g (SD=1.21), and in none of the considered bacterial pathogens, such as *L. monocytogenes*, *E. coli*, and *Salmonella* spp., were they ever quantitatively and/or qualitatively isolated from the tested food matrices. More in detail, the discovered mesophilic bacterial loads presented the following values/per region: 2.39 log CFU/g (SD=1.02) Abruzzo, 2.63 log CFU/g (SD=1.48) Campania, 3.05 log CFU/g (SD=0.54) Lazio, 2.48 log CFU/g (SD=0.76) Marche, 1.74 log CFU/g (SD=0.62) Molise, and 3.22 log CFU/g (SD=1.35) Puglia. Based on the mentioned evidence, the highest value was observed in Puglia canteens and the lowest in the Molise region. The performed two-tailed p-value tests permitted us to find out many statistically significant differences, comparing the obtained TMC values among the 6 Italian regions reported as follows: Abruzzo-Puglia $p=0.038$, Campania-Marche $p<0.001$, Lazio-Campania $p=0.04$, and Puglia-Molise $p<0.001$. The raincloud plot graphs in Figure 2 illustrate the observed analyses.

Based on the food matrix types, the calculated TMC presented these general mean values: 3.23 log CFU/g (SD=0.86) for animal-origin foods and 2.56 log CFU/g (SD=1.16) for plant-based ones. The *t*-test showed a statistically significant difference, with $p<0.001$. More specifically, the discovered TMC per tested foodstuffs were as follow: 2.48 log CFU/g (SD=1.26) for vegetables, 3.51 log CFU/g (SD=1.07) pasta, 2.55 log CFU/g (SD=1.03) pork-based products, 3.01 log CFU/g (SD=1.06) for poultry-based ones, 2.61 log CFU/g (SD=0.99) egg-based, and 4.13 log CFU/g (SD=0.11) for cheese. The distributions of the obtained TMC (reporting the μ and SD values) per regions and food types are illustrated in detail in Table 1.

The statistical analyses revealed no significant differences, making comparisons of the TMC values: plant-based products – pasta ($p=0.039$), pasta – poultry-based products ($p=0.049$), pork-based foods – cheese ($p<0.001$), and poultry-based ones – cheese ($p=0.009$).

Focusing on the regional data, the two-tailed *t*-tests permitted the discovery of the following differences per food matrices: TMC_{PASTA}-$TMC_{PORK\text{-}BASED}$ $p=0.049$ in Abruzzo, TMC_{VEG}-TMC_{PASTA} $p=0.013$ in Campania, and $TMC_{POULTRY}$-TMC_{PASTA} $p=0.013$ in the Puglia region.

Finally, a total of 16 different commensal and/or telluric bacterial genera were isolated and identified from the food matrices, as schematically represented in Table 2.

Discussion

The collective catering and/or canteen restaurant forms can represent crucial surveillance points for possible foodborne outbreaks due to the coexistence of many variables (*e.g.*, food storage, handling, treatments, technological processes, *etc.*), which can lead to possible hygienic and sanitary implications for final consumers (Marcotrigiano *et al*., 2023). More in detail, this is the first study which focused on hygienic and sanitary parameters of foodstuffs normally administrated to the Carabinieri Corps collected from 23 military canteen distributed in 6 Italian regions. The present investigation is a consequential step continuously to a previous one which developed and discussed the operational and training activities of food safety pillars among the Carabinieri military contingents (Pulze *et al*., 2023).

The adopted microbiological approach, belonging to this study, permitted the general observation of high hygienic levels; indeed, the discovered TMC medium value was 2.65 log CFU/g, resulting in a lower than an analogous investigation conducted in the European scenario such as: 5.12 log CFU/g observed in meals consumed by patients in a military hospital in Turkey (Ayçiçek *et al*., 2004). In both cases, no strictly bacterial pathogens were identified from all tested food matrices. Based on the poor scientific literature concerning the military canteen, the observed data (2.65 log CFU/g), from a comparative perspective, was also compared to civil collective canteens, resulting in line with TMC 2.77 log CFU/g from food served in an Italian hospital (Umbria region) described by Lupattelli *et al.* (2022). In the European scenario, the discovered TMC values, in this study, were also in line with 2.47 log CFU/g observed from foodstuffs in a Portuguese university canteen (Soares *et al*., 2020) and 2.89 log CFU/g from meals served in secondary schools in Budapest (Hungary) (Tóth *et al*., 2018).

More in detail, the described statistically significant differences (belonging to this investigation): TMC_{PASTA}-$TMC_{PORK\text{-}BASED}$ p=0.049, TMC_{VEG}-TMC_{PASTA} p=0.013, and $TMC_{POULTRY}$-TMC_{PASTA} p=0.013 were similarly reported by Soares *et al.* (2020), presenting p<0.01 by comparing $TMC_{PORK\ MEAT}$- TMC_{PASTA}, TMC_{PASTA}- TMC_{VEG}, and $TMC_{POULTRY\ MEAT}$- TMC_{PASTA}.
Based on food types, the highest TMC values observed in the screened Carabinieri military canteens were cheese specimens (average value: 3.92 log CFU/g) and pasta ones (average value: 3.05 log CFU/g). Concerning cheese and dairy products, Ayçiçek *et al.* (2004) discovered TMC of 3.29 log CFU/g in a military hospital in Turkey, and concerning the TMC_{PASTA}, it was in line with 2.47 log CFU/g reported by Soares *et al.* (2020) in Portugal. On the contrary, vegetable meals presented the lowest TMC values than the other considered food matrices, both in this study (TMC_{VEG}: 2.43 log CFU/g) and the other similar ones: TMC_{VEG}: 2.53 log CFU/g (Soares *et al*., 2020), TMC_{VEG}: 2.37 log CFU/g (Ayçiçek *et al.*, 2004), and TMC_{VEG}: 2.45 log CFU/g (Tóth *et al*., 2018).
The scientific explanations for the cited data and studies converge on the concept of different food management practices and their direct correlation with food-handling procedures performed by food operators (Osimani and Clementi, 2016). It consequentially implies and involves the gained hygienic levels of a specific collective canteen, and if these adopted measures result conform to the declared procedures in the anticontrol manuals (referring to the so-called good hygiene practices, as described by the European Regulation No. 852/2004), it should lead to food safe conditions for consumers. Finally, the possible differences in bacterial loads per food matrix types can also be justified by the possible thermal exposure obtained during the cooking processes, before serving (Osimani and Clementi, 2016). Indeed, in this study and in the before mentioned studies, cheese meals were usually administered raw, whereas vegetable ones were boiled or microwaved.
The importance of hygienic measures has also been demonstrated by the discovery of bacterial genera from the screened food matrices, as illustrated in Table 2. Indeed, the detection of negative coagulase *Staphylococci*, *Sphingomonas* spp., and *Kocuria* spp. identified both from processed animal origin and vegetables, indicated improper applications of food handling procedures. Concerning the *Pantotea* genus and *Achromobacter xylosoxidans*, their detection has been carried out by spices added during the vegetable meal preparations. Although these bacteria are not pathogenic for humans, this further highlights the importance of constant monitoring of qualified food suppliers with special regard to ingredients that can be added, and which can be considered as *external* to the conform applied hygienic measures in the collective kitchens.
Finally, the absence of the considered bacterial pathogens (*e.g*., *L. monocytogenes*, *Salmonella* spp.) indicates the high attention to and efficacy of sanitation procedures applied in the Carabinieri canteens, consistent with those in civil facilities (Osimani and Clementi, 2016). However, the discovered bacterial loads and the identified ones (described in the results section) were commensal strains; these data represent the evidence of contamination of the different analyzed food matrices related to the food handling and assembling procedure before administration to the military consumers.

Conclusions

This study involved 23 Carabinieri Corps canteens distributed in six regions in southern central Italy, and for the first time, a military force took part in a hygienic surveillance study about the food matrices normally administered to the consumers. The crucial importance of the correct application of hygienic measures still represents the main endpoint for foodborne illnesses prevention, as observed in this study. However, the harboring of high loads of commensal bacteria, usually coming from humans, is strictly related to improper food handling performed by operators or to not-well qualified raw material suppliers. Therefore, constant and efficacious surveillance of these two variables will be useful to improve productive standards in the collective military canteens.

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Table 1. Detailed distribution of total mesophilic count values (log CFU/g) among the tested foods in many Italian regions.

Carabinieri canteens	Total mesophilic count (log CFU/g): μ and SD					
Italian regions	**VEG.**	**PORK**	**POULTRY**	**EGG**	**CHEESE**	**PASTA**
Abruzzo	μ=1.53 SD=0.82	μ=2.57 SD=0.74	μ=3.08 SD=1.64	μ=2.66 SD=0.99	μ=4.16 SD=1.01	μ=1.87 SD=0.37
Campania	μ=2.55 SD=1.36	μ=2.48 SD=1.20	μ=2.36 SD=1.48	μ=1.56 SD=0.48	μ=3.71 SD=1.02	μ=3.15 SD=1.29
Lazio	μ=3.11 SD=1.05	μ=3.04 SD=0.19	μ=3.02 SD=1.25	μ=2.97 SD=0.31	μ=4.17 SD=0.72	μ=3.52 SD=1.28
Marche	μ=2.13 SD=0.62	μ=1.70 SD=0.37	μ=2.42 SD=0.65	μ=2.76 SD=1.05	μ=4.02 SD=0.56	μ=2.82 SD=0.77
Molise	μ=1.70 SD=0.88	μ=1.81 SD=1.03	μ=2.91 SD=0.97	μ=3.01 SD=1.27	μ=3.18 SD=0.72	μ=3.72 SD=0.73
Puglia	μ=3.54 SD=1.48	μ=2.80 SD=1.35	μ=3.39 SD=1.45	μ=1.51 SD=0.33	μ=4.23 SD=1.03	μ=3.18 SD=1.71

μ, mean value; SD, standard deviation.

Table 2. Schematic illustration of the mainly identified bacterial genera from food matrices collected in the Carabinieri canteens.

Italian regions	**Food matrices**	**Bacterial ID**
Abruzzo	Pork-based (mortadella)	*Serratia fonticola*
Campania	Vegetable (salad)	*Achromobacter xylosoxidans*
		Leuconostoc pseudomesenteroides
	Pork-based (grilled chop)	*Pantotea agglomerans*
	Poultry-based (grilled chicken)	*Staphylococcus sciurii*
	Vegetable (carrots)	*Enterococcus cloacae*
		Kocuria rhizophila
	Pasta	*Pseudomonas luteola*
	Poultry-based (grilled turkey)	*Pantotea* spp.
		Klebsiella pneumoniae
	Vegetable (spinaches)	*Sphingomonas paucimobilis*
Lazio	Vegetable (carrots)	*Burkholderia gladioli*
Marche	Vegetable (potatoes)	*Rhizobium radiobacter*
	Egg-based (omelet)	*Sphingomonas paucimobilis*
	Vegetable (spinaches)	*Pantotea* spp.
Puglia	Poultry-based (grilled chicken)	*Pasteurella pneumotropica*

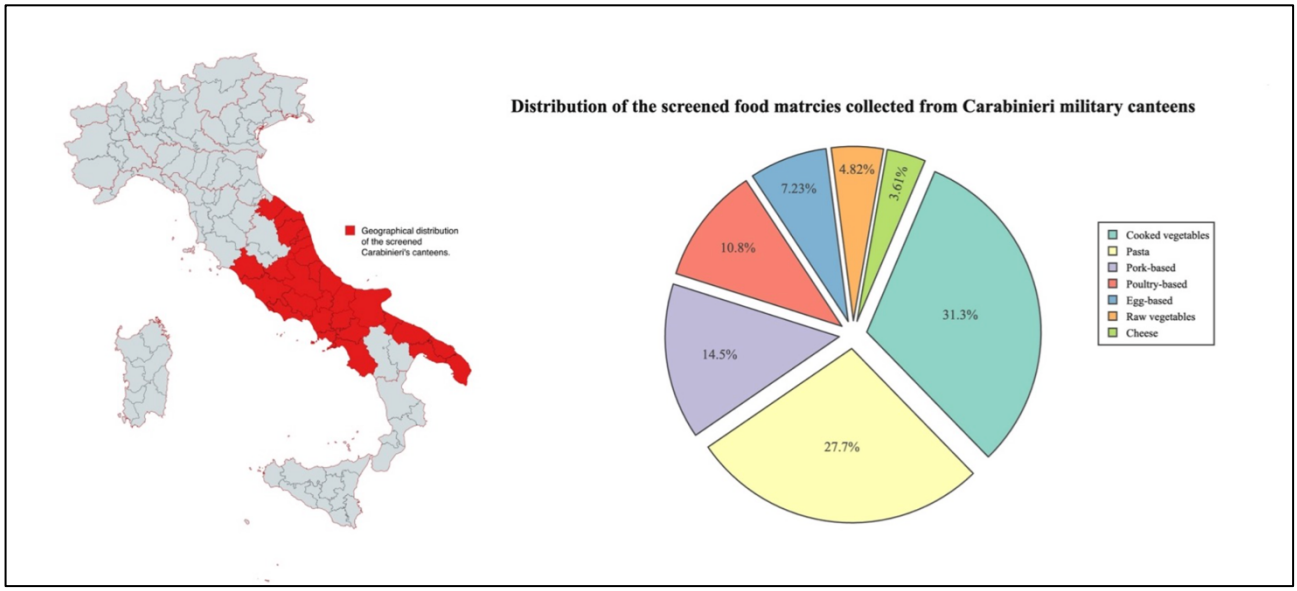

Figure 1. Geographical distribution of the screened Carabinieri canteens and the collected foodstuffs.

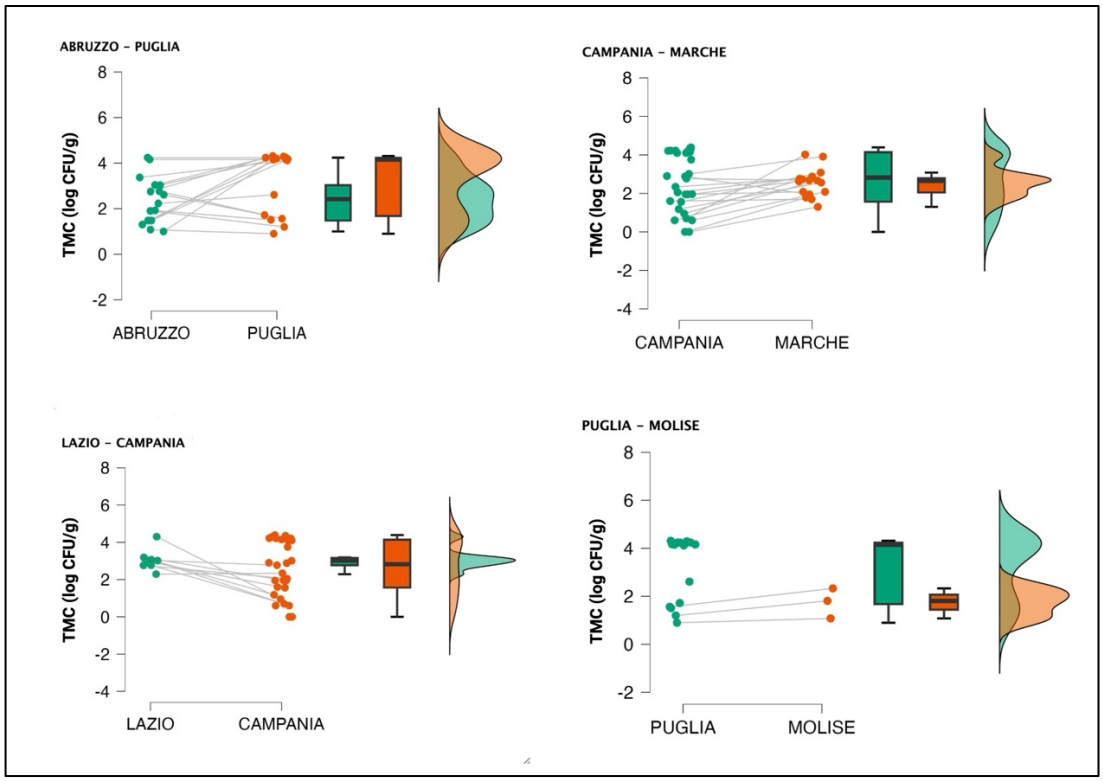

Figure 2. Raincloud plots of significant statistical differences comparing the obtained total mesophilic count results.