ORIGINAL ARTICLE

Occurrence of *Salmonella* and *Listeria* spp. on retail poultry products in South Italy and comparison of conventional and rapid methods for their detection

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Key words

Salmonella • Listeria • Poultry meat

Summary

Salmonella and Listeria spp. are frequently detected in poultry meats. Conventional isolation and identification methods to detect these microrganisms in food are laborious and time-consuming. In the present study the occurrence of Salmonellae and Listeriae on 362 samples of retail poultry in Caserta, South Italy was evaluated and standard microbiological and rapid methods were compared. Furthermore, the samples were collected and analyzed twice a week, on Monday and Friday to establish their possible variability from storage. Both methods showed a strong contamination of samples by Listeria spp. (about 50% for both methods) with 12% Listeria monocytogenes while the contamination of Salmonella was poorer (14-15%). The two procedures showed a good agreement for the detection of Listeriae while the sensitivity of the Rapid test for Salmonellae was poorer (75%). Data about sampling on Monday and Friday highlighted a significant increase in Listeria spp. at the end of the week.

Introduction

Salmonellosis and listeriosis are recognized as two of the most important food borne diseases in many countries as well as in Italy [1-3].

Annually, approximately 40,000 salmonellosis cases are reported to the Centres for Disease Control and Prevention (CDC) but taking into account the degree of under-reporting, 1.4 million cases have been estimated each year in the US alone [4]. Despite Italy's adoption of the HACCP (Hazard Analysis of Critical Control Points) and the drop in the number of reported illnesses, thousands of episodes occur each year. According to the available data from the Epidemiology Bulletin of the Italian Ministry of Health, an annual average of 15,000 cases was notified during the period 1993-2002 [5].

In contrast, listeriosis is less frequent but more severe. It has an incidence below 10 cases per million with high mortality rate (approximately 20% mortality with an increase up to 75% in high-risk individuals [6, 7].

Chicken products are a frequent source of *Salmonella* and *Listeria* contamination in several countries [8-11]. Although raw poultry is rarely implicated in human listeriosis, the risk of cross-contamination from other food is high.

Conventional isolation and identification methods for detection of Salmonellae and *Listeria* spp. in food are labour intensive and time-consuming lasting up to 7-10 days. This time is unacceptable to large sectors of food industry that handle highly perishable products such as poultry.

In the present study the conventional microbiological procedures were used to detect the two pathogens in parallel with the Salmonella Rapid Test and Listeria Rapid Test developed by Oxoid (Hampshire, UK). The former Rapid Test is based on the enrichment, selective growth and motility of *Salmonella* species in culture vessels, the latter is a rapid immunoassay using monoclonal antibodies for *Listeria* species except *L. grayi*. These assays present advantages in terms of rapidity (approximately 42 h), low cost and simplicity. Few studies have been carried out on the sensitivity and specificity of these systems [12, 13].

The aims of the present study were to determine the occurrence of Salmonellae and *Listeria* spp. in chicken carcasses, chicken parts (wings and legs) and processed products such as sausages and hamburgers made with poultry and sold at different supermarkets in Caserta, South Italy and to compare the conventional and rapid analytical methods for their detection. Furthermore, the samples were collected and analyzed twice a week, on Monday and Friday to establish their possible variability since it was assumed that products not sold on Monday were stored through the week and it is well known that often meat contamination is correlated to unsanitary food production or storage practices.

Materials and methods

Samples

Ninetysix chicken carcasses, 176 chicken parts (86 wings, 90 legs) and 90 further processed chicken products (50 sausages and 40 hamburgers) were sampled at the beginning and at the end of the week for six months for a total of 362 samples. All retail products were prepared and packaged by companies of Caserta province.

Each sample was placed in a single sterile plastic bag and was brought to the laboratory on ice, tested upon arrival or kept at 4 °C and analyzed within 4 h for *Salmonella* and *Listeria* spp. A 25 g sample of each product was taken aseptically by scalpel excision. For the carcasses the 25 g portion was formed by samples of breast, wing, leg, neck, dorsal and ventral skin. All media utilized in this study were purchased from Oxoid, Italy.

Microbiological methods

The presence of Salmonellae was evaluated according to standard procedures [14, 15] using a stomacher (Stomacher 400, A.J. Seward, London, UK) to macerate the samples. The homogenates (obtained with 225 mL of Buffered Peptone Water) were incubated for 18 ± 2 h at 37 °C. Next, 0.1 and 1 mL were transferred to 10 mL of Rappaport Vassiliadis Broth (42 °C for 24 h) and to Selenite-Cystine Broth (37 °C for 24 h), respectively. Both enrichment cultures were streaked on Brilliant Green Agar (BGA) and Desoxycholate Citrate Agar (DCA). After incubation for 24 h at 37 °C, plates were examined for typical colonies (pink-red on BGA and a dull colour with the black centre on DCA), sub-cultured on Nutrient Agar for purity and confirmed as Salmonellae by inoculation of Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA) tubes. The strains exhibiting typical reactions (red alkaline slants, yellow acid butts with gas formation and production of hydrogen sulphide for TSI and LIA and purple colour for LIA) after approximately 24 h of incubation at 37 °C were subjected to serological test using polyvalent somatic O antiserum (Polyvalent II, Dade Behring SA, Paris, France).
The microbiological detection method for *Listeria* spp. was performed according to ISSN 96/35 procedure [16] homogenizing the samples in stomacher with 225 mL of Half Fraser Broth (1 min at 230 rpm) and incubating at 30 °C for 24-48 h. Next, 0.1 mL of this primary enrichment was transferred to 10 mL of Fraser Broth and incubated at 30 °C for 24-48 h. The primary and secondary broths were sub-cultured onto Palcam Agar and Listeria Selective Agar (Oxford formulation). All plates were incubated at 37 °C for 24-48 h; Palcam plates were incubated under microaerophilic atmosphere (5-12% CO_2; 5-16% O_2; 75% N_2). One suspect *Listeria* spp. colony from each plate (very small black colonies) was chosen and purified on tryptone soy agar with 0.6% yeast extract for further characterization. Presumptive *Listeria* colonies were confirmed by catalase and oxydase tests, Gram staining, β-haemolysis and further biochemically identified at species level by API *Listeria* (BioMerieux, France).

Salmonella and Listeria Rapid Tests

Salmonella and Listeria Rapid Tests were applied following the manufacturers' instructions (Oxoid).
For *Salmonella* detection 1 mL of the pre-enrichment culture in Buffered Peptone Water was added to the culture vessel of the kit equipped with two tubes. Each tube contained a *Salmonella* selective medium and an upper indicator medium separated by a porous partition. Only motile salmonellae (limitation of this test that does not detect non-motile salmonellae) migrated actively through the lower medium to the upper indicator where their presence was indicated by a colour change. Therefore, after incubation of the system at 41 °C for 24 h the tubes were examined for colour changes and those which showed positive reactions were further tested with the agglutination assay Salmonella Latex Test (Oxoid). Samples giving positive results with this test have been reported as presumptively containing Salmonellae.
For Listeriae detection 0.1 mL of the culture in Half Fraser Broth was transferred to 10 mL of Buffered Listeria Enrichment Broth (BLEB) and incubated at 30 °C for 21 h. Next, an aliquot (2 mL) of inoculated BLEB was heated at 80 °C for 20 min in a water bath to release flagellin protein. Finally, this aliquot was cooled at room temperature and 135 μL were added to the blue sample window of the *Listeria* test device where a membrane binds a soluble anti-flagellin antibody bound to blue latex particles. When flagellin was present, it was detected by a line of a second antibody placed in the result window. When the blue line appeared in this location after 20 min, the sample was taken as presumptively containing Listeriae.
In order to verify the ability to detect *Salmonella* spp. and *Listeria monocytogenes* with the standard and rapid methods described, food samples were also artificially contaminated by *Salmonella enteritidis* (ATCC 13076) and *Listeria monocytogenes* (ATCC 7644) into control flasks of the pre-enrichment medium. Negative controls were proceeded with positive controls as for test cultures.

Data analysis

Data from 362 samples were subjected to chi-square statistical method to determine the uncertainty of total positive samples for Salmonellae and Listeriae with the two mentioned methods. The same method was also applied on positive samples taken on Monday and Friday to verify the storage time influence on the results. If there were significant differences, Yates's adjustment was also calculated. The results of the Rapid Tests were evaluated calculating the sensitivity (the capability to avoid false negative results), specificity (the capability to not generate false positive results), efficiency (the possibility of a given result being exact), predictive values and k coefficient of the method and described by the following equations [17]:
Sensitivity = a/(a + c)
Specificity = d/(b + d)
Efficiency = (a + d)/(a + b + c + d)
Predictive value of a positive or negative test = a/(a + b) or d/(c + d)
k coefficient = (efficiency – $_X$)/(1-$_X$) with $_X$ = [(a + b/n) (a + c/n) + (c + d/n) (b + d/n)]
Considering the conventional microbiological method as the condition showing the true contamination of samples, in this study a sample was considered true positive (a) when presumptive positive results in rapid

test were confirmed by the microbiological method. A sample was considered a false positive (b) when it was positive in the rapid test but was not confirmed in classic microbiological procedure. A sample was considered as a false negative (c) when it showed a negative result in rapid test but it was positive in conventional microbiological method. A sample was considered a true negative (d) when it was negative by the two methods used. In this calculation, the predictive value of a positive or negative test corresponded to the likelihood that a positive or negative test was exact while k coefficient compared the classical microbiological test to the Rapid Test.

Results

Table I summarizes the results obtained. Both the conventional microbiological method and the Listeria Rapid Test showed a strong contamination of poultry meat from *Listeria* spp. (51% *vs*. 53%) while the contamination of Salmonellae was always lower (14% in the standard method *vs*. 15% in the Rapid Test). However, in the present study the comparison between the positive results of 362 samples tested with the two methods did not show significant differences. The incidence of *Salmonella* in other studies varied between 0 and 100% [11, 18-20] while the occurrence of *L. monocytogenes* was lower (2-9%) for some Authors [21] and higher (15% for poultry skin samples; 36% raw poultry) for others [9, 17] who investigated poultry. It should be pointed out that the isolation rates for these microrganisms depend on the type of tissue sampled (meat or skin) and on the country of the study [17, 20].

In the present study the contamination of chicken carcasses from *Salmonella* was higher (24-25%) than that of chicken parts (14-16%) and further processed products (2-3%), since only three of the 90 samples of sausages and hamburgers investigated resulted positive for *Salmonella* in the conventional method and two in the Rapid Test. Our results agree with those of Capita et al. [20] who explained their findings with heavily contamination of breast and neck skin of chicken carcasses due to slaughter and evisceration. However, even if muscle tissues of healthy animals are generally free of microorganisms, they may become contaminated by faecal material during processing procedures in slaughter plants. In the present study, the good quality results obtained for sausages and hamburgers might be explained by the fact that, although the significant handling increases the risk in concern, these products contain NaCl, spices and herbs that, as supported by researchers who tested the same cured products [20], modify the physico-chemical characteristics of the processed products making them a poor substrate for pathogens growth. The same trend of contamination for the different variety of poultry was found for Listeriae but the percentage of positive samples was always more elevated than the percentage found for Salmonellae. Several samples (20%) were contaminated with more than one species of *Listeria*, and 22% contained unidentified Listeriae. *L. monocytogenes*, the causal agent of listeriosis, was found in 12% of the samples analyzed. The high distribution of the microrganism in poultry would suggest a high incidence of disease, although the minimal infective dose for listeriosis is unknown and the level of poultry contamination is not considered a risk to the consumer because the food is expected to be sufficiently heated before its consumption. *L. welshimeri* was the most extensively distributed species (21%) but, as *L. innocua*, it is not associated with diseases [22].

The comparison between the results of Rapid Tests and microbiological methods are shown in Table II where the estimation of specificity, sensitivity, efficiency, predictive values and k coefficient correlated to the classical procedures is also reported. The Salmonella Rapid Test showed more positive samples (54 on 362) compared to the microbiological method (52 on 362) although only 39 of them were confirmed by the conventional culture method. As already reported *Listeria* contamination was not statistically different in the two systems used (51% in conventional test *vs*. 53% in rapid

Tab. I. Rates of ***Salmonella*** and ***Listeria*** contamination in poultry products using the conventional method and the Rapid Tests.

Type of sample (n)	*Salmonella*		*Listeria*	
	Conventional Method	Salmonella Rapid Test	Conventional method	Listeria Rapid Test
Chicken carcasses (96)	24 (25%)[a]	23 (24%)[a]	62 (64%)[b]	64 (67%)[b]
Chicken parts (176)	25 (14%)	29 (16%)	91 (52%)	91 (52%)
Wings (86)	9 (10%)	11 (13%)	43 (50%)	42 (49%)
Legs (90)	16 (18%)	18 (20%)	48 (54%)	49 (54%)
Processed chicken products (90)	3 (3%)	2 (2%)	32 (36%)	37 (41%)
Sausages (50)	2 (4%)	2 (4%)	22 (44%)	25 (50%)
Hamburgers (40)	1 (3%)	0	10 (25%)	12 (30%)
Total (362)	52 (14%)	54 (15%)	185 (51%)	192 (53%)

n: number of samples tested; [a]: number of *Salmonella*-positive samples (percentage); [b]: number of *Listeria*-positive samples (percentage)

test). However, twenty positive samples for Listeria Rapid Test were negative using the microbiological procedure, while the number of positive samples for both methods was 172 (47.5%). The Rapid Tests are quick and simple to use but, they are not a complete advantage over traditional methods because also in the present study they gave false positive and negative results and furthermore, they do not let the identification to species level. The parameters reported in Table II allow us to better appreciate the differences in results between the two methods. A weak agreement (0.68) was found between the standard microbiological procedure for Salmonellae and the Rapid Test, as revealed by the k coefficient that indicates a perfect agreement when it equals 1. The sensitivity of the rapid procedure was 75% with a predictive value of a positive test equal to 72%, indicating that there is low probability that a positive result is exact. Instead, for the *Listeria* a good agreement was found between the two tests (k = 0.82) with high values of sensitivity (93%), specificity (89%), efficiency (91%) and predictivity (90% positive test, 92% negative test). Our results agree with those of other Authors [14] who used Palcam culture medium to isolate Listeriae. These Authors conclude that the results obtained comparing a new test with a standard test depend on sensitivity and selectivity of the medium used for the isolation. The difference in the responses of *Salmonella* and *Listeria* may be explained by the fact that the Salmonella Rapid Test combines enrichment and selective growth in a single culture vessel reducing only the time taken by traditional culture method, while the Listeria Rapid Test modifies the approach of detection of this microrganism being based on the immunoassay technology.

Finally, we reported the results of poultry sampled on Monday and Friday for six months in Table III to compare the level of contamination, supposing that in the products not sold on Monday and stored through the week the number of the bacteria investigated increased. For this purpose the packaging date of the products in the shelf was considered to distinguish fresh packed products from stored ones. This comparison regards only the results of the conventional procedure. The trend found indicates an increase in positive results from Monday to Friday both for *Salmonella* and *Listeria* spp. The total positive results for *Salmonella* were increased of 4.9% (not significant difference at chi-square test) while the presence of Listeriae on total samples shifted from 44% on Monday to 58% on Friday with a significant difference with chi-square = 6.86 (p = 1%) confirmed by Yates correction (p = 5%). This might be

Tab. II. *Salmonella* and *Listeria* Rapid Test results and their statistical evaluation in comparison with microbiological test for 362 poultry samples.

	Rapid Test results	Microbiological method result		Sensitivity (%)	Specificity (%)	Efficiency (%)	Predictive value (%)		k coefficient
		+	-				Positive test	Negative test	
Salmonella	+ (54)	39 (a)	15 (b)	75	95	92	72	96	0.68
	- (308)	13 (c)	295 (d)						
Listeria	+ (192)	172 (a)	20 (b)	93	89	91	90	92	0.82
	- (170)	13 (c)	157 (d)						

(a) true positive; (b) false positive; (c) false negative; (d) true negative

Tab. III. Presence of *Salmonella* spp. and *Listeria* spp. in the poultry products sampled on Monday and on Friday using the conventional method.

Type of sample	Tested samples	Positive samples (percentage)	
		Salmonella	*Listeria*
Chicken carcasses			
Monday	50	8 (16%)	29 (58%)
Friday	46	16 (35%)	33 (72%)
Chicken parts			
(wings and legs)			
Monday	88	10 (11%)	39 (44%)
Friday	88	15 (17%)	52 (59%)
Processed chicken products			
(sausages and hamburgers)			
Monday	39	3 (8%)	10 (26%)
Friday	51	0	22 (43%)

due to listeria's ability to survive in refrigerated conditions for long time. In particular, the contamination rate of the carcasses and chicken parts increased during the week both for *Salmonella* (from 16 to 35% for carcasses and from 11 to 17% for chicken parts) and *Listeria* (from 58 to 72% and 44 to 59% respectively) while the contamination of the further processed products increased only for *Listeria* (from 26 to 43%), according to the hypothesis above reported on processed samples. In fact, the detection of Salmonellae on Monday was of 8% and decreased to 0% on Friday demonstrating that the survival of this pathogen is strongly conditioned by factors such as the water activity (NaCl) and the antimicrobials (spices and herbs) used in the production of sausages and hamburgers.

In conclusion, the results of the present study, in addition to the above discussion on the evaluation and comparison of cultural and rapid methods, indicate that poultry products could still be considered a potential risk for human health. Good hygienic practices should be observed at all times of slaughter, processing and delivery of poultry. Proper hygiene should minimize exposure of carcasses to faecal materials and pathogens such as *Salmonella* and *L. monocytogenes*, among others, and reduce opportunities for the extent of occurrence of contamination and cross-contamination. Furthermore, efforts should be made to inform consumers about storage time and temperature, handling and correct cooking to avoid that poultry product may be at risk of illness.

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