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EFFECT OF DISINFECTANTS ON DIFFERENT STRAINS OF *LISTERIA MONOCYTOGENES*

RADISLAVA TEODOROVIĆ, MIRJANA BUGARSKI, V. TEODOROVIĆ, BRANA RADENKOVIĆ AND M. MIRILOVIĆ

Faculty of Veterinary Medicine, Belgrade, Yugoslavia

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The objective of this work was to determine which of the examined disinfectants represents the agent of choice for isolates of L. monocytogenes originating from foodstuffs of animal origin and if there are differences regarding sensitivity associated with specific serotypes.

Disinfectant A (sodium hypochlorite + phosphates + alkali) did not exhibit a listericidal effect on any examined serotype, while disinfectants B (10 % hydrochloride of 1- dodecyl - 1.4.7- triazooctane - 8- carbonic) and C (acidic iodoform with tenside- iodine complex)) exhibited a listericidal effect against most strains. Desinfectant B had the greatest effect at the highest working concentration (2 %) with a very significant difference (p<0.01) in comparason with the listericidal effect of disinfectant C.

Analysis of the sensitivity of different serotypes of L. monocytogenes showed that, despite evident higher or lower sensitivity to disinfectant B, the antigenic structure (seroype) was not related to the sensitivity of the examined strains. However, with disinfectant C, the greatest listericidal effect was exhibited on serotypes 3a and 1/2b, while it was smaller for other serotypes of L. monocytogenes. Statistically very significant differences (p<0.01) and significant differences (p<0.05) were detected between the mean inhibition zones of most serotypes of L. monocytogents.

Therefore, the sensitivity of different strains of L. monocytogenes originating from foodstuffs of animal origin to disinfectants can be related to the antigenic structure, actually the serotype (C) or not (disinfectant B)

Key words: Listeria monocytogenes, disinfectants, serotypisation, sensitivity.

INTRODUCTION

The first incidence of listeriosis in humans was described in 1929 (James, 1995). With a series of outbreaks of several epidemics in North America in the eighties, it was generally accepted that food is the main source of this disease and the vector of the infection in humans (Gahan et al., 1991, Pinner et al., 1992, Schuchar et al., 1992). It is known that *L. monocytogenes* not only causes listeriosis, but also creates a predisposition for the occurrence of other diseases as secondary infections. Healthy individuals can also be carriers of Listeria (Farber and Petercin, 1991). The incidence of listeriosis in humans occurring through foodstuffs of animal origin is linked with insufficient knowledge of pathogenic bacteria which can be conveyed through food and cause diseases with a high mortality rate. These bacteria have the ability to be widely distributed in raw products (Bunčić, 1991, MacGowan et al., 1994, Sheridan et al, 1994, Dimitrijević, 1998), to grow at low temperatures (Dimitrijević el al., 1999), as well as to appear in different places of the production process. Their presence in food can result from any of the following: postprocess contamination in the production environment, cross-contamination due to manipulation of food, contact with surfaces contaminated by other food in the area of storage.

The wide distribution of *L. monocytogenes* in nature, probably makes it unrealistic completely to rule out initial contamination of foodstuffs of animal origin with this microorganisam. This indicates the extreme importance of sound knowledge of the factors which can reducte its presence in food.

With the objective to reduce as much as possible listeria that might be present in the production environment and on contaminated surfaces, we tried to provide two answers in this work. Firstly, which of the chosen disinfectants (of different chemical nature) would represent the agent of choice for listeria isolates originating from foodstuffs of animal origin. Secondly, to determine whether there are differences, and if so, their magnitude, between different serotypes of *L. monocytogenes,* regarding sensitivity to the chosen disinfectants.

MATERIALS AND METHODS

The examination included disinfecting agents: A (sodium hypochlorite+phosphates+alkali); B (10 % hydrochloride of 1-dodecyl-1,4,7-triazooctane-8-carbonic acid); C (acidic iodoform with tenside-iodine complex) in concentrations as manufactured. They were applied to 48 strains selected from a collection of *L. monocytogenes* strains isolated from food of animal origin. The strains were isolated and identified from foodstuffs of animal origin by the method of McLain and Lee (1989). The standard agar gel diffusion method (with wells) was used as the inhibition test. Every type was assessed in triplicate in order to provide adequate accuracy and reproducibility of the data. The results were expressed as the mean values for inhibition zone diameter (mm) for each strain. The statistical significance of differences between procedures and strain origin was calculated by the random plan method using statistical software (Statgrafics, 5,0; Statistical Graphic Corporation USA).

RESULTS AND DISCUSSION

A collection of different strains of *L. monocytogenes* was made from isolates of foodstuffs of animal origin. This collection (Table 1) included 48 strains originating from: ground meat (pork, beef, mixed) vacuum packed frankfurters, raw sausages (pork-beef), chicken, fish, fresh milk, and cheeses.

The sensitivity of *L. monocytogenes* strains to the tested disinfectants is presented in Table 1.

Table 1. Sensitivity of *L. monocytogenes strains,* foodstuffs to the selected disinfecting agents shown by inhibition zone diameter (mm)

Code	B_1	B_2	B_3	C
	zone ± SD	zone ± SD	zone ± SD	zone ± SD
48	2.67 ± 0.52	3.67 ± 0.52	4.67 ± 0.51	8.67 ± 0.51
53	3.00 ± 0.00	3.50 ± 0.55	4.50 ± 0.55	2.67 ± 0.52
54	4.00 ± 0.00	5.67 ± 0.52	7.00 ± 0.00	3.00 ± 0.00
55	2.83 ± 0.41	4.83 ± 0.41	5.67 ± 0.52	7.00 ± 0.00
56	3.33 ± 0.52	4.67 ± 0.52	4.83 ± 0.98	11.17 ± 0.98
57	3.33 ± 0.52	4.50 ± 0.55	5.67 ± 0.82	4.60 ± 0.55
58	3.00 ± 0.00	3.83 ± 0.41	6.00 ± 0.00	4.66 ± 0.82
59	4.00 ± 0.00	4.50 ± 0.55	4.66 ± 0.52	3.00 ± 0.00
60	3.50 ± 1.05	5.00 ± 1.10	7.20 ± 0.75	4.66 ± 0.82
61	3.50 ± 0.55	5.16 ± 0.75	7.00 ± 0.89	2.33 ± 0.82
62	5.00 ± 0.00	4.66 ± 0.82	6.00 ± 0.00	0.00 ± 0.00
63	3.00 ± 0.63	3.50 ± 0.55	3.83 ± 0.75	4.00 ± 0.00
66	5.00 ± 0.00	5.83 ± 0.75	7.00 ± 0.00	0.00 ± 0.00
67	2.16 ± 0.41	3.33 ± 0.82	4.50 ± 0.55	6.50 ± 0.55
68	4.00 ± 0.00	3.83 ± 0.41	5.66 ± 0.82	10.16 ± 0.41
69	4.00 ± 0.00	5.33 ± 0.52	6.00 ± 0.00	2.66 ± 0.52
70	3.50 ± 0.84	5.00 ± 0.00	5.66 ± 0.82	3.00 ± 0.00
71	4.00 ± 0.00	3.83 ± 0.41	7.00 ± 0.00	0.00 ± 0.00
72	3.00 ± 0.00	3.33 ± 0.52	5.33 ± 0.52	0.00 ± 0.00
74	2.50 ± 0.84	3.17 ± 0.41	5.00 ± 0.00	6.50 ± 0.55
77	3.00 ± 0.00	4.00 ± 0.00	4.66 ± 0.82	0.00 ± 0.00
78	2.83 ± 0.75	3.83 v 0.41	5.33 ± 0.52	3.00 ± 0.00
79	4.00 ± 0.00	4.66 ± 0.52	5.00 ± 0.63	2.00 ± 0.00
82	3.00 ± 0.63	4.00 ± 0.00	5.00 ± 0.63	0.00 ± 0.00
83	4.66 ± 0.51	5.00 ± 0.00	5.50 ± 1.05	2.33 ± 0.52
84	5.00 ± 0.00	6.50 ± 0.55	6.00 ± 0.00	7.16 ± 0.75
90	4.00 ± 0.63	5.50 ± 0.55	6.16 ± 0.75	2.16 ± 0.41
91	3.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	2.33 ± 0.52
92	2.66 ± 0.82	4.83 ± 0.75	5.50 ± 0.55	7.50 ± 0.55
125	3.00 ± 0.00	4.33 ± 1.03	6.00 ± 0.00	7.00 ± 0.00

127	5.00 ± 0.00	4.16 ± 0.75	5.33 ± 0.52	7.33 ± 0.52
130	3.00 ± 0.00	4.33 ± 0.52	4.33 ± 0.52	3.00 ± 0.00
134	3.33 ± 0.52	5.00 ± 0.00	5.33 ± 0.52	7.00 ± 0.00
167	5.00 ± 0.00	6.00 ± 0.00	5.50 ± 1.05	4.66 ± 0.52
168	4.33 ± 0.52	5.00 ± 0.00	5.33 ± 1.03	0.00 ± 0.00
169	4.00 ± 0.00	7.16 ± 0.98	8.00 ± 0.00	2.00 ± 0.00
170	4.00 ± 0.00	4.33 ± 0.52	4.33 ± 0.52	0.00 ± 0.00
171	2.50 ± 0.55	5.00 ± 0.00	4.50 ± 0.55	6.00 ± 0.00
70172	4.00 ± 0.00	5.00 ± 0.00	6.00 ± 0.00	2.33 ± 0.52
70313	4.00 ± 0.89	4.33 ± 0.52	5.00 ± 0.00	0.00 ± 0.00
70550	5.00 ± 0.00	5.33 ± 1.03	7.33 ± 1.03	0.00 ± 0.00
70592	2.66 ± 0.82	3.00 ± 0.00	4.00 ± 0.00	0.00 ± 0.00
70620	4.00 ± 0.00	4.50 ± 0.55	5.50 ± 0.55	2.50 ± 0.55
70708	3.00 ± 0.00	4.33 ± 0.52	6.00 ± 0.00	0.00 ± 0.00
70722	3.14 ± 0.69	5.67 ± 0.82	5.33 ± 0.52	4.50 ± 0.55
70841	5.00 ± 0.00	5.67 ± 0.82	6.33 ± 0.52	2.00 ± 0.00
70916	5.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	3.00 ± 0.00
71062	6.00 ± 0.00	4.33 ± 0.52	4.50 ± 1.05	2.67 ± 0.52
	3.69 ± 0.96	4.61 ± 0.99	5.53 ± 1.07	4.56 ± 2.48

B1 - 0,5% working solution;
B2 - 1.% working solution;
B3 - 2% working solution;
C - 0.3% working solution;

Disinfectant A did not have an inhibitory effect on any examined strain, so no data are presented in the table. The other disinfectants showed a listericidal effect on a different number of strains.

Disinfectant B exhibited a listericidal effect on all examined strains. With an increased concentration of the working solution, the listericidal effect also increased, and the highest effects (5.53 ± 1.07) were achieved with the highest concentration (2 %). Statistical analysis of the results obtained for listericidal effect of 0,5 %, 1 % and 2 % working solutions of disinfectant B showed very significant differences ($p<0.01$). Contrary to disinfectant B, disinfectant C exhibited an inhibitory effect only on 36 strains of *L. monocytogenes.* The average inhibition zone for sensitive listeria strains was 4,56 ± 2,48.

A comparison of the results for the highest inhibitory effects of disinfectant B (2% solution) and disinfectant C (0.3% solution) showed a very significant difference ($p<0.01$) between the mean diameters of inhibition zones.

In order to identify sources of infection in epidemiological - epizootiological investigations, numerous techniques of listeria typization have been developed for food, as well as other possible sources of these bacteria. Of all typization methods, the greatest attention is paid to serological typization which essentially defines the basic characteristics of the antigens present on and in listeria. Numerous authors have developed reference methods for serotypization of listeria, and have also presented the main characteristics of antigens present in *Listeria spp.* (Seeliger and Hones, 1986, Boerlin and Piffaretti, 1991, Norrung and

Gerner-Sidt, 1993). With different combinations of somatic and flagellated antigens, 16 serotypes of *L. monocytogenes* and related species have been defined.

In these investigations, eight serotypes were detected for isolates of L. monocytogenes originating from foodstuffs (Table 2).

Most strains belonged to serotype 1/2a (43.75%) and serotype 1 (22.92%), while the arrangement of the other strains did not differ essentially.

Table 2. Serotypization of L. monocytogenes strains from foodstuffs

Serotyp	Code				Number of strains	%
	54	60	70	127		
1	56	66	74	130	11	22.92
	59	67	125			
	48	77	90	70620		
	53	78	91	70708		
1/2a	57	79	92	70916	21	43.75
	61	82	134			
	62	83	70172			
	71	84	70313			
1/2b	171	70550			2	4.17
1/2c	169	70592		71062	3	6.25
3a	55				1	2.08
3b	167				1	2.08
	58	68	72			
4					5	10.41
	63	69				
	168	70722		70841		
4b					4	8.33
	170					

An analysis of the sensitivity of different serotypes of *L. monocytogenes* showed that all serotypes were more or less sensitive to disinfectants B and C. Regarding sensitivity to disinfectant B (2% solution) there were statistically significant differences ($p<0.05$) between strains, which were evidently big, but the arithmetic means of inhibition zones for serotypes were equal (Table 3).

It is thus evident that the antigenic structure (serotype) has no significance, when disinfectant B is concerned, regarding sensitivity of the examined strains of *L. monocytogenes* to its action.

With disinfectant C, where a listericidal effect was recorded in 36 strains, greater variations were found regarding sensitivity. Disinfectant C showed the greatest inhibitory effect on serotype 3a and 1/2b, while the smallest inhibitory effect was exhibited on serotype 1/2c. Statistical processing of data (using the LSD test) showed that there were very significant differences ($p<0.01$) and significant differences ($p<0.05$) between arithmetic means of inhibition zone diameters for most serotypes (Table 4).

Table 3. Non-significant differencesion between the sensitivity of *L. monocytogenes* serotypes to disinfectant B (2% solution)

Croups	X	4b	4	1/2c	3b	1/2a	1	3a	1/2b
1/2b	5.92	0.58	0.55	0.42	0.42	0.40	0.33	0.25	-
3a	5.67	0.33	0.30	0.15	0.17	0.15	0.08	-	
1	5.59	0.26	0.22	0.09	0.09	0.07	-		
1/2a	5.52	0.18	0.14	0.02	0.02	-			
3b	5.50	0.17	0.13	0.00	-				
1/2c	5.50	0.17	0.13	-					
4	5.37	0.03	-						
4b	5.33	-							

Table 4. Statistically significant dofferencesi of *L. monocytogenes* serotypes sensitivity to disinfectant C (3% solution)

Croup	X	1/2c	4b	1/2a	3b	4	1	1/2b	3a
3a	7.00	4.67**	3.75**	3.03**	2.33**	1.83*	1.48	1.00	-
1/2b	6.00	3.67**	2.75**	2.03**	1.33*	0.82	0.48	-	
1	5.52	3.18**	2.67**	1.55*	0.85	0.34	-		
4	5.17	2.84**	1.92*	1.21	0.51	-			
3b	4.67	2.33*	1.41	0.70	-				
1/2a	3.97	1.63*	0.72	-					
4b	3.25	0.92	-						
1/2c	2.33	-							

The greatest differences between these means ($p<0.01$) were found between serotypes 3a and 1/2c, 3a and 4b, 3a and 1/2a, 3a and 3b. Thus, when this disinfectant is concerned, antigenic structure has significance regarding sensitivity of the examined strains of *L.monocytogenes.* However, one should have in mind that the greatest sensitivity to disinfectant C was registered in those serotypes which are relatively rarely present in foodstuffs (3a and 1/2b), while a significantly smaller sensitivity was registered in those serotypes which cover the largest number of strains of L. monocytogenes occuring in foodstuffs of animal origin (1/2a, 1).

On the basic of the analyzed results, it can be said that the sensitivity of different strains of *L. monocytogenes* originating from foodstuffs of animal origin can be determined both by the disinfectant which is used (B), and with its antigenic structure, or serotype (C). On the grounds of the type, its could be possible in advance to determine possibilities for applying corresponding antiisterial agents in order to prevent possible contamination, or infection.

In order to reduce to a minimum the risk to humans of listeriosis originating from foodstuffs of animal origin, or to neutralize it completely, it is necessary to apply the HACCP concept (Hazard Analysis Critical Control Point) in production, which identifies and controls every " critical point", i. e. place in the production process which could be contaminated with a pathogenic microorganisam, where disinfection also has an important place (Van den Elzen aznd Snijders, 1993, Dimitrijević, 1998).

REFERENCES

1. *Boerlin P., Piffaretti J. C.*, 1991. Typing of human, animal food and environmental isolates of Listeria monocytogenes by multilocus enzyme elestrophoresis. Appl. Envirom. Microbiol., 57, 1629-1642.

2. *Bunčić S.*, 1991. The incidence of Listeria monocytogenes in slaughtered animales, in meat, and in meat products in Yugoslavia. Int. J. Food. Microbol., 12, 173-180.

3. *Dimitrijević M.* 1998. Ispitivanje varijacija između sojeva Listeria monocytogenes u pogledu osetljivosti prema bakteriocinima mlečnokiselinskih bakterija. Magistarska teza, Beograd.

4. *Dimitrijević Mirjana, Teodorović V., Baltić M., Mirilović M.,* 1999. Variations in the sensitivity of listeria monocytogenes types to lactic acid bacteria bacteriocins. Acta Veterinaria, 49 (1), 49-56.

5. *Farber J. M., Petrkin P. I.,* 1991. Liisteria monocytogenes, a foodborne pathogen. Microbio. Rev., 55, 476-511.

6. *Gahan C. G. M., Collins J. K.,* 1991. Listeriosis: biology and implications for the food industry. Trends Food Sci. Technol., 2, 89-93.

7. *MacGowan A. P., Bowker K., McLauchlin J., et al.,* 1994. The occurence and seasonal changes in the isolation of Listeria spp. in shop bought food stuffs, human feces sewage and soil from urban sources. Int. J. Food. Microbio., 21, 325-334.

8. *McLain D., Lee W., H.,* 1989. Isolation and identification of Listeria momocytogenes from meat. USDA (FSLS) Microbiol. Dcu. Ib. Comm. N° 57.

9. *Norrung B., Gerner-Sidt P.*, 1993. Comparation of multilocus enzyme electrooophoresis (MEE), ribotyping, restriction enzyme analysis (REA) and phage typing of Listeria monocytogenes. Epidemiology and Infection, 111, 71-89.

10. *Pinner R. W., Schuchat A., Swaminathan B., Hayes P. S., Deaver K.a., Weaver R. E., Plikaytis B. D., Reeves M., Broome C. V. and Wenegwr J.* D., 1992. Role of foods in sporadic listeriosis. II Microbiologic and epidemiologic investigation. JAMA 267,2046-2050.

11. *Schuchat A., Deaver K. A.,Wenger J. D., Plikaytis B. D., Mascola L., Pinner R. W., Reingold A. L. and Broome G. V.,* 1992. Role of foods in sporadic listeriosis.I case-control study of dietary risk factors. JAMA 267, 2041-2045.

12. *Seeliger H. P. R., Jones D.*, 1986. Listeria.Bergeys Manuel of Systematic Bacteriology (Eds Sneath, P. H. A., N. S., Mair and Sharpe), Williams and Wilkins, Baltimore, MD, USA, Vol.2, 1235-1245.

13. *Sheridan J. J., Duffy G., McDowell D. A. and Blair I. S.,* 1994. The occurrence and initial numbers of Listeria in Irish meat and fish products and the recovery of injured sells from frozen products. Int. J. Food Microbiol., 22, 105-113.

14. *Van den Elzen A. M. G., Snijders, J. M. A.,* 1993. Critical points in meat production lines regarding the introduction of Listeria monocytogenes. Vet. Q., 15 m, 143-145.

UTICAJ DEZIFICIJENSA NA RAZLIČITE SOJEVE *LISTERIA MONOCYTOGENES*

RADISLAVA TEODOROVIĆ, MIRJANA BUGARSKI, V. TEODOROVIĆ,
BRANA RADENKOVIĆ i M. MIRILOVIĆ

SADRŽAJ

Cilj ovog rada je da odgovori na dva vrlo značajna pitanja: prvo, koji od ispitivanih dezinficijenasa ispoljava najbolji dezinfekcioni efekat na sojeve Listeria monocytogenes, poreklom iz namirnica animalnog porekla i drugo, da li u pogledu osetljivosti *L. monocytogenes* prema ispitivanim dezinficijensima postoje razlike uslovljene serotipskom pripadnošću.

Dezificijens A (natrijum hypochlorit+phosphati+alkalija) nije ispoljio listericidni efekat ni prema jednom ispitivanom serotipu, dok su dezificijensi B (10% hydrochloride 1-dodecil-1,4,7-triazooctan-8-carbonska kiselina) i C (kiseli iodoform sa tensid-iodnim kompleksom) ispoljili listericidni efekat prema većem broju sojeva. Dezificijens B ispoljio je najviši baktericidni efekat pri najvišoj radnoj koncentraciji sa statistički vrlo značajnom razlikom ($p<0,01$) u odnosu na listericidni efekat dezificijensa C.

Analizom rezultata osetljivosti različitih sojeva *Listeria monocytogenes* utvrđeno je da i pored evidentne ispoljene osetljivosti listerija prema dezificijensu B, antigensa struktura odnosno, serotip nema većeg značaja u pogledu osetljivosti ispitivanih sojeva. Međutim dezificijens C, najveći listericidni efekat ispoljio je prema listerijama serotipa 3a i 1/2b, dok je prema ostalim serotipovima bio manji. Između aritmetičkih sredina zona inhibicije većine serotipova, utvrđene su statistički vrlo značajne razlike ($p<0,01$), odnosno značajne razlike ($p<0,05$).

Na osnovu dobijenih rezultata može se zaključiti da je osetljivost različitih sojeva *L. monocytogenes* poreklom iz namirnica animalnog porekla bila ispoljena prema dezinficijensu B, kao i da osetljivost listerija prema ispitivanim dezinficijensima zavisi od njihove antigenske strukture ondosno serotipske pripadnosti.