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Isolation of *Listeria monocytogenes* from Smoked Cheese

Nageb Al-Zoreky[1]

Department of Food Science, Faculty of Agriculture, Sana'a University, Sana'a, Yemen

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Abstract. Samples of smoked, salted cheese were collected from local markets of the city of Taiz. They were tested for the prescence of *Listeria monocytogenes* . Data revealed that the pathogen was only recovered from 12.5% of cheese samples analyzed under this study. Other listerial species were not isolated from cheese, directly plated onto selective agar media . Direct plating gave very low counts (20 CFU/g or less) of *L. monocytogenes*, naturally contaminated smoked cheese. Selective enrichment of samples at 37°C for 48 hr was necessary for isolating *Listeria spp.*, present in cheese along with other co-contaminating microflora. The natural microflora of smoked cheese, especially Enterococci and Staphylococci, grew or developed diagnostic traits similar to those of *L. monocytogenes* in Listerial selective agar (Oxoid) . When increased volumes (>0.2ml) of samples were spread, Alzoreky- Sandine Listeria Medium (ASLM) recovered contaminated listeriae while inhibiting indigenous microorganisms of cheese. Results of this investigation emphasize the need to adopt a standard procedure for manufacturing smoked cheese in Yemen.

Introduction

Food-borne diseases of humans by *L. monocytogenes* was recogonized for the first time in the 1980's [1; 2], before which the bacterium was only known as a pathogen for animals. The organism has psychrotrophic properties enabling it to grow and multiply under refrigeration temperatures (2°-5°C). As a matter of fact, cold enrichment technique is used in laboratories as a selective method to isolate the pathogen from contaminated food [3; 4]. Being widely distributed in nature, *L. monocytogenes* has been isolated from water, soil, sewage, food plants & restaurants [5; 6]. Furthermore, many types of food such as dairy products, vegetables, meat and seafood have been incriminated as vehicles of *L. monocytogenes* [7- 9]. Ironically, it was clearly stated that *L. monocytogenes* survived pasteurization of milk when cells were engulfed by leucocytes but later on well-monitored, laboratory tests proved that the organism was not

[1] Corresponding address: Ma'aeen Post Office, P.O. Box 13367, Sana'a, Yemen

viable after proper pasteurization [10]. Pearson & Marth [2] and Farber & Peterkin [11] discussed in details the properties of *L. monocytogenes* .

Symptoms of Listeriosis include flue-like symptoms, abortion , septicemia and meningitis . The mortality rate was reported to be as high as 30 % and could be even higher in immunocompromised people such as infants, pregnant women and cancer & AIDS patients. In fact, foodborne illnesses cause loss of productivity and outstanding expenses for health care [12] . Even though contamination of food with *L. monocytogenes* could be minimized by enforcing food standards & specifications, traditional food industry in Yemen is not regulated by appropriate safety guidelines . Information is also not available on incidents of *L. monocytogenes* in traditional food attracting consumers. Therefore, the objective of this study was to screen a traditional cheese for presence of *L. monocytogenes* . It is made primarily from goats' milk (raw) by some villagers under unsanitary conditions. The product is considered as a semi-hard cheese with about 40% moisture content and characterized as a salted cheese with an attractive light brown color imposed by smoking.

Materials and Methods

Eight samples of smoked, salted cheese were collected over a three-month period from local markets in Taiz. Samples were transported to the laboratory and tested immediately or kept refrigerated overnight before analyses. Twenty-five gram portions of each representative sample were aseptically weighed and homogenized with 0.1 % sterile, peptone water (Difco, USA) in a pre-sterilized Waring blender (model 34PL99, Dynamic Corp., U.S.A.). Serial dilutions were prepared using the aforementioned diluent. Recovery of *L. monocytogenes* as well as other *Listeria spp.* from cheese was carried out using direct plating (0.1- 0.5 ml) of diluted samples onto Listeria Selective Medium (Oxoid, UK) and ASLM [13; 14]. Selective enrichments of cheese in enrichment broth [15, p. 5] with no alkaline treatment were incubated at 37°C for seven days, during which samples were spread onto listerial, isolation agar mentioned earlier . Five suspected colonies were further purified by streaking onto tryptic soy agar and then identification was confirmed according to the procedure of Lovett [15, p. 5], using *L. monocytogenes* (Jalisco) as a control. Aerobic plate count (APC) of cheese was also made using the method of Messer *et al.* [16, p. 133]. Statistical analyses of the obtained results were done using the Minitab program [17] .

Results and Discussion

Data presented in Table 1 show incidents of listerial isolates in all samples tested. Only 12.5% of samples were positive for presence of persumptive *L. monocytogenes* and the level of contamination was 20 CFU/g. On the other hand, numbers of cells in the remaining samples screened were below detection level (<10 CFU/g). Variability of *L.*

monocytogenes counts among samples could be justified since samples were derived from a commodity made by different producers under unspecified standards . Stress or sub-lethal injury of listerial contamination could be responsible for underestimation of *L. monocytogenes*. Accordingly, that may suggest ineffectiveness of direct plating in recovering *L. monocytogenes* from cheese containing unculturable listeriae. Some investigators experienced also unsuccessful, direct isolation of *Listeria spp.* from specific types of cheeses without enrichment of tested samples [18; 19]. Obviously, no correlation existed between incidents of listerial organisms and APCs of samples (Table 1). Irrespective of the aerobic counts, Tran *et al.* [20] indicated that detection sensetivities of *L. monocytogenes* from food were dependent on types of mesophilic microorganisms out-numbering *L. monocytogenes*. Meanwhile, differences in APCs (10^5->10^7 CFU/g) were not uncommon since production of smoked cheese was carried out by manufacturers that did not follow the same method of production . It should be emphasized that direct microscopic examination of suspected colonies grown on listeria selective agars was misleading because cells of *L. monocytogenes* exhibited abnormal morphology . Similar observations were reported by Loessner *et al.*[21].

Table 1. Distributions of *Listeria monocytogenes* in smoked cheese, directly plated onto selective media[a]

Samples	*Listeria monocytogenes* (CFU/g)	Aerobic plate count (CFU/g)[c]
1	ND[b]	$3.1x10^7$
2	ND	$5.2x10^6$
3	20	$4.7x10^6$
4	ND	$2.6x10^7$
5	ND	$2.9x10^6$
6	ND	$2.25x10^7$
7	ND	$4.5x10^6$
8	ND	$9.6x10^5$

[a] 0.1- 0.5 ml spread onto Oxoid and Alzoreky- Sandine Listeria Medium
[b] not detected (<10 CFU/g)
[c] average of triplicates

Table 2 summarizes outcomes of selective enrichment of screened samples on recovery of *L. monocytogenes* in prescence of competitive organisms . Selective enrichments examined after 24 hr resulted in better isolation ($P < 0.05$) of *Listeria spp.* (37.5% positive samples) in comparison with direct plating alone (12.5%). However, persumptive *L. monocytogenes* was only detected in 25% of tested cheese (Table 2) . Even though they were not the same product, selective enrichment provided lower listerial, isolating rates from ewe and goat cheeses [18; 19]. Interestingly, *L. grayi* was recovered from a sample of cheese tested (Table 2).

Table 2. Isolation pattern of *L. monocytogenes*, naturally contaminated smoked cheese[a]

Listeria species	% Positive samples[b]
L. monocytogenes	25
Other *Listeria spp.* [c]	12.5
Total	37.5

[a] Samples were subjected to selective enrichment at 37°C for 48 hr before spread plating onto Oxoid & ASLM
[b] Samples had <10 CFU/g *L. monocytogenes* prior to selective enrichment
[c] *L. grayi* isolate

It was evident that smoked cheese harbored variable levels and species of listeriae , depending on sources of cheese and exposure to cross contamination. Selective enrichment of cheese for 48 hr gave inflated counts of persumptive *L. monocytogenes* on Oxoid agar (data not shown). Oxoid medium has recently been used by the FDA for better recovery of *L. monocytogenes* from food . However, previous works indicated occasional growth of co-contaminating bacteria of food viz. Enterococci and Staphylococci on Oxoid agar [4; 7; 13; 14; 18]. On the other hand, ASLM inhibited non-listeriae of cheese but lower counts of *L. monocytogenes* were not detected when reduced volumes of samples (<0.2ml) were used.

In general, lower counts of *L. monocytogenes* in smoked cheese screened under this study may be related to intrinsic factors of this type of food e.g.% moisture, pH, % salt and bacteriocins of background flora . In addition, the cheese was subjected to smoke which was stated to contain antilisterial factors [1; 11]. It is worthwhile to mention that failure in detecting *L. monocytogenes* in some samples may not ruled out the possibility of contamination of cheese with certain listeriae inhibited in both solid and liquid media [3; 4; 7; 13] used for isolation. Finally, it can be concluded that a standard method should be followed by cheese makers for production of improved and safer cheese in accordance with it's traditional characteristics.

References

[1] El-Gazzar, F.E. and Marth, E.H. " *Listeria monocytogenes* and Listeriosis Related to Milk and Milk Products and Dairy Ingredients." *Milkwissenschaft.*, 46, No. 2 (1991), 82-86.

[2] Pearson, L.J. and Marth, E.H. " *Listeria monocytogenes* - Threat to a Safe Food Supply: A Review." *J.Dairy Science,* 73, No. 4 (1990), 912-928.

[3] Cassiday, P.K. and Brackett, R.E. " Methods and Media to Isolate and Enumerate *L. monocytogenes* : A Review." *J. Food Protection,* 52, No. 3 (1989), 207-214.

[4] Schiemann, D.A., Shope, S.R. and Brown, M.J. " Development of New Enrichment Broths and Plating Agars for Isolation of Hemolytic Species of *Listeria*." *J. Food Safety,* 10 (1990), 233-252.

[5] Cox, L.J., Kleiss, T. and Cordier, J.L. "*Listeria* in Food Processing, Non-Food and Domestic Environments." *Food Microbiology,* 6, No. 1 (1989), 49-61.

[6] Kozak, J.J." Regulatory Responses to the Problems of Pathogenic Bacteria in the Dairy Industry." *J.Dairy Science*, 71, No. 10 (1988), 2817-2819.

[7] Northolt, M.D. " Recovery of *L. monocytogenes* From Dairy Products Using the TNCB-TNSA Method and the Draft IDF Methods." *Neth. Milk Dairy J.*, 43 (1989), 299-310.

[8] Al-Mohizea, I.S. " Microbiological Studies on Some Salad Vegetables in Local Markets." *J. King Saud Univ., 8, Agric. Sci.*,(1) (1996), 99-106.

[9] Destro, M.T., Serrano, A.deMelo and Kabuki, D.Y. " Isolation of *Listeria* Species From Some Brazilian Meat and Dairy Products." *Food Control,* 2, No. 2 (1991), 110-112.

[10] Lovett, J., Wesley, I.V., Donnely, C.W. and Messer, J.W. " High-Temperature Short Time Pasteurization Inactivates *L. monocytogenes*." *J. Food Protection,* 53, No. 9 (1990), 734-738.

[11] Farber, J.M. and Peterkin, P.I. "*Listeria monocytogenes*, A Food-Borne Pathogen." *Microbiol. Reviews,* 55, No. 3 (1991), 476-511.

[12] Roberts, T. "Bacterial Food-Borne Illnesses Costs Put At $ 4.8 Billion." *Food Chemical News,* 31, No. 22 (1989), 41-42.

[13] Curtis, G.D., Mitchel, R.G., Andrea, F.K. and Emma, J. " A Selective Differential Medium for the Isolation of *L. monocytogenes* ." *Letters in Appl. Microbiology*, 8 (1989), 95-98.

[14] Al-Zoreky, N. and Sandine, W.E. "Highly Selective Medium For Isolation of *L. monocytogenes* from Food." *Appl. and Environmental Microbiology*, 56, No. 10 (1990), 3154-3157.

[15] Lovett, J. "*Listeria* Isolation." *U.S. FDA Bacteriological Analytical Manual*, Association of Official Analytical Chemists (Suppl.), Arlington, Virginia, 1987.

[16] Messer, J.W., Behney, H.M. and Leudecke, L.O. "Microbiological Count Methods."*Standard Methods For the Examination of Dairy Products*." G.H. Richardson (Ed.). Washington, D.C.: American Public Health Association, 1985.

[17] Minitab Statistical Program. *Reference Manual, Macintosh Version.* Quickset Inc., Rosemont, PA, USA, 1991.

[18] Van Netten, P., Perales.I., Curtis, G.D. and Mossel,D.A. "Liquid and Solid Selective, Differential Media For the Detection and Enumeration of *L. monocytogenes* and Other *Listeria spp.*"*Int. J. Food Microbiology*, 8 (1989), 299-316.

[19] Greenwood, M.H., Roberts, D. and Burden, P. "The Occurrence of *Listeria spp.* in Milk And Dairy Products : A National Survey in England and Wales." *Int. J. Food Microbiology*, 12 (1991), 197-206.

[20] Tran, T.T., Stephenson, P. and Hitchins, A.D. "The Effect of Aerobic Mesophilic Microfloral Levels on the Isolation of Inoculated *L. monocytogenes* Strain LM 82 From Selected Foods." *J. Food Safety*, 10 (1990), 267-275.

[21] Loessner, M.J., Bell, R.H., Jay, J.M. and Shelef, L.A."Comparison of Seven Plating Media For Enumeration of *Listeria. spp.*"*Appl. and Environ. Microbiology*, 54, No. 12 (1988), 3003-3007.

عزل ليستيريا مونوسيتوجينيس من الجبنة المدخنة

نجيب الزريقي

قسم علوم الأغذية، كلية الزراعة، جامعة صنعاء

صنعاء، الجمهورية اليمنية

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ملخص البحث. تم جمع عينات من الجبنة المدخنة (المملحة) من الأسواق المحلية لمدينة تعز. أجريت الاختبارات على الجبنة للكشف عن ليستيريا مونوسيتوجينيس. كشفت البيانات بأن البكتيريا الممرضة وجـــدت في ١٢,٥٪ مـــن عينات الجبنة التي تم اختبارها في هذه الدراسة. لم يتم عزل أنواع أخرى لليستيريا من الجبنة التي تم زرعها مبـــاشرة على البيئات الاختيارية. أعطى الزرع المباشر أعداد منخفضة جداً (٢٠أو أقل وحده مكونه للمستعمرة لكل حرام) من ليستيريا مونوسيتوجينيس. كان من الضروري إثراء العينـــــات اختياريا على درجة حرارة ٣٧°م لمدة ٤٨ ساعه لعزل الليستيريا الملوثة للجبنة مع ميكروبات أخرى. نمت الميكروبات الملوثة للجبنة المدخنة وبصفة خاصـــة الأنتيروكوكاي والأيستافيلوكوكاي في البيئة الإختياريه لليستيريا (أوكسويد) أو أن هذه الميكروبات أعطت مميزات تشخيصية مماثلة لتلك التي تميز الليستيريا مونوسيتوجينيس في البيئة. عندما استخدمت أحجام متزايدة (< ٢,. مل) من العينات التي تم زرعها، استردت بيئة الزريقي وساندين (ا.س.ل.م) الليستيريا الملوثة للجبنة في حين أنه تم تثبيط الميكروبات الموجودة طبيعيا في الجبنه. أكدت نتائج هذا البحث الاحتياج إلى تبني طريقه قياسية لتصنيـــع الجبنـــة المدخنة في اليمن.