SEROLOGICAL IDENTIFICATION OF *LISTERIA MONOCYTOGENES* ISOLATED FROM FOOD USING SLIDE AGGLUTINATION TEST

SEROLOŠKA KLASIFIKACIJA SEVOV *LISTERIA MONOCYTOGENES,* IZOLIRANIH IZ ŽIVIL, Z UPORABO SEROAGLUTINACIJSKEGA TESTA

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Abstract

Listeria monocytogenes is subdivided into 13 serotypes on the basis of somatic (O) and flagellar (H) antigens. Because a vast majority of human disease are caused by only three serotypes (1/2a, 1/2b and 4b), serotyping is of limited value in epidemiologic investigations. Strains of Listeria monocytogenes isolated from food have been collected at the Department of Sanitary Microbiology since 1994. Pure cultures of Listeria recovered from foods were confirmed with the api-Listeria test (bioMerieux). Serological slide agglutination test was done on all Listeria monocytogenes isolates, using commercial antisera Bacto - Listeria O Antisera Types 1,4 and Poly (Difco). The 56 strains obtained were classified into 2 serovars. There were 46 strains of Listeria monocytogenes serotype 1, and six strains of serotype 4; the test for four strains was negative. The aim of subtyping Listeria monocytogenes isolates was to identify serotypes that had been most frequently isolated from food samples.

Key words: Listeriosis, detection, serotyping, agglutination

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Izvleček

Listeria monocytogenes ima 13 serotipov, serološko klasificiranih z aglutinacijskimi testi z antiserumi proti somatskim (O) in flagelarnim (H) antigenom. Večina opisanih okužb s povzročiteljem Listeria monocytogenes se pri ljudeh zgodi s serotipi 1/2a, 1/2b in 4b. Pri epidemioloških raziskavah ima serotipizacija le omejeno vrednost. V laboratoriju Oddelka za sanitarno mikrobiologijo od leta 1994 zbiramo in hranimo seve povzročitelja Listeria monocytogenes, izolirane iz živil. Pri biokemični potrditvi smo uporabili test api-Listeria (bioMerieux). Seroaglutinacijo sevov smo izvedli s komercialno pripravljenim testom Bacto- Listeria O Antisera Types 1,4 in Poly (Difco). Vseh 56 izoliranih sevov smo serotipizirali v 2 serotipa. Večina, 46 testiranih sevov je serotip 1, šest sevov je serotip 4, ostali štirje sevi pa niso aglutinirali z nobenim antiserumom. Namen našega dela je bil pregledati, kateri serotipi so najpogosteje prisotni pri izolatih povzročitelja Listeria monocytogenes iz živil.

Ključne besede: listerioza, izolacija, seroaglutinacija

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1 Introduction

Listeria monocytogenes is the major pathogenic species of the genus *Listeria. Listeria monocytogenes* is a ubiquitous pathogenic bacterium that may cause listeriosis in high-risk popularion grops, including newborns, elderly and immunocompromised persons and pregnant women (1).Human listeriosis is a serious systemic infection, although subclinical infection may also occur as a mild gastrointestinal or influenza-like illness (2).
Since 1985, when it was identified as the causative agent in a large food-borne outbreak involving Mexican-style soft cheese, *Listeria monocytogenes* has been a constant concern of the food industry and regulatory agencies. In addition to its carrying a serious threat to human health, this pathogen has a considerable economic impact (3).
Listeria monocytogenes is a Gram-positive nonsporeforming, facultatively anaerobic bacterium, which is motile by means of peritrichous flagella. It has been isolated from soil, sewage, dust, water and foods, such as raw milk, cheeses, ice cream, raw vegetables, raw meats, raw and smoked fish and others (3). Because *Listeria monocytogenes* is widely present in the environment, it would be impossible to prevent people and animals from coming in contact with the bacteria. However, farmers, animal producers, food processors and food handlers can all take steps to reduce contamination and keep food safe from *Listeria monocytogenes*.
Confirmatory identification of *Listeria monocytogenes* is based on preliminary confirmation by biochemical tests (4). The CAMP test and genetic tests may be required in certain circumstances (4). Pure cultures are required for all confirmatory identification tests. Serological testing may be employed to further characterize isolates already confirmed to be *Listeria monocytogenes*. *Listeria* O Types (somatic antigen) 1, 4 and poly antisera are available from Difco Laboratories.

2 Methods

Bacteriological examinations were performed according to the procedure described in the International Standard SIST EN ISO 11290-1:1996 (5).
Serological identification of *Listeria monocytogenes* was done using commercially prepared antisera Bacto- Listeria O Antisera Types 1,4 and Poly (Difco) according to the producer's instructions.

2.1 Determination and biochemical identification

A given mass or volume of the product was tested for the presence of *Listeria monocytogenes*. A selective primary enrichment medium (Half Fraser broth) was inoculated with 25g or 25ml of the sample. The test portion was incubated at 30`C for 24h. 10ml of secondary enrichment (Fraser broth) was inoculated with 0.1ml of culture from the primary enrichment medium after incubation. It was incubated at 37`C for 48h. The culture from the secondary enrichment was plated out on the selective solid media (Oxford and PALCAM agar), incubated at 37`C for 24 to 48h. The presence of characteristic colonies was checked after 48h. *L. monocytogenes* was confirmed by physiological and biochemical tests.

2.2 Serological identification

Suspension of pure *Listeria monocytogenes* culture was prepared in the FA buffer. The organism suspension was heated to 80`C for 1h and centrifuged. The bulk was removed from the supernatant fluid and the organisms were resuspended in the remaining portion of the fluid. One drop of the prepared organism suspension was added to one drop of antiserum diluted in saline solution (1:20) on a glass plate, and rocked for 1 to 2 minutes. Similarly, one drop of the prepared organism suspension was added to one drop of negative control buffer solution and rocked for 1-2 minutes. Positive (Listeria O Antiserum plus homologous O Antigen) and negative (FA Buffer Dried rehydrated containing 0.3 ml of formaldehyde per 100 ml plus a drop of the organism suspension) controls were also used.
The organism suspension was not agglutinated in the control saline but in the homologous antisera.

3 Results

More than 50% of food samples from which *Listeria monocytogenes* was recovered were obtained from ready-to-eat foods. Most of the isolates (46 of 56) belonged to serotype 1, and only six isolates to serotype 4. Four of the 56 isolates were not typeable by means of the commercial kit used.

4 Discussion

Listeria monocytogenes is a ubiquitous pathogenic bacterium that may cause listeriosis in high risk

individuals, including neonates, elderly and immunocompromised persons, and pregnant women. Various methods for detecting and typing Listeria monocytogenes in food have been evaluated. Serotyping is still routinely used in outbreaks of listeriosis, yet it has a relatively poor discriminatory value. Some isolates may prove untypeable with standard typing antisera. Antibody- based methods tend to have low specificity, resulting in false-positive results (1, 6). The main advantages of polymerase chain reaction (PCR) include high specificity, sensitivity,and rapidity(6). In the future, new PCR methods for detection and typing of Listeria monocytogenes will be introduced in this laboratory with the aim of enhancing the accuracy and rapidity of food intoxication analysis.

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