COMPARISON OF TWO SEROTYPING METHODS OF L. MONOCYTOGENES ISOLATED FROM HUMAN CASES OF LISTERIOSIS, FOOD AND FOOD PLANTS ENVIRONMENT

Almeida GN, Magalhães RS, Santos IR, Carneiro LM, Oliveira BD, Teixeira PC and Hogg TA*

Introduction

Listeria monocytogenes is the causative agent of serious epidemic and sporadic food-borne listeriosis. Although rare when compared to other food-borne diseases, a significant feature of listeriosis is the high lethality rate (about 30%), which makes *L. monocytogenes* a pathogen of considerable concern.

Apparently, not all strains of *L. monocytogenes* are capable of causing disease in humans. Of the 13 serovars only 4 (1/2a, 1/2c, 1/2b, and 4b) are associated with more than 98% of known reported human cases of listeriosis.

Despite it's poor discriminatory power, with only four serotypes being found in foods or involved in cases of human listeriosis, serotyping is usually the first step in a typing scheme. When an outbreak or contamination route, are under investigation, serotype information allows investigators to exclude strains from further study.

Serotyping by conventional methods requires several days (3-5) before final results are available, whilst using multiplex PCR, results can be obtained in 6 hours.

The aim of this study was to compare the performance of these two methods using strains from clinical, food or from food plant environments deposited at the *Listeria* strain collection at Escola Superior de Biotecnologia.

Materials and Methods

A total of 185 strains - 39 of food origin, 55 from food plant environments and 91 from human clinical cases - were used in this study.

Classical agglutination serotyping was performed using anti O and anti H antisera according to the manufacturers' instructions (Denka Seiken Co., Ltd, Tokyo, Japan) and the modifications described by Lukinmaa (2003). Multiplex PCR "serotyping" was performed according to Doumith et al (2004).

Results

Table 1 shows the results obtained by both methods tested. Three different serotypes were identified by the agglutination methodology: 19 strains were serotype 1/2a, 97 were serotype 1/2b and 69 were serotype 4b. By multiplex-PCR strains were grouped in three groups: 14 strains were 1/2a-3a, 103 were 1/2b-3b and 68 were 4b-4d-4e.

Table 1 – Typing results obtained by multiplex PCR and agglutination Denka commercial antisera

Agglutination / PCR	1/2a – 3a	1/2b – 3b	4b – 4d – 4e
1/2a	13	5	1
1/2b	0	95	2
4b	1	3	65

In Figure 1 the linear regression obtained for both methodologies is shown. Figure 2 show an example of the multiplex assay profiles

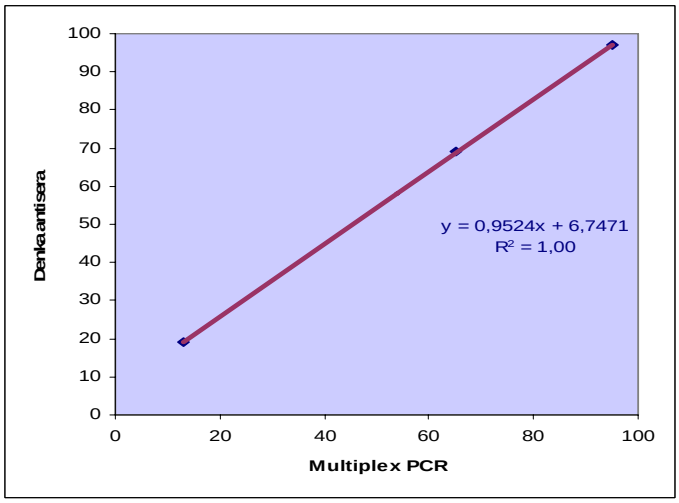

Figure 1 – Linear regression obtained for typing by multiplex PCR *vs* classical agglutination.

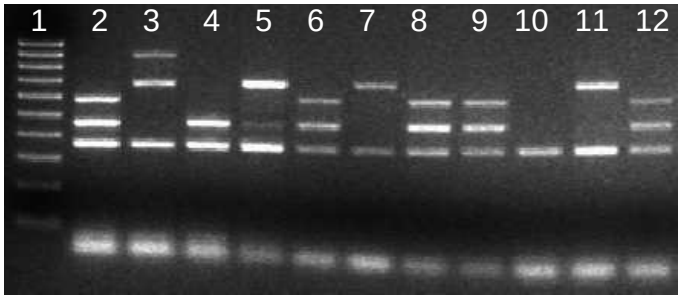

Figure 2 –Lane 1 DNA ladder (Biorad, EZ Load™ Molecular Rulers), lanes 2 – 5 control strain profiles for multiplex groups: 4b-4d-4e, 1/2c-3c, 1/2b-3b-7, 1/2a-3a, respectively, lanes 8 -12 profiles obtained from isolates.

Conclusion

A linear relationship was found between results obtained using classical agglutination serotyping (Denka Seiken antisera) and multiplex-PCR: Classical agglutination = 0,95 multiplex-PCR+ 6,75; R^2= 1,00.

The overall concordance between the two methods was 94%. For serotypes 1/2a, 1/2b and 4b it was 68%, 98% and 94%, respectively.

It was demonstrated that molecular serotyping can be a useful rapid method although it does not distinguish strains 1/2b from 3b and 7, 1/2c from 3c and 4b from 4e and 4d. PCR patterns are, however, less subjective to interpret than the agglutination method.

References

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Universidade Católica Portuguesa · Escola Superior de Biotecnologia
Gonçalo Nieto Almeida
Rua Dr. António Bernardino de Almeida · 4200-072 PORTO
Tel.: + 351 225 580 00 81 · Fax: + 351 225 580 111
E-mail: gnalmeida@esb.ucp.pt