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EVALUATION OF PHENOTYPIC MARKERS ASSOCIATED WITH PATHOGENICITY IN THE GENUS *Listeria*

Zilma das Graças NUNES & Ernesto HOFER (1)

SUMMARY

A total of 130 *Listeria* strains were tested in order to evaluate lecithinase production and capacity for Congo red adsorption as markers of pathogenicity. The strains were identified according to acid production from sugars and by the CAMP test and the data were correlated with the ability to produce keratoconjunctivitis in guinea pigs.

L. monocytogenes cultures presented 51.8% and 88.8% positivity rates for Congo red adsorption and lecithinase production, respectively, whereas 80.8% and 100% for *L. innocua* cultures were negative for the two test, respectively.

KEYWORDS: *Listeria monocytogenes*; Hemolysin; Lecithinase; Congo red adsorption; Keratoconjunctivitis.

INTRODUCTION

The mechanisms of pathogenicity of *Listeria monocytogenes* definitely represent one of the more relevant problems in the study of listeriosis. Some aspects indeed provide useful traits for the differential diagnosis of pathogenic strains and are consequently applicable to taxonomy.

Previously reported markers of *Listeria* virulence are hemolysin, pathogenicity for chick embryos, production of keratoconjunctivitis in guinea pigs, lipase, serotype, phagotype, biotype, and production of oxygen radicals, among others[16, 20]

ROCOURT et al.[17] have suggested the use of the CAMP test together with acid production from sugars and antigen composition for the differentiation of *Listeria* species. However, hemolysin production is extremely variable in *Listeria* and is often difficult to detect *in vitro* [11]. In addition, hemolytic tests are subject to a series of extrinsic factors that may lead to erroneous results[1, 18]. It should also be pointed out that *Listeria seeligeri* has no pathogenicity even though it produces hemolysin.

Hemolysin has been associated with lecithinase production3, 9, 10, 12, but conflicting results have been obtained. In this respect, the objective of the present study was to determine the real validity of this marker in the distinction between *L. monocytogenes* and L. innocua and to determine the correlation between strain virulence and ability to adsorb the Congo red dye, as observed in *Shigella flexneri* by MAURELLI et al.[14] and by SASAKAWA et al.[19].

(1) Department of Bacteriology, Oswaldo Cruz Institute, FIOCRUZ, Av. Brasil, 4365; Manguinhos; 21045-900 Rio de Janeiro, RJ, Brasil.

MATERIAL AND METHODS

Sampling: A total of 130 cultures from the *Listeria* Sector of the Department of Bacteriology, Oswaldo Cruz Institute, were used. These strains were isolated from different sources (human and animal) and from different regions between 1974 and 1985. The bacterial species were identified by the criterion of ROCOURT et al.[17] based on the test of acid production from sugars D-xylose, L-rhamnose, α-methyl-D-mannoside, and D-mannitol and on the CAMP test.

Anton test: Forty-eight strains (27 of *L. monocytogenes* and 21 of *L. innocua*) were selected at random. Albino guinea pigs (*Cavia porcellus*) were employed for the test using the methodology of HOFER[7].

Congo red adsorption: Strains were inoculated into tryptic agar plates (Difco) containing the Congo red dye (MERCK) at a final concentration of 0.015 g% and incubated at 37°C for 48h. Culture were considered to be positive when they presented isolated colonies of reddish color after incubation[19].

Lecithinase production: Agar tryptic soy (Difco) medium with egg yolk added at 5% concentration was used [3]. The strains were grown on tryptic phosphate broth (Difco) at 37°C for 24 hours and inoculated by deposition on the plate surface with a platinum loop.

Reactions were considered to be positive in the presence of an opaque around the bacterial growth after incubation for 24 and 48 hours at 37°C.

RESULTS

The biochemical characterization of the 130 *Listeria* strains permitted the identification of two species: *Listeria monocytogenes* (47.6%) and *Listeria innocua* (46.9%). *L. monocytogenes* was prevalent in the strains from human sources (69.3%), and *L. innocua* in strains of animal origin (81.8%) (Table 1). Seven strains that could not be assigned to *L. monocytogenes* because of the absence of hemolysis, or to *L. innocua* because of serotype and pathogenicity, were denoted *Listeria* sp.

One culture that was unable to utilize rhamnose, a result more compatible with *L. innocua* [17] was classified as *L. monocytogenes* because of positivity to the CAMP test.

Distribution of these species according to human source (Table 2) showed that most of strains classified as *L. monocytogenes* (96.0%) were associated with symptomatic individuals. Conversely, all *L. innocua* cultures were isolated from asymptomatic persons.

Analysis of *in vivo* pathogenicity by the Anton test showed that 96.2% of the *L. monocytogenes* strains were positive, in striking contrast to the negative result obtained for the *L. innocua* strains (Table 3).

The Congo red adsorption test (Table 4) demonstrated that 32.2% of the *L. monocytogenes* strains adsorbed the dye, while 80.8% of the *L. innocua* cultures did not show this property. Taking these data togheter with those obtained by

TABLE 1

Numerical and percent distribution of *Listeria* species detected in strains from human and animal sources.

SOURCE	*L. monocytogenes*	L. innocua	Listeria sp	TOTAL
HUMAN	52 (69.3)*	16 (21.3)	7 (9.3)	75 (99.9)
ANIMAL	10 (18.1)	45 (81.8)	-	55 (99.9)
TOTAL	62 (47.6)	61 (46.9)	7 (5.3)	130 (99.9)

* The numbers in parentheses are percentages.

TABLE 2

Numerical and percent frequency of *Listeria* species isolated from different human sources according to the presence of symptoms in the individuals studied.

ORIGIN	*L. monocytogenes* (n=52)		*L. innocua* (n=16)	*Listeria sp* (n=7)
	SYMPTOMATIC	ASYMPTOMATIC	ASYMPTOMATIC	SYMPTOMATIC
CSF[1]	41 (78.8)(3)	-	-	5 (71.4)
BLOOD	7 (13.4)	-	1 (6.2)	1 (14.2)
PLACENTA	2 (3.8)	-	9 (56.2)	-
VAG. SEC.[2]	-	-	2 (12.5)	-
FECES	-	2 (3.8)	4 (25.0)	1 (14.2)
TOTAL	50 (96.0)	2 (3.8)	16 (99.9)	7 (99.8)

1 - Cerebrospinal fluid

TABLE 3

Behavior of *Listeria* species in terms of the Anton test (Keratoconjunctivitis).

Species	Keratoconjunctivitis		Total
	Positive	Negative	
L. monocytogenes	26 (96.2)	1 (3.7)	27 (99.9)
L. innocua	-	21 (100.0)	21 (100.0)

* The numbers in parentheses are percentages.

TABLE 4

Behavior of *Listeria* species in terms of Congo red adsorption.

Species	Adsorption		Total
	Positive	Negative	
L. monocytogenes	20 (32.2)*	42 (67.7)	62 (99.9)
L. innocua	13 (19.1)	55 (80.8)	68 (99.9)
TOTAL	33 (25.3)	97 (74.6)	130 (99.9)

* The numbers in parentheses are percentages.

the Anton test, it can be seen that 51.8% of the *L. monocytogenes* strains that produced keratoconjunctivitis adsorbed the dye, whereas 80.9% of the *L. innocua* strains were negative to both tests.

It should also be pointed out that the single *L. monocytogenes* strain that was negative to the Anton test did not adsorb the dye (Table 5).

In the lecithinase production test, only 3 of the 62 *L. monocytogenes* strains presented negative results, corresponding to a 95.1% rate of positivity for production of the enzyme. In contrast, 98.5% of the *L. innocua* strains did not produce lecithinase (Table 6).

Correlation of these data with those obtained by the Anton test (Table 7) shows that 88.8% of

TABLE 5

Relation between the capacity for Congo red adsorption and pathogenicity (Anton test) as a function of *Listeria* species and source.

ORIGIN	*L. monocytogenes* (n=27)				*L. innocua* (n=21)			
	(++)*	(- -)	(+-)	(-+)	(++)	(- -)	(+-)	(-+)
HUMAN	11	1	-	8	-	5	1	-
	(40.7)	(3.7)		(29.6)		(23.8)	(4.7)	
ANIMAL	3	-	-	4	-	12	3	-
	(11.1)			(14.8)		(57.1)	(14.2)	
	14	1	-	12	-	17	4	-
TOTAL	(51.8)	(3.7)		(44.4)		(80.9)	(18.9)	

* Profile corresponding to the results of the Congo Red adsorption tests and the Anton test.

** The numbers in parentheses are percentages.

TABLE 6

Behavior of *Listeria* species with respect to lecithinase.

Species	Lecithinase		Total
	Positive	Negative	
L. monocytogenes	59 (95.1)*	3 (4.8)	62 (99.9)
L. innocua	1 (1.4)	67 (98.5)	68 (99.9)
TOTAL	60 (46.1)	70 (53.8)	130 (99.9)

* The numbers in parentheses are percentages.

TABLE 7

Relation between lecithinase and pathogenicity (Anton test) as a function of *Listeria* species and source.

ORIGIN	*L. monocytogenes* (n=27)				*L. innocua* (n=21)			
	(++)*	(- -)	(+-)	(-+)	(++)	(- -)	(+-)	(-+)
HUMAN	17	1	-	1	-	5	-	-
	(62.9)**	(3.7)		(3.7)		(23.8)		
ANIMAL	7	-	-	1	-	16	-	-
	(25.9)			(3.7)		(76.1)		
	24	1	-	2	-	21	-	-
TOTAL	(88.8)	(3.7)		(7.4)		(99.9)		

* Profile corresponding to the results of the lecithinase test and of the Anton test.

** The numbers in parentheses are percentages.

the *L. monocytogenes* strains were simultaneously positive to the *in vivo* pathogenicity test and to the lecithinase production test, whereas the *L. innocua* strains were negative to both tests. It should also be pointed out that the single *L. monocytogenes* strain that did not produce keratoconjunctivitis did not produce lecithinase.

DISCUSSION

The fermentative action on carbohydrates was effective in characterizing the samples and aided the identification of *L. monocytogenes* and *L. innocua*. As to the differentation of the two species by the CAMP tests, it should be mentioned that some cultures presented very low levels of hemolysin production and may have been considered negative [1, 11, 18]. In this respect, the composition and preparation of the culture medium proved to be factors of extreme importance for obtaining reliable results. Thus, the amount of blood added to the basic medium or the thickness of the culture medium on the Petri dishes were important factors.

It should also be pointed out that the expansion of the hemolytic activity of *L. monocytogenes* was often more clear in the presence of the protein excreted by *Rhodococcus equi* than in the presence of the hemolysin of *Staphylococcus aureus*, in contrast to data reported by ROCOURT el al. [17] and MCLAUCHLIN [15]. It may be proposed that this effect was due to the fact that the *R. equi* culture was not hemolytic, thus facilitating the reading of the results. However, FRASER[2] demonstrated the potentiation of *L. monocytogenes* hemolysis by *R. equi*, as later confirmed by SKALKA el al. [22, 23] and by RODRIGUEZ et al.[18].

Several investigators have stated that all clinical isolates share the hemolytic activity and virulence characteristic of *L. monocytogenes* [4, 5, 6, 11, 13]. In this respect, the high rate of *L. monocytogenes* strains isolated from human sources that were related to clinical cases (96.0%) confirms the strict association between this species and the development of the disease (Table 2). Also, the occurrence of this species in 3.8% of strains from asymptomatic individuals and in 18.1% of cattle cultures (Table 2) reflects the possibility that these hosts acts as carriers of the pathogenic species [8].

Similary, all strains classified as *L. innocua* were isolated from asymptomatic individuals (Table 2), demonstrating the absence of virulence of this species [21].

The strains denominated *Listeria* sp were isolated from symptomatic individuals. Thus, we propose that a loss of the ability to produce hemolysin may have occurred over successive passages through culture media or due to a long permanence in conservation media, which impaired the identification of these cultures as *L. monocytogenes*.

Correlation of the results of the Anton test with those of Congo red adsorption (Table 5) revealed a strict association between these markers and the pathogenicity of *Listeria*. The high negativity rates presented by *L. innocua* (80.8% and 100% respectively) reinforce this hypothesis.

A single hemolytic strain did not produce keratoconjunctivitis in guinea pigs, nor did it produce lecithinase or adsorb Congo red. The loss of these markers is in agreement with the avirulence of the strain, and it may be assumed that the long permanence in preservation media may have favored this alteration.

The present data suggest that these tests could be used as accessory markers in the identification of *Listeria* species. In addition, improved methods for the detection of dye adsorption, mainly to minimize the effects of false results, will definitely contribute to higher positivity rates in *L. monocytogenes* strains.

RESUMO

Avaliação de marcadores fenotípicos associados à patogenicidade no gênero *Listeria*.

Com propósito de avaliar a produção de lecitinase e a capacidade de adsorção do

corante vermelho Congo como marcadores de patogenicidade, foram estudadas 130 amostras de *Listeria*. Estas amostras foram identificadas segundo a produção de ácido a partir de açúcares aliada ao teste CAMP, correlacionando-se estes dados à capacidade de produção de ceratoconjuntivite em cobaio.

As culturas de *L. monocytogenes* apresentaram taxas de positividade para a adsorção do corante e produção de lecitinase de 51,8 e 88,8%, respectivamente, enquanto 80,8% e 100% das culturas de *L. innocua* foram negativas para os referidos testes.

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