Ann. Fac. Medic. Vet. di Parma (Vol. XXVII, 2007) pag. 157 - pag. 164

ANTIMICROBIAL SUSCEPTIBILITY OF LISTERIA MONOCYTOGENES ISOLATED FROM FOOD AND FOOD-PROCESSING ENVIRONMENT

Conter Mauro[1], Paludi Domenico[2], D'Orio Vincenzo[2], Vergara Alberto[2], Ianieri Adriana[1]

Key words:
Listeria monocytogenes; antibiotic resistance; food; environment.

Parole chiave:
Listeria monocytogenes; resistenza antimicrobica; alimenti; ambiente

ABSTRACT

The objective of this study was to evaluate the susceptibility of 38 *L. monocytogenes* strains isolated from 542 food and food-processing environmental samples to 22 antibiotics currently used in veterinary and human therapy. Susceptibility tests were performed by an agar plate antibiotic disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. At least 97.4% of strains resulted resistant to oxacillin, lincomycin, flumequine, and clindamycin, regardless of both source and serotype. Sulphafurazole resulted significantly more active against environmental isolates than to meat- and fish-isolates (63.7% vs 41.2% and 30%, respectively). With regard to serotype, both 4b and 1/2c strains resulted significantly more resistant to sulphafurazole, compared to the other serotypes found. This study shows that *L. monocytogenes* strains from food and food-environments are susceptible to the antibiotics commonly used in veterinary and human listeriosis treatment. Considering that *L. monocytogenes* is slowly becoming antibiotic resistant, a continued surveillance of emerging antimicrobial resistance of this pathogen is important to ensure effective treatment of human listeriosis. These data are useful in improving background data on antibiotic resistance of strains isolated from food and food environment.

RIASSUNTO

L'obiettivo di questo studio è stato quello di valutare la sensibilità agli antibiotici di 38 ceppi di *L. monocytogenes* isolati da 542 campioni provenienti da alimenti e ambienti di lavorazione, nei confronti di 22 antibiotici comunemente impiegati in terapia umana e veterinaria. La sensibilità è stata valutata in vitro mediante il metodo della diffusione in agar, in accordo alle linee guida del Clinical and Laboratory Standards Institute (CLSI). Il 97.4% dei ceppi si è dimostrato resistente a oxacil-

1 Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti, Università degli Studi di Parma
2 Dipartimento di Scienze degli Alimenti. Università degli Studi di Teramo

lina, lincomicina, fumechina e clindamicina, indipendentemente dalla provenienza o dal sierotipo. Il 55,3% dei ceppi si è rivelato resistente al sulfafurazolo che si rivelava maggiormente attivo verso gli isolati ambientali rispetto ai ceppi isolati dalle carni o dai prodotti ittici (63,7% contro 41,2% e 30%, rispettivamente). Relativamente al sierotipo, sia i ceppi appartenenti ai sierotipi 4b e 1/2c risultavano significativamente più resistenti al sulfafurazolo rispetto agli altri sierotipi. Questo studio mostra che i ceppi isolati di *L. monocytogenes* sono risultati sensibili agli antibiotici comunemente impiegati in terapia per il trattamento delle listeriosi. Considerando che la resistenza di *Listeria monocytogenes* agli antibiotici sta lentamente aumentando, risulta fondamentale una continua sorveglianza di questo patogeno per monitorare l'efficacia dei trattamenti delle listeriosi. Questi dati possono essere utilizzati per migliorare le conoscenze sull'antibiotico resistenza dei ceppi isolati dagli alimenti e dai relativi ambienti di lavorazione.

INTRODUCTION

The genus *Listeria* consists of a group of Gram-positive bacteria of low G + C content. Six species have been identified: *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, and *L. grayi*, but only *L. monocytogenes* and L. ivanovii are considered virulent. *L. monocytogenes* is an important foodborne pathogen (Swaminathan, 2001) and has been isolated from a variety of food products. Meat, poultry, dairy, and vegetable products have all been implicated as vehicles of listeriosis (Schlech et al., 1983, Bula et al., 1995; Kiss et al., 2006; Thévenot et al., 2006; Arslan et al., 2008). Since foodborne listeriosis was first reported in 1981 (Schlech et al., 1983), numerous foodborne outbreaks of *L. monocytogenes* have been documented worldwide (Farber and Peterkin, 1991; Jacquet et al., 2002).

L. monocytogenes is usually susceptible to a wide range of antibiotics (Hof et al., 1997), but since the first isolation of a multiresistant strain in France in 1988 (Poyart-Salmeron et al., 1990), other strains resistant to one or more antibiotics have been recovered from food, environment and from sporadic cases of human listeriosis (Hadorn et al., 1993; Franco Abuin et al., 1994; Charpentier et al., 1995). Antibiotics to which some *L. monocytogenes* are resistant include tetracycline, gentamicin, penicillin, ampicillin, streptomycin, erythromycin, kanamycin, sulphonamide, trimethoprim, and rifampicin (Charpentier and Courvalin, 1999). Little information is available on the antimicrobial susceptibility of *L. monocytogenes*, particularly on strains isolated from food and food environment, indicating the necessity of monitoring the antimicrobial susceptibility of this pathogen. Moreover, apart from penicillins and folate pathway inhibitors, the Clinical and Laboratory Standards Institute (CLSI) does not provide any clinical breakpoints for Listeria susceptibility testing.

The present study aimed to evaluate the susceptibility of 38 *L. monocytogenes* strains isolated from food and food-processing environments to 22 antibiotics currently used in veterinary and human therapy.

MATERIALS AND METHODS

Strains were isolated from 542 food and environmental samples (44.7% from meat, 26.3% from fish, and 29% form food-processing environments). Strain isolation and identification were performed according to the ISO 11290-1 method. *Listeria* isolates were then analyzed by a multiplex PCR using a modification of the method described by Border et al. (1990). Primers LI1 and U1 are based on 16S rRNA sequence data and is specific to the genus *Listeria*, while primers LM1 and LM2 are based on listeriolysin O gene sequence data and is specific for *L. monocytogenes* (Border et al., 1990). Strain serotyping was conducted by the ELISA-based method and confirmed by multiplex PCR amplification technique according to Doumith et al. (2004). Stock cultures were maintained at -80°C in Microbank bead vials (Biolife Italiana, Milan, Italy) until use. As needed, stocks were thawed and subcultured twice on Trypticase Soy Agar (TSA) (Oxoid, Garbagnate Milanese, Milan, Italy) before use.

Susceptibility tests were performed by standard disk diffusion method on Mueller Hinton Agar (MHA) (Oxoid), following the procedures recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006a and 2006b). *Staphylococcus aureus* ATCC 29213 was used as control strain. Twenty-two antibiotics were chosen: Amikacin 30 µg, Amoxicillin 10 µg, Ampicillin 10 µg, Clindamicin 2 µg, Chloramphenicol 30 µg, Cephalotin 30 µg, Erythromycin 15 µg, Enrofloxacin 5 µg, Flumequine 30 µg, Gentamicin 10 µg, Lincomycin 2 µg, Oxacillin 1 µg, Penicillin 10 µg, Rifampicin 5 µg, Spiramicin 100 µg, Streptomycin 10 µg, Sulphafurazole 300 µg, Tetracycline 30 µg, Tobramycin 10 µg, Trimet + Sulf 1.25/23.75 µg, Vancomycin 30 µg, Kanamycin 30 µg. All antibiotic disks used were obtained from Oxoid.

All statistical analysis was made using SPSS (Chicago, IL) ver. 13.0.

RESULTS AND DISCUSSION

A total of 542 samples food and environmental samples were examined for the presence of *Listeria*. *Listeria* monocytogenes was isolated from 38 (7.01%) samples. In particular, 44.7% of the strains were isolated from meat, 26.3% from fish, and 29% form food-processing environments. Isolated strains belonged to serotypes 1/2a (42%), 1/2b (29%), 1/2c (18%), and 4b (11%).

Antimicrobial susceptibility of the 38 isolated strains of *Listeria* monocytogenes was then evaluated by the standard disk diffusion method. Figure 1 summarizes the antimicrobial susceptibility profiles of the isolated strains: resistance to 6 out of 22 antimicrobial agents tested was observed. Overall, the majority of the strains were resistant to oxacillin, lincomycin, flumequine, and clindamycin, whereas some half of the strains were resistant to sulphafurazole. Only one strain was resistant to rifampicin. All tested isolates were sensitive to the other antibiotics.

Sulphafurazole resulted significantly more active against environmental isolates than to meat- and fish-isolates (63.7% vs 41.2% and 30%, respectively; $P < 0.001$) (Figures 2, 3 and 4). With regard to serotype, both 4b and 1/2c strains resulted significantly ($P < 0.01$) more resistant to sulphafurazole, compared to other serotypes

found. Chloramphenicol exhibited decreased activity against 25% of 4b strains only.

Ampicillin, rifampin, or penicillin plus gentamicin remain the treatment of choice for most manifestations of listeriosis. Co-trimoxazole is considered to be a second-choice therapy. Vancomycin and erythromycin are also used, respectively, to treat bacteremia and pregnant women diagnosed with listeriosis (Charpentier E. and Courvalin P., 1999). In general, most *L. monocytogenes* strains isolated in this study are susceptible to the antibiotics commonly used in veterinary and human listeriosis treatment. The main exception was one strain resistant to rifampicin and sulphafurazole. A similar pattern of resistance was also been found by other authors (Facinelli et al., 1991; Morse et al., 1999) suggesting the worldwide increase in antibiotic resistance.

Considering that *L. monocytogenes* is slowly becoming antibiotic resistant by acquisition of known antibiotic resistance genes from gram-positive bacteria, a continued surveillance of emerging antimicrobial resistance of this pathogen is important to ensure effective treatment of human listeriosis. These data can be used to improve background data on antibiotic resistance of strains isolated from food and food environment and for epidemiological and public health studies of *L. monocytogenes*.

References

1) Arslan S., Ozdemir F., 2008. Prevalence and antimicrobial resistance of Listeria spp. In homemade white cheese. Food Control 19, 360-363.
2) Border, P.M., Howard J.J., Plastow G.S. and Siggens K.W., 1990. Detection of Listeria species and Listeria monocytogenes using polimerase chain reaction. Letters in Applied Microbiology 1, 158-162
3) Bula C.J., Bille J. and Glauser M.P., 1995. An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults. Clinical Infectious Diseases 20, 66–72.
4) Charpentier E., Gerbaud G., Jacquet C., Rocourt J. and Courvalin P., 1995. Incidence of antibiotic resistance in Listeria species. Journal of Infectious Diseases 172, 277–281.
5) Charpentier E. and Courvalin P., 1999. Antibiotic resistance in Listeria spp., Antimicrobial Agents and Chemotherapy 43, 2103–2108.
6) Clinical and Laboratory Standards Institute (CLSI) 2006a. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved guideline (M45-A). Clinical and Laboratory Standards Institute, Wayne, PA.
7) Clinical and Laboratory Standards Institute (CLSI) 2006b. Performance standards for antimicrobial disk susceptibility tests; Approved standard – Ninth edition (M2-A9). Clinical and Laboratory Standards Institute, Wayne, PA.
8) Doumith, M., Buchrieser C., Glaser P., Jacquet J. and Martin P., 2004. Differentiation of the major Listeria monocytogenes serovars by multiplex PCR. Journal of Clinical Microbiology 42, 3819-3822.
9) Facinelli B., Giovanetti E., Varaldo P.E., Casolari P., Fabio U., 1991. Antibiotic

resistance in foodborne Listeria. The Lancet 338, 1272.
10) Farber J.M. and Peterkin P.I., 1991. Listeria monocytogenes, a food-borne pathogen, Microbiological Reviews 55, 476–511
11) Franco Abuin C.M., Quinto Fernandez E.J., Fente Sampayo C., Rodriguez Otero J.L., Dominguez Rodriguez L. and Cepeda Saez A., 1994. Susceptibilities of Listeria species isolated from food to nine antimicrobial agents. Antimicrobial Agents and Chemotherapy 38, 1655–1657.
12) Hadorn K., Hachler H., Schaffner A. and Kayser F.H., 1993. Genetic characterization of plasmid-encoded multiple antibiotic resistance in a strain of Listeria monocytogenes causing endocarditis. European Journal of Clinical Microbiology and Infectious Diseases 12, 928–937.
13) Hof H., Nichterlein T. and Kretschmar M., 1997. Management of listeriosis, Clinical Microbiology Reviews 10, 345–357
14) Jacquet C., Gouin E., Jeannel D., Cossart P. and Rocourt J., 2002. Expression of ActA, Ami, InlB, and listeriolysin O in Listeria monocytogenes of human and food origin. Applied and Environmental Microbiology 68, 616–622.
15) Kiss R., Tirczkab T., Szitac G., Bernáthd S., Csikó G., 2006. Listeria monocytogenes food monitoring data and incidence of human listeriosis in Hungary, 2004. International Journal of Food Microbiology 112, 71-74.
16) Morse R., O'Hanlon K., Virji M., Collins M.D., 1999. Isolation of rifampin-resistant mutants of Listeria monocytogenes and their characterization by rpoB gene sequencing, temperature sensitivity for growth, and interaction with an epithelial cell line. Journal of Clinical Microbiology 37, 2913-2919.
17) Poyart-Salmeron C., Carlier C., Trieu-Cuot P., Courtieu A.L. and Courvalin P., 1990. Transferable plasmid-mediated antibiotic resistance in Listeria monocytogenes. Lancet 335, 1422–1426.
18) Schlech W.F., Lavigne III P.M., Bortolussi R.A., Allen A.C., Haldane E.V., Wort A.J., Hightower A.W., Johnson S.E., King S.H., Nicholls E.S. and Broome C.V., 1983. Epidemic listeriosis - evidence for transmission by food. New England Journal of Medicine 308, 203–206.
19) Swaminathan B., 2001. Foodborne pathogenic bacteria: Listeria monocytogenes. In: M.P. Doyle, L.R. Beuchat and T.J. Montville, Editors, Food Microbiology: Fundamentals and Frontiers (2nd ed.), ASM Press, Washington, DC, 337–352.
20) Thévenot D., Dernburg A., Vernozy-Rozand C., 2006. An updated review of Listeria monocytogenes in pork meat industry and its products. Journal of Applied Microbiology 101, 7-17.

Figure 1: Overall results of antimicrobial resistance

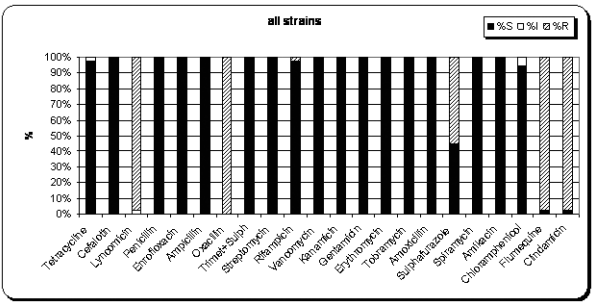

Figure 2: Antimicrobial resistance of meat isolates

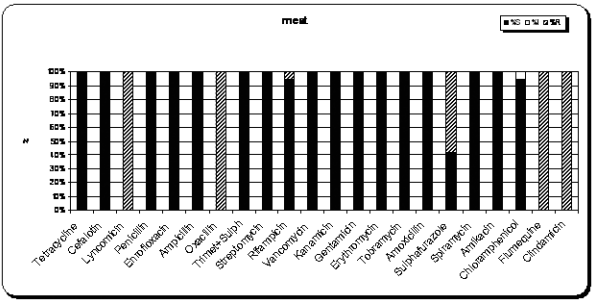

Fig. 3: Antimicrobial resistance of fish isolates

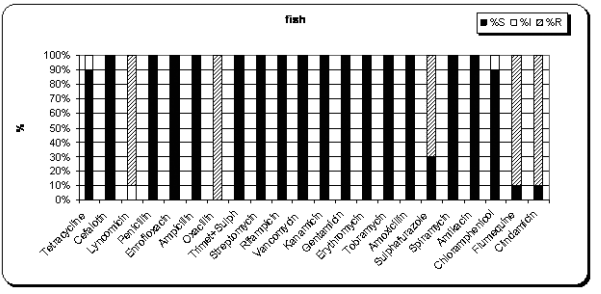

Fig. 4: Antimicrobial resistance of environmental isolates