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DETECTION OF ANTIMICROBIAL SENSITIVENESS OF ISOLATES OF LISTERIA MONOCYTOGENES FROM FOOD CHAIN USING VITEK 2 COMPACT BIOMERIEUX

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ABSTRACT

Sensitivity of 26 Listeria monocytogenes isolates toward 18 antimicrobial substances used in veterinary and human medicine was examined using the automated VITEK 2 Compact system bioMerieux. The obtained results indicate that L. monocytogenes strains isolated from food and food processing environment had resistance to several or more antimicrobial substances that are commonly used in the treatment in animals and humans. Results showed resistance of all 26 (100%) isolates toward Benzylpenicilin, Ampicilin/Sublactam, Oxacillin, Imipenem and Fosfomycine. Also 7 of the isolates (26.9%) were resistant to Clindamiycin, 3 (11.5%) to Quinupristion/ Dalfopristin and 1 strain to Teicoplanin, Vancomycin, Tetracycline and Fusic acid, respectively.

Key words: *Listeria monocytogenes, antimicrobial resistance, phenotypic similarity, MIC, VITEK 2*

INTRODUCTION

Genus Listeria is a group of Gram-positive bacteria. So far there are six identified species: *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 pathogen that comes from food, but is also isolated from different foodstuffs. Since the first recorded case of listeriosis caused by food occurred in 1981 (1), this foodborne infection is documented in numerous published papers on listeriosis from food around the world (2, 3). Meat, poultry meat, dairy products and vegetable products have been identified as vectors of listeriosis (4, 5, 6). *L. monocytogenes* is usually sensitive to broad spectrum of antibiotics (7), but after the first isolation of multiresistant strain in France in 1988 (9), other resistant strains were isolated from food, food producing premises environment, as well as, sporadic strains from cases of human listeriosis. The use of antimicrobial substances in animals and humans makes selection of resistant bacteria populations. In feed, despite EC ban on certain antibiotics in veterinary and human medicine (10), antibiotics are being used to control and treatment of diseases caused by bacteria but also as growth promoters (8). Undesirable consequence of use of antibiotics in animals is the potential development of antimicrobial-resistant zoonotic pathogens in food and their

spread as contaminants in food (12). Furthermore, spontaneous mutation of pathogenic bacteria from food, or spread of resistant bacteria in the absence of selection pressure also may also contribute to the emergence of antimicrobial resistant Listeria in food (11). Lately, there are established new antibiotic resistant phenotypes of many pathogens including *L. monocytogenes.* Many bacteria resistant to antibiotics are found in digestive tract as saprophytes or commensals in nature. They pose resistance genes that could be transferred through mobile genetic elements like plasmids and transpons (9) to other pathogenic bacteria, including *L. monocytogenes* (13). The most frequent source for resistance genes *L. monocytogenes* is likely to be *Enterococcus* spp. and *Streptococcus spp.* (14). There are few data on antimicrobial susceptibility of *L. monocytogenes,* especially for strains isolated from food and food production environment, which is an indicator for necessity of implementation of monitoring methods and tracing the transfer of antibiotic resistance (15). Determination of antimicrobial resistance is a tool for determination of phenotypic similarity between isolates. With phenotypic comparisons we get the opportunity to locate the sources of contamination in the food producing plants or along the food chain (17, 18).

This study aimed to evaluate the susceptibility of 26 strains L. monocytogenes isolated from food and environment for food production to 18 antimicrobials currently used in human and veterinary medicine, and thus determining their phenotypic similarity based upon Vitek 2 bioMerieux, ASE (Advanced expert system) data base.

MATERIAL AND METHODS

The survey covered 26 strains of *L. monocytogenes.* Of these, 3 were isolated from beef meat, 4 from chicken meat, 1 from dairy product, 4 from the surface of carcasses, 7 from the surface equipment, one from beef products, one from eggs grading facilities and 4 from raw cow's milk. Isolation and identification of strains was performed according to ISO 11290-1 method. Cultures were stored and kept in vials with 0,8 ml BPW + 0,2 ml glycerol at -80^{0}C until use. We used *Listeria monocytogenes* control reference strains for quality control.

Antimicrobial susceptibility testing was performed by VITEK 2 Compact system, bioMerieux and AES database, that is integral part of equipment. To prepare bacterial inoculum, we used pure bacterial isolates on nutrient agar after aerobic incubation 37^{0}C for 24 hours. Cultures were suspended in 0.45% saline solution equivalent to the standard of 0,45 McFarland. This suspension was used for filling the AST (Antimicrobial susceptibility test, bioMerieux) cards according to the manufacturer's instructions. Then, the cards were automatically loaded, sealed and stored in the instrument for further incubation and reading. We used card AST-P535 for *Staphylococcus* and *Enterococcus,* due to the absence of a specific card for *Listeria monocytogenes.* The composition of the card and the MIC range are shown in Table 1. It should be noted that clinical point of sensitivity for Listeria spp. in not defined. Apart from penicillin (penicillin and ampicilin) and folate pathway inhibitors (trimerhoprim-sulphometoxazol), for which clinical breakpoint for L. monocytogenes susceptibility testing are defined according to Clinical and Laboratory Standards Institute (19). In present study CLSI criteria *Staphylococcus* were applied. For those antibiotics for which neither *Listeria* nor *Staphylococcus* assessment criteria were available, brakepoints for sensitivity were define as follow: Fosfomycin ≤64 mg/L; Fusic acid ≤2 mg/L (20). As control strains we used: S. *aureus, L. monocytogenes,* and *E. faecalis.*

RESULT AND DISCUSSION

From 18 used antimicrobials, results showed resistance of all 26 (100%) isolates toward Benzylpenicilin, Ampicilin / Sublactam, Oxacillin, Imipenem and Fosfomycine. Also 7 of the isolates (26.9%) were resistant to Clindamiycin, 3 (11.5%) to Quinupristion/Dalfopristin and 1 strain to Teicoplanin, Vancomycin, Tetracycline and Fusic acid, respectively. This survey showed multi-resistance to most of the isolates from food and food producing facilities toward most frequently used antimicrobial substances in veterinary and human medicine. Of the 26 tested strains 1 (3.8%) showed resistance to nine antimicrobials, two isolates (7.6%) showed re-

sistance to eight antimicrobials, two (7.6%) isolates were resistant to seven, four strains were resistant to 6, (15,3%) and 14 strains resistance toward 5 antimicrobials contained in the AST-P535 card.

done having into account their phenotypic similarity based to other used antimicrobials in AST-P535 cards. Similarity match was done according to AES classification taking into account only sensitivity,

Table 1. Composition of AST-P535 card, MIC range, and sensitivity to certain antimicrobial substances

antimicrobial substance	MIC range (µg/ml)	Number of isolates and sensitivity		
		R	I	S
Benzylpenicilin	0,03-0,5	26	0	0
Ampicilin/Sublactam	2-32	26	0	0
Oxacillin	0,25-4	26	0	0
Imipenem	1-16	26	0	0
Gentamicin	0,5-16	0	0	26
Ciprofloxacin	0,5-8	0	14	12
Moxifoxacin	0,25-8	0	0	26
Erythromycin	0,25-8	0	0	26
Clindamycin	0,25-8	7	0	19
Quinupristion/Dalfopristin	0,25-16	3	0	23
Linezolid	0,5-8	4	0	22
Teicoplanin	0,25-32	1	0	25
Vancomycin	1-32	1	0	25
Tetracycline	1-16	1	0	25
Fosfomycine	8-128	26	0	0
Fusic acid	0,5-32	1	24	1
Rifampicin	0,5-32	0	1	25
Trimethoprim/sulfomethoxazole	10-320	0	0	26

All tested strains showed resistance toward Benzylpenicilin, Amp./Sublactam, Oxacilln, Imipenem and Fosfomycine therefore we excluded those antimicrobials that showed same sensitivity or resistance in tested strains. Classification of isolates was

resistance and intermediate sensitivity toward specified antimicrobials. The sensitivity of individual isolates and their origin according to the place from which they are isolated is presented in Table 2.

Table 2. Antimicrobial resistance of isolates of Listeria monocytogenes to various antimicrobial substances

Origin of Listeria monocytogenes isolates	Ciprofloxacin	Clindamycin	Quinupristion/ Dalfopristin	Linezolid	Teicoplanin	Vancomycin	Tetracycline	Fusic acid	Rifampicin
Cluster I									
Raw cow milk	1	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Raw cow milk	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Cluster II									
Swab dairy surface	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	2	≤0,5
Raw poultry steak	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	4	≤0,5
Raw poultry steak	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Raw poultry steak	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Kashkaval	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Swab pig carcass	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Swab pig carcass	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Swab equipment poultry slaughterhouse	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Swab equipment poultry slaughterhouse	≤0,5	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Cluster III									
Swab equipment poultry slaughterhouse	1	≥ 8	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Eggs grading facility	≤0,5	≥ 8	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Swab meat processing plant	1	4	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Minced beef	1	≥ 8	0,5	4	≤0,5	≤ 1	≤ 1	16	≤0,5
Cluster IV									
Swab lamb carcass	1	≤0,25	0,5	2	≤0,5	≤ 1	≤ 1	8	≤0,5
Swab lamb carcass	1	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Swab equipment lamb slaughterhouse	1	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Beef sausage fermented	1	≤0,25	0,5	4	≤0,5	≤ 1	≤ 1	8	≤0,5
Other strains									
Raw cow milk	1	≤0,25	1	4	≤0,5	≤ 1	≤ 1	8	≤0,5

Legend: *colorless squares-sensitive (S), yellow squares-intermediate (I), red square-resistant (R)*

According to data from Table 2, there are several exact phenotypic matches of isolates that can be put in the same cluster. There is four clusters of phenotypes and seven different strains that cannot be classified in any of given groups. Two strains in first cluster expressed intermediate resistance to Ciprofloxacin and Fusic acid. They originated from same matrix, raw cow milk. Next cluster is the largest comprised of nine strains from different type of matrices such poultry meat, poultry, pig and lamb slaughterhouses, minced beef and dairy products. This group of isolates showed intermediate resistance only to Fusic acid. Third cluster is comprised from strains obtained from swabs of three surfaces, poultry slaughterhouse, eggs grading facilities and meat processing plant. They expressed resistance to Clindamycin and intermediate resistance to Ciprofloxacin. Fourth cluster had four strains from which 2 were from swabs from lamb carcasses, one from surface in lamb slaughterhouse and one from beef fermented sausage with intermediate resistance to Ciprofloxacin and Fusic acid. All other strains can't be included in any existing cluster, however, they had some closeness regarding to resistance. Finally, it is obvious that strain from poultry MRM showed highest resistance compared to all tested strains.

CONCLUSION

Uncontrolled use of antimicrobial substances in the production process of food of animal origin is the reason for the high or multiple resistance to commonly used antimicrobial substances in veterinary and human medicine. The objective of the food operators should be production of food that is microbiologically safe for consumption during the shelf life, including the criteria for *Listeria monocytogenes* (15). This microorganism is usually located in biofilms on surfaces in the food producing plants and is a source of contamination of finished products (15, 16). Elimination of *Listeria monocytogenes* in the plant or in the food chain have to be priority of food business operator (16). To trace the source of Listeria by its location in the food chain, phenotypic similarity can be obtained with use of automated systems for antimicrobial resistance as VITEK 2 Compact bioMerieux. Also, in this study we obtained useful data regarding the medication to be used in the treatment of listeriosis.

REFERENCES

1. Schlech, W.F.. Lavigne III, P.M., Bortolussi, RA, Allen. A.C, Haldane, E.V., Wort, A.J., Hightower. A.W., Johnson, S.E., King. S.H., Nicholls, E.S., Broome, C.V.. 1983. Epidemic listeriosis — evidence for transmission by food. New England Journal of Medicine 308, 203-206.

2. Denny, J., McLauchlin. J., 2008. Human *Listeria monocytogenes* infections in Europe — an opportunity for improved European surveillance. Eurosurveillance 13 Available online: http://www.eurosurveiIlance.org/ViewArticle.aspx?ArticleId=s8082.

3. Farber, J.M., Peterkin, P.I., 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiological Reviews 55, 476-511.

4. Arslan, S., Ozdemir, F, 2008. Prevalence and antimicrobial resistance of *Listeria* spp. in homemade white cheese. Food Control 19, 360-363.

5. EFSA (European Food Safety Authority), 2007. Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness — Scientific Opinion of the Panel on Biological Hazards. The EFSA Journal 599,1-42.

6. Gianfranceschi, M.V., Gattuso, A., D'Ottavio, M.C, Fokas, S., Aureli, P., 2007. Results of a 12-month long enhanced surveillance of listeriosis in Italy. Eurosurveillance 12 Available online: http://www.eurasurveiIlance.org/ViewArticle.aspx7Articleld-746.

7. Hof, H., Nichterlein, T., Kretschmar, M., 1997. Management of listeriosis. Clinical Microbiology Reviews 10, 345-.357. Hof, H., 2003. History and epidemiology of listeriosis. FEMS Immunology and Medical

8. Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C, Jones, R., Nightingale, C, Preston, R., Waddell, J., 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. Journal of Antimicrobial Chemotherapy 23,28-52.

9. Poyart-Salmeron, C, Carlier, C, Trieu-Cuot, P., Courtieu, A.L., Courvalin, P., 1990. Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes.* The Lancet 335,1422-1426. Roberts, M.C, Facinelli, B., Giovanetti, E., Varaldo, P.E., 1996.

10. European Commission, 1998. Council Regulation (EC) No 2821/98 of 17 December 1998 amending, as regards withdrawal of the authorisation of certain antibiotics, Directive 70/524/EEC concerning additives in feedingstuffs. Official Journal of the European Community L351,4-8.

11. EFSA (European Food Safety Authority), 2008b. Scientific Opinion of the Panel on Biological Hazards on a request from the Health and Consumer Protection, Directorate General, European Commission on Microbiological Risk Assessment in feedingstuffs for food-producing animals. The EFSA Journal (2008) 720,1-84.

12. EFSA (European Food Safety Authority), 2008a. Draft Opinion on Antimicrobial Resistance in Food. Available online: http://www.efsa.eti.int/EFSA/DocurnentSet/biohaz..public... cons_amr_en.pdf.

13. Perreten, V., Schwarz, F., Cresta, L, Boeglin, M., Dasen, G., Teuber, M., 1997. Antibiotic resistance spread in the food. Nature 389, 801-802.

14. Biavasco, F., Giovanetti, E., Miele, A., Vignaioli, C, Facinelli, B., Varaldo, P.E., 1996. In vitro conjugative transfer of Van A vancomycin resistance between enterococci and listeriae of different species. European Journal of Clinical and Microbiological Infectious Disease 15, 50-59.

15. Dean Jankuloski, *Listeria monocytogenes* contamination of the facilities for production and processing of food from animal origin in Republic of Macedonia, Mr. sci thesis 2007

16. Dean Jankuloski, Pavle Sekulovski, Risto Prodanov, Zehra Hajrulai Musliu, Biljana Stojanovska Dimzoska, *Listeria monocytogenes* contamination of the environment and surfaces of the equipment in the meat processing plants in Republic of Macedonia, Macedonian veterinary review, Vol. 30, no. 1, 33-39, 2007

17. Pavle Sekulovski, Slavco Mrenoski, Dean Jankuloski. Survival of *Listeria monocytogenes* during manufacture and ripening of sheep brined cheese (ovco belo sirenje), FIL-IDF World diary summit & exhibition, Mexico 2008

18. Nikolovska V., Sekulovski P., Jankuloski D., (2009) *Listeria monocytogenes* in raw meat and meat products, 6th Balkan Congress of Microbiology, 28-31 October, 2009, Ohrid, Macedonia

19. 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.

20. Troxler, R., von Graevenitz, A., Funke, G., Wiedemann, B., Stock, 1., 2000. Natural antibiotic susceptibility of Listeria species: *L grayi, L innocua, L ivanovii, L monocytogenes, L seeligeri* and *L welshimeri strains.* Clinical Microbiology and Infection 6,525-535.