

ANTIBIOTIC RESISTANCE PROFILE OF *L. MONOCYTOGENES* ISOLATED FROM FOOD SOURCES

J.A. Khan^{*1,2}, R.S. Rathore², S. Khan³ and I. Ahmad¹

¹Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University (AMU), Aligarh-202002, India.

²Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, India

³Department of Biotechnology, Doon (PG) Paramedical College and Hospital, Dehradun - 248001, Uttarakhand, India.

ABSTRACT

The infection caused by *L. monocytogenes* is commonly known as listeriosis. The disease occurs in humans and a variety of animals. The bacterium *L. monocytogenes* has considered as an important foodborne pathogen in all over the world. The occurrence of antibiotic resistance among *L. monocytogenes* is posing problem in treatment of the disease. In this study, the *in vitro* testing of antibiotic susceptibility among twenty four food isolates of *L. monocytogenes* showed highest (33.3%) resistance to penicillin. Further a slightly lower (20.8%) resistance to chloramphenicol and tetracycline each was observed. To the ampicillin, ciprofloxacin, cephalothin, gentamycin and trimethoprim a lower range of resistance from 4.1-12.5% was observed. In this study, ciprofloxacin and gentamycin were observed as most effective antibiotic against tested isolates to which only 4.1% and 8.3% resistance were observed respectively. Apart from this, the multiple drug resistant among 3 (8.3%) *L. monocytogenes* isolates was also observed. The presence of *L. monocytogenes* in foodstuffs is a serious problem. This study indicates significant resistance among isolates of *L. monocytogenes* from food sources. That can pose the problem in treatment of *L. monocytogenes* infections as resistant strains may also transfer the resistance to other microorganisms. Therefore, the multiple drug resistance among *L. monocytogenes* is alarming an increase potential threat to human health posed by this pathogen through the consumption of contaminated meat, milk and milk products in Bareilly city, India.

KeyWords: Food; *L. monocytogenes*; Antibiotic resistance; multiple drug resistance.

INTRODUCTION

Listeria monocytogenes is a gram positive, facultative anaerobic, non-acid fast, and a non-spore forming rod that expresses a typical tumbling motility at 20-25°C. The bacterium is a highly adaptable food-borne pathogen that causes the life threatening illness listeriosis in infected individuals (Kaur et al. 2007; Adzitey and Huda 2010). The organism

has been isolated from various animal food products such as meat, milk and their products associated with many listeriosis outbreaks. Therefore, contaminated foods are considered a primary source of transmission of infection in sporadic cases as well as outbreaks (Dumen et al. 2008; Latorre et al. 2009; Jami et al. 2010).

The monitoring of foodborne pathogens presence in different type of foods is primary

* Corresponding author: javed_bacteriologist@yahoo.com

tool for the implementation of food safety systems. It is necessary to monitor the antimicrobial resistance of foodborne pathogens for effective food safety planning and targeted interventions. It is well known that food may play an important role in the transfer of antibiotic resistance in term of antibiotic residues or transfer of resistant genes from food microflora to pathogenic bacteria. The prevalence of antimicrobial drug resistance among foodborne pathogens has increased due to its use in human therapy and animal farming for therapeutic and prophylactic purposes. Consequently, the multidrug resistance among these foodborne pathogens including *L. monocytogenes* has been observed (Wong et al. 2012). It is well known that the treatment of infections caused by multidrug resistant bacteria is more difficult as compare to normal bacteria. Such strains are more dangerous and of great food safety concern. The increase in the prevalence of multi-drug resistant *L. monocytogenes*, particularly resistance to ampicillin, penicillins, aminoglycosides and sulphonamides are an emerging problem worldwide (Morobe et al. 2009). Therefore the objective of this study is to reveal the antibiotic profile of *L. monocytogenes* in the retail food sources comprising raw meat, raw milk and their products in Bareilly, India.

MATERIAL AND METHODS

A total of twenty four isolates of *L. monocytogenes* were obtained from 650 commercial food samples (400 from raw meat and 250 from raw milk and milk products). These food samples were randomly collected from eight butchereries, two dairy farms and three dairy/confectionery shops at various locations in the Bareilly city, Uttar Pradesh, India. The *L. monocytogenes* isolates from different food samples were isolated as per USFDA/BAM/CFSAN method (Khan et al. 2013).

Antibiotic resistance profile of these *L. monocytogenes* isolates was obtained by testing the susceptibility to commonly used antibiotics by disc diffusion method described Bauer et al. (1966). The antibiotics were procured from Hi Media (India). Briefly, 0.1 mL of test bacterial suspension (10^8 cfu/ml) was prepared in BHI broth (Pronadisa, Spain) and was inoculated

onto Muller Hinton (MH) agar plates by using sterile glass spreader. The agar plates were allowed to dry for 10 min. After drying the plates, antibiotic disc were placed on agar plates and by using sterile forcep. The plates were incubated overnight at 37°C. The results were taken after 18 h.

RESULTS

Table 1 showed the antibiotic resistance among twenty four *L. monocytogenes* isolates. The highest (33.3%) resistance to penicillin was observed. However a slightly lower (20.8%) resistance to chloramphenicol and tetracycline each was observed. A lower range of resistance (4.1-12.5%) was observed against ampicillin, ciprofloxacin, cephalothin, gentamycin and trimethoprim.

Table 1: Resistance (%) of *L. monocytogenes* isolates to various antibiotics obtained from various food sources in this study.

S. No.	Antibiotic	Potency (µg per disc)	Resistance (%) among <i>L. monocytogenes</i> isolates
1.	Ampicillin (Am)	10	8.3
2.	Cephalothin (Ch)	30	12.5
3.	Chloramphenicol (Cl)	30	20.8
4.	Ciprofloxacin (Cf)	5	4.1
5.	Gentamycin (G)	10	8.0
6.	Penicillin G (P)	10*	33.3
7.	Trimethoprim (Tm)	5	8.3
8.	Tetracycline (T)	30	20.8

Findings also revealed that ciprofloxacin and gentamycin were observed as most effective antibiotic against tested isolates to which only 4.1% and 8.3% resistance were observed respectively. The resistance of *L. monocytogenes* to the various antibiotics isolated from various food sources has shown in figure 1.

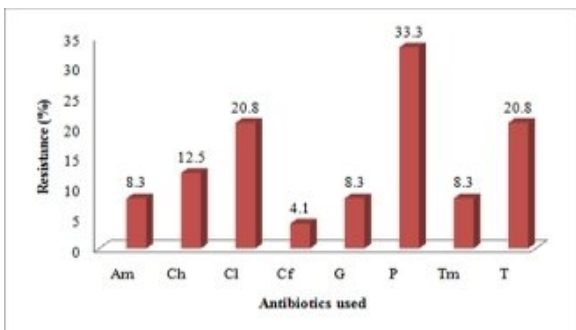
Table 2: *In vitro* antibiotic resistance profile of *L. monocytogenes* isolates.

Isolate No.	Antibiotic resistance profile	Resistant to antibiotics (numbers)
MLM-31	Am	1
BLM-17 BLM-20	Ch	1
BLM-35, CLM-24, CLM-43	Cl	1
CLM-111	Cf	1
BLM-32, FLM-45, CLM-6, LM-19, CLM-116	P	1
FLM-173	Tm	1
FLM-13	Am, G	2
BLM-3	Ch, T	2
FLM-147	G, T	2
CULM-19, MLM-47	P, Cl, T	3
FLM-21	P, Cf, T, Tm	4
FLM-44, FLM-148, CLM-2, CLM-5, CLM-129	Sensitive to all antibiotic tested	Sensitive

BLM- *L. monocytogenes* from beef, FLM- *L. monocytogenes* from fish meat, CLM- *L. monocytogenes* from raw chicken, MLM- *L. monocytogenes* from raw milk, CULM- *L. monocytogenes* from curd. Am- Ampicillin, Ch- Cephalothin, Cl- Chloramphenicol, Cf- Ciprofloxacin, G- Gentamycin, P- Penicillin G, T- Tetracycline, Tm- Trimethoprim.

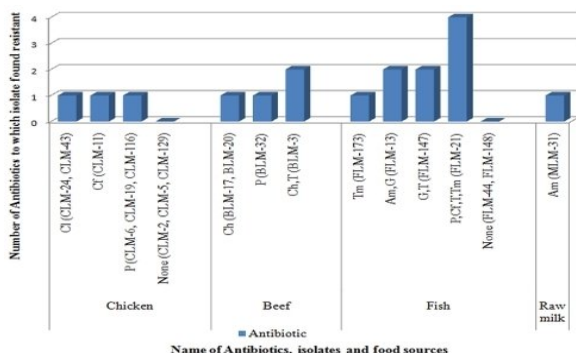
The resistance pattern of *L. monocytogenes* isolates has depicted in table 2.

Figure1: Antibiotic resistance (%) among *L. monocytogenes* isolated from various food sources.



Analysis revealed that three isolates (CULM-19; MLM-47; FLM-21) were resistant to 3 or more antibiotics. Multiple drug resistant (resistance to ≥ 3 antibiotics) among these 3 (8.3%) *L. monocytogenes* isolates was observed. The detail of multiple drug resistant in *L. monocytogenes* isolates from various food sources has shown in figure 2.

Figure 2. Detail of multidrug antibiotic resistance among isolates of *L. monocytogenes* isolated from various food sources.



DISCUSSION

This study revealed the antibiotic resistance pattern of twenty four isolates of *L. monocytogenes* against 8 antibiotics. Overall, a lower resistance observed against each antibiotics viz. ampicillin, cephalothin, ciprofloxacin, gentamycin and trimethoprim. In our observations, isolates have shown a higher susceptibility towards ciprofloxacin and gentamycin.

The drug of choice for treating listeriosis is ampicillin or penicillin G combined with an aminoglycoside, classically gentamicin. The

combination of trimethoprim with a sulfonamide, such as sulfamethoxazole in co-trimoxazole, is considered to be a second choice of therapy in listeriosis (Charpentier and Courvalin 1999). The higher rate of resistance towards the penicillin obtained in our study has also been observed earlier. Whereas close resistant to gentamycin and ampicillin has been also reported (Morobe et al. 2009). In previous other findings, Yucel et al. (2005) indicated a high percentage of resistant strains (66%) of *L. monocytogenes*. In another study, Aureli et al. (2003) showed the occurrence of moderately susceptible *L. monocytogenes* strains (49.1%) in poultry and totally (100%) susceptible *L. monocytogenes* strains in meat. The report supports the occurrence of high rate of susceptible strains observed in our study also towards used antibiotics. The resistance towards the chloramphenicol, trimethoprim and tetracycline obtained in this study was in close agreement as obtained in India recently (Nayak et al. 2012).

In this study, most of the isolates were found resistance to one or more antibiotics. It supports the fact that resistance among foodborne pathogens may increasing due to heavy use as growth promoters in livestock husbandry. It is found that many drugs, antibiotics, and hormones are applied in the livestock industry to get maximum benefit from their meat and by producing more milk (Wong et al. 2012).

The antibiotic resistant pattern also evaluated that 3 isolates (MLM-47, CULM-19 & FLM-21) showed multidrug resistant against 3 or more antibiotics used in this study. The multiple drug resistant also has been reported in India (Nayak et al. 2012; Sharma et al. 2012).

CONCLUSION

As in this study and from other studies highlighted above, it is observed that a considerable resistance among *L. monocytogenes* has been occurred, unfortunately. Therefore, the antibiotic sensitivity pattern of the *L. monocytogenes* in the present findings indicate the presence of multiple drug resistance among *L. monocytogenes* alarming evidence of the emergence of multi resistant *Listeria* strains.

pointing to an increase in the potential threat to human health posed by this pathogen through the consumption of contaminated meat, milk and milk products. The emergence of antibiotic resistant *L. monocytogenes* strains may pose the possibility of clinical treatment failure for listeriosis in future.

ACKNOWLEDGEMENT

I would like to thank Director, IVRI, for granting permission to carry the work at IVRI. I am also very thankful to Dr. S.V.S. Malik (Principal Scientist, Division of VPH, IVRI, Bareilly, India) and Dr. K.N. Bhilegaonkar (Principal Scientist, Division of VPH, IVRI, Bareilly, India) for their constant guidance and sharing information on *L. monocytogenes*.

REFERENCES

- Adzitey, F. and Huda, N. (2010). *Listeria monocytogenes* in foods: incidences and possible control measures. *African Journal of Microbiology Research* **4**: 2848-2855.
- Aureli, P., Ferrini, A.M. and Mannoni, V. (2003). Susceptibility of *Listeria monocytogenes* isolated from food in Italy to antibiotics. *International Journal of Food Microbiology* **83**:325–330.
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. (1966). Antibiotic Susceptibility testing by a standardized single disk method. *The American Journal of Clinical Pathology* **45**: 493-496.
- Charpentier E. and Courvalin P. (1999). Antibiotic resistance in *Listeria* spp. *Antimicrobial Agents Chemotherapy* **43**: 2103-2108.
- Dumen, E, Baca, A.U. and Dumen, E. (2008). Comparative detection of *Listeria monocytogenes* in raw milk by microbiological method and PCR. *Medycyna Weterynaryjna* **64**: 59-63.
- Jami, S., Jamshidi, A. and Khanzadi, S. (2010). The presence of *Listeria monocytogenes* in raw milk samples in Mashhad, Iran. *Iranian Journal of Veterinary Research* **11**: 4-33.
- Kaur, S., Malik, S.V.S., Vaidya, V.M. and Barbuddhe, S.B. (2007). *Listeria monocytogenes* in spontaneous abortions in humans and its detection by multiplex PCR. *Journal of Applied Microbiology* **103**:1889-1896.
- Khan, J.A., Rathore, R.S., Ahmad, I. and Khan, S. (2013). *In vitro* detection of pathogenic *Listeria monocytogenes* from food sources by conventional, molecular and cell culture method. *Brazilian Journal of Microbiology* **44**: 751-758.
- Latorre, A.A., Van Kessel, J.A.S., Karns, J.S., Zurakowski, M.J., Pradhan, A.K., Zadoks, R.N., Boor, K.J. and Schukken, Y.H. (2009). Molecular ecology of *Listeria monocytogenes*: Evidence for a reservoir in milking equipment on a dairy farm. *Applied and Environmental Microbiology* **75**: 1315-1323.
- Morobe, I. C., Obi, C. L., Nyila, M. A., Gashe, B. A. and Matsheka, M. I. (2009). Prevalence, antimicrobial resistance profiles of *Listeria monocytogenes* from various foods in Gaborone, Botswana. *African Journal of Biotechnology* **8**: 6383-6387.
- Nayak, J.B., Brahmabhatt, M.N. and Sadariya, K.A. (2012). Studies on *in vitro* sensitivity and resistance pattern of *Listeria* spp. from raw meat against antibacterial drugs. *Pharma Science Monitor* **3**: 103-117.
- Sharma, D., Sharma, P. K., Saharan, B. S., and Malik A., (2012). Isolation, identification and antibiotic susceptibility profiling of antimicrobial resistant *Listeria monocytogenes* from dairy farm. *International Journal of Microbial Resource Technology* **1**: 1-4.
- Wong, W.C., Pui, C.F., Tunung, R., Ubong, A., Noor Hidayah, M.S., Farinazleen, M.G., Noorlis, A., Cheah, Y.K. and Son, R. (2012). Antibiogram pattern among cultures of *Listeria monocytogenes* isolated from frozen burger patties in Malaysia. *Tropical Agricultural Science* **35**: 793-804.
- Yucel, N., Citak, S., Onder, M., (2005). Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. *Food Microbiology* **22**: 241–245.