

Table 1. Physico-chemical properties of seaweed-supplemented yoghurt

Parameter	Value
Moisture	77.2%
Fat	3.2%
Protein	3.9%
Ash	0.2%
Carbohydrate	15.5%
Hunter color parameters	
	L* 67.2
	a* 2.8
	b* 13.6
pH	4.6
acidity	0.8%
DPPH Activity	80.2%
Metal chelating activity	67.2%
Reducing power	0.5

supplemented yoghurt is a unique attempt to utilize the salubrious seaweed along with goodness of yoghurt. Usage of fucoidan as health

promoting ingredient is well justified considering its health beneficial aspects. The functional benefits of seaweed can be utilized in human diet using yoghurt as supplementation vehicle.

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## Molecular detection of antibiotic resistance genes in multidrug resistant *Listeria monocytogenes* isolated from fish retail markets

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*Listeria monocytogenes* is a ubiquitous pathogen with an ability to contaminate a variety of foods during pre- and/or post-processing (Olaimat et al., 2018). Due to its ability to resist wide environmental conditions such as pH (4.7 to 9.2), high salinity (10% NaCl) and temperature (0.5 to 45°C), *L. monocytogenes* is recognized

as significant food safety hazard, especially in ready-to-eat (RTE) foods. Owing to its high mortality rate (20 to 30%) and hospitalization rates of *Listeria* infection, the Food and Drug Administration of the United States implemented zero tolerance approach for *L. monocytogenes* in all the RTE foods (Hitchins, 1998). This

organism causes severe human illness such as human listeriosis, which results in meningitis, meningoencephalitis, septicemia, and other serious complications during pregnancy such as abortions and stillbirth (Scallan et al., 2011). *L. monocytogenes* is usually susceptible to a broad spectrum of antibiotics that are generally employed effectively against gram positive bacteria but majority of strains show native resistance to fosfomycin, cefepime and cefotaxime (Hof et al., 1997). However, reports on increasing number of multidrug resistant *L. monocytogenes* strains is a major concern in humans and animal health care (Charpentier and Courvalin, 1999). The emergence of the antibiotic resistant strains of *Listeria* spp. might be due to the increased selective pressure of antibiotics or may be due to mutations or acquisition of mobile genetic elements like plasmids and conjugative transposons (Poyart et al. 1990; Charpentier and Courvalin, 1999) through conjugation methods (Perichon and Courvalin, 2009). The present study is aimed to detect the presence of molecular determinants of antibiotic resistance in *L. monocytogenes* isolated from fish and fishery environment which exhibited phenotypic antibiotic resistance.

Isolation of *Listeria monocytogenes* was carried out following USDA method as described by McClain and Lee (1998). Ten isolates of *L. monocytogenes* from fish and fishery environment showing phenotypic resistance to  $\beta$ -lactams, macrolides and tetracyclines were subjected to PCR amplification of antibiotic resistant genes viz., *blaZ* gene for penicillin (Olsen et al., 2006), *ampC* for ampicillin (Dallenne et al., 2010) *ermA*, *ermB*, and *ermC* for erythromycin (Sutcliffe et al., 1996) and *tet* genes *tetA* (Randall et al., 2004), *tetB* (Van et al. 2008), *tetK* (Strommenger et al., 2003), *tetL* (Escolar et al., 2017), *tetM* (Ng et al., 2001) and *tetS* (Charpentier et al., 1993) for tetracyclines. The

results of phenotypic and genotypic resistance of *L. monocytogenes* isolates are provided in Table 1. The results showed that 40% of *L. monocytogenes* strains revealed the presence of two molecular determinants of antibiotic resistance. A high prevalence of *blaZ* (90%) was found in *L. monocytogenes* (Fig.1) followed by *tetS* (40%). The detection of *blaZ* genes in 90% of *L. monocytogenes* isolates suggests that *blaZ* is the chief means of penicillin resistance. The resistance of *L. monocytogenes* to penicillin may be transcribed through the production of the enzyme  $\beta$ -lactamase, controlled by *blaZ*, *blaI*, and *blaR* cluster (Firth and Skurray, 2000). It is reported that *L. monocytogenes* may have attained *blaZ* gene through selection pressure of penicillin or acquired from other bacteria through horizontal gene transfer. Conjugative transfer of antibiotic resistance from the plasmids of enterococci and streptococci to the *Listeria* spp., and the successive movement of such mobile genetic element to *L. monocytogenes* was reported (Charpentier and Courvalin, 1999). All the *L. monocytogenes* isolates showed phenotypic resistance to ampicillin and tetracycline, but none of the ten isolates harbored either *ampC* or *tet* genes, except *tetS*. The present study found that incidence of various antimicrobial resistance determinants did not constantly associate with the phenotypical antibiotic resistance demonstrated by *L. monocytogenes*. This implies that alternate mechanisms such as reduced permeability of outer membrane proteins (Farmer et al., 1992), activation of antibiotic efflux pump (Charvalos et al., 1995), transformation in a gene associated with ribosomal protein (Yan and Taylor, 1991) or co-resistance and cross-resistance may be important drivers of antibiotic resistance. Further work to understand the true mechanisms that contribute to the antibiotic resistance in phenotypic resistant *L. monocytogenes* is necessary.

Table 1. Comparison of phenotypic and genotypic resistance in *L. monocytogenes* isolates

Antibiotic	Interpretive criteria		Phenotypic resistant isolates*	ARG	Amplicon size (bp)	ARG positive isolates*
	Sensitive	Resistant				
Penicillin (10 IU) <sup>a</sup>	13	<13	10	<i>blaZ</i>	377	9
Ampicillin (10 µg) <sup>a</sup>	16	<16	10	<i>ampC</i>	630	0
Erythromycin (15 µg) <sup>a</sup>	25	<25	10	<i>ermA</i>	645	0
				<i>ermB</i>	639	0
				<i>ermC</i>	642	0
Tetracycline (30 µg) <sup>b</sup>	>14	14	10	<i>tetB</i>	773	0
				<i>tetK</i>	360	0
				<i>tetL</i>	739	0
				<i>tetM</i>	406	0
				<i>tetS</i>	573	4

\*number of isolates; <sup>a</sup>: EUCAST breakpoints; <sup>b</sup>: WHONET 5.6

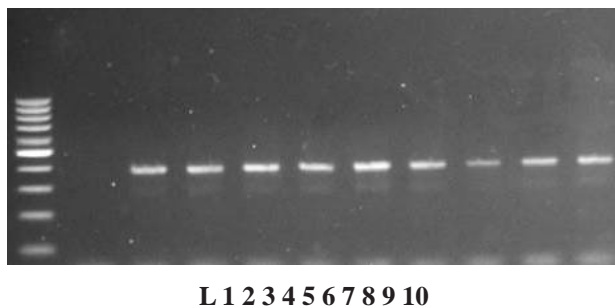


Fig 1. PCR amplification of *blaZ* gene (377 bp) in *L. monocytogenes* isolates. Lane L: 100 bp ladder, Lane 1: negative control, lane: 2-10: *L. monocytogenes* isolates of retail fish market

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