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Antibiogram Profiles of *Listeria monocytogenes* isolated from foods

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***Abstract*— *Listeria monocytogenes* is one of the major foodborne pathogen with an opportunistic character that is able to cause severe human listeriosis worldwide. A total of 23 *L. monocytogenes* strains that were isolated from foods sold in local markets in Selangor, Malaysia were tested for their susceptibility against eight commonly used antimicrobial agents using disc diffusion assay method. The highest resistance in *L. monocytogenes* isolates was noted against ampicillin (100%) and penicillin G (100%) and all of the strains are suspceptible to streptomyocin. More than half of the *L. monocytogenes* strains were shown to be susceptible to chloramphenicol and erythromyocin. The results obtained suggest that there is an emergence of antibiotic multi-resistant Listeria in the environment.**

Keywords-component; Listeria monocytogenes, foodborne pathogens, local markets, antimicrobial agents, disc diffusion assay

I. INTRODUCTION

Listeria monocytogenes is a food-borne pathogen that is widely distributed in the environment and also can be found in the gastrointestinal tract of individuals who remain as asymptomatic carriers. It can cause sporadic and epidemic outbreaks worldwide which is commonly known as *Listeriosis* as a result of consumption of the contaminated foods [1, 2]. Among foods that easily contaminated with *L. monocytogenes* are ready-to-eat (RTE) foods (sausage, burger, etc.), unpasteurized dairy foods (cheese and milk), cured and raw meats (hot dogs, undercooked chicken), prepared seafood salads, and raw and unprocessed meats [3].

The non invasive listeriosis that occurs in healthy adults can only cause gastrointestinal illness, fever, vomiting and diarrhea, where the degree of severity is dependent on the characteristics of the host and the organism's environment. The more severe form of listeriosis, which is known as an invasive listeriosis occurs among the newborn, pregnant women, the elderly and immunocompromised patients [4]. With the onset of epidemics such as HIV/AIDS, there has been an increase in the size of the population at risk of morbidity and mortality due to this type of listeriosis [3]. The disease can manifest as central nervous system (CNS) disease, sepsis, endocarditis, gastroenteritis, focal infections and can cause still births and abortions [5].

In the US, it is estimated that there are 76 million cases of food-borne illnesses each year. According to Mead et al. [6], the incidence of listeriosis is considered low with an average of 2500 infections yearly. However, a mortality rate is considerably higher than the more common infections from other food-borne pathogens such as *Escherichia coli O157:H7 (E. coli)*, *Campylobacter* spp. and *Salmonella* spp. where it can be as high as 20-30% regardless of antimicrobial treatment. Thus, it indicates that the presence of *Listeria* in foods poses a significant danger.

A combination of ampicillin and an aminoglycoside has been known to treat listeriosis [7]. Other antibiotics that can be used to treat the disease are vancomycin, sulfamethoxazole-trimetophrim (SXT) and rifampin antibiotics. Cephalosporins [3] and tetracycline 1 - 5% [8], however are not effective for treating listeriosis. The effective dosage of antibiotics for listeriosis treatment however, has not yet been definitively determined since it is dependent on the immunity of the infected individual and the strain of the organism. Overall, antimicrobial treatment against listeriosis showed susceptibility to most antimicrobials but it can be slowed and may even be untreatable or persistent [9].

II. MATERIALS AND METHODS

In this study, a total of 23 *L. monocytogenes* strains that were isolated from processed meat (which includes sausages, burger, minced meat and chicken) were tested against eight antimicrobial discs that are ampicillin, chloramphenicol, erythromycin, penicillin G, rifampicin, streptomycin, sulfametoxazole-trimetophrim (SXT) and tetracycline. Antimicrobial susceptibility of isolated strains was tested using disc diffusion assay. *Escherichia coli* ATCC 25922 were used as a control strain.

Four colonies of *L. monocytogenes* strains from an overnight Triptic soy agar (TSA) were transferred into 10 ml of Mueller Hinton broth (MHB). Tubes were incubated for 24 hours at 37°C until visibly turbid. Then the cultures were swabbed onto Mueller Hinton agar (MHA) in triplicate using sterile cotton swab. Discs contained each antimicrobial agent were placed onto MHA using antimicrobial susceptibility test system (Oxoid). The plates were incubated for 24 hours at 37°C and then the diameter of clear zone was measured. The diameter of clear zone was measured in millimeter (mm) and only the disc with a diameter of 6 mm was used in this study. Results obtained were then analyzed according to National Committee for Clinical Laboratory Standards (NCCLS) (Performance Standards for Antimicrobial Disc Susceptibility Testing). A susceptibility category of each data was assigned based on breakpoints criteria for *L. monocytogenes* or *Staphylococci*. Table 1 shows the zone diameter interpretive chart for used antibiotics and control zone diameter limits for *E. coli* ATCC 25922 in mm unit.

TABLE 1 ZONE DIAMETER INTERPRETIVE CHART FOR USED ANTIBIOTICS AND CONTROL ZONE DIAMETER LIMITS.

Antibiotics	Zone diameter interpretive standards (mm)			Control Zone diameter limits (mm) for *E. coli* ATCC 25922
	S	I	R	
Ampicillin	≥20	-	≤19	16-22
Chloramphenicol	≥18	13-17	≤12	21-27
Erythromycin	≥23	14-22	≤13	-
Penicillin G	≥28	20-27	≤19	-
Rifampicin	≥20	17-19	≤16	8-10
Streptomycin	≥10	7-9	6	-
Sulphametoxazole-trimetophrim	≥16	11-15	≤10	23-29
Tetracycline	≥19	15-18	≤14	18-25

Note: S = Susceptible; I = intermediate; R = resistant.
The dash (-) indicates that no acceptable range has been established.

III. RESULTS AND DISCUSSION

Table 2 and 3 show the diameters of clear zone of 23 isolated strains against eight antibiotics. The diameters of clear zone for ampicillin are ranging from 0 to 4.0 mm. Clear zone diameters of chloramphenicol were found to range from 3.5 to 28.0 mm. For erythromycin, the diameters of clear zone are ranging from 17.5 to 35.5 mm while for penicillin G ranging from 1.0 to 19.0 mm. The diameters of clear zone for rifampicin are ranging from 9.5 to 20.0 mm. A wide range of clear zone diameters were obtained with streptomycin which ranging from 12.5 to 43.0 mm. For SXT and tetracycline, the diameters of clear zone are ranging from 1.0 to 24.5 mm and 6.0 to 31.5 mm, respectively. In this study, *L. monocytogenes* ATCC 19155 and *E. coli* ATCC 25922 were used as control. The diameters of clear zone for both controls are shown in the tables below.

TABLE 2 DIAMETER OF CLEAR ZONE (MM) OF ISOLATED *L. MONOCYTOGENES* STRAINS AGAINST 8 ANTIBIOTICS TESTED BY DISC DIFFUSION ASSAY.

Isolates	AMP	C	E	P
LM1	1.0	13.5	20.0	15.5
LM2	1.0	15.0	17.5	17.5
LM3	1.0	14.0	19.0	15.0
LM4	1.0	17.0	17.5	17.0
LM5	2.5	19.0	24.5	19.0
LM6	1.0	15.5	22.0	15.0
LM7	0	14.5	21.0	16.5
LM8	4.0	18.0	24.0	18.0
LM9	1.0	18.5	23.5	1.5
LM10	1.5	18.5	25.5	1.5
LM11	2.5	17.5	28.0	1.0
LM12	1.5	18.5	25.0	1.5
LM13	3.0	26.0	35.5	2.0
LM14	1.0	28.0	31.5	2.0
LM15	1.5	22.0	31.0	2.5
LM16	2.0	19.0	23.5	1.5
LM17	2.5	18.5	23.0	1.5
LM18	2.0	21.0	24.0	2.0
LM19	1.0	19.0	21.5	1.0
LM20	1.0	15.0	20.0	1.0
LM21	2.0	16.0	22.0	1.5
LM22	1.0	17.0	24.0	1.5
LM23	1.5	16.5	21.0	5.0
L. monocytogenes ATCC 19155	30.0	19.0	28.0	28.0
E. coli ATCC 25922	17.5	26.5	12.0	1.5

TABLE 3 (CONT.) DIAMETER OF CLEAR ZONE (MM) OF ISOLATED *L. MONOCYTOGENES* STRAINs against 8 antibiotics tested by disc diffusion assay.

Isolates	RD	S	SXT	TE
LM1	11.5	23.5	1.0	8.0
LM2	12.5	21.5	15.0	6.0
LM3	11.0	18.5	14.0	7.0
LM4	13.0	25.5	15.5	8.0
LM5	14.5	24.0	15.0	8.5
LM6	13.0	22.5	13.0	7.5
LM7	12.0	21.5	13.0	7.5
LM8	14.0	29.0	20.5	7.5
LM9	11.5	31.5	21.5	31.5
LM10	13.0	30.5	24.5	30.5
LM11	13.0	32.5	22.0	22.0
LM12	11.0	29.5	3.5	9.0
LM13	20.0	32.0	3.0	13.9
LM14	17.5	43.0	3.5	14.0
LM15	16.7	39.0	3.5	14.0
LM16	11.5	31.0	3.5	9.5
LM17	9.5	27.5	3.5	9.5
LM18	11.0	30.0	4.5	7.0
LM19	16.0	25.0	15.5	9.5
LM20	13.0	12.5	13.0	8.5
LM21	14.0	24.5	12.0	7.0
LM22	20.0	24.5	22.0	9.5
LM23	15.5	25.5	4.0	8.5
L. monocytogenes ATCC 19155	27.5	26.0	31.5	21.5
E. coli ATCC 25922	9.0	17.5	25.5	14.0

Note: AMP = ampicillin; C = chloramphenicol; E = erythromycin; P = penicillin G; RD = rifampicin; S = streptomycin; SXT = sulfametoxazole-trimetophrim; TE = tetracycline.

Table 4 shows the number of isolates for each category (with percentage) to antibiotics tested. More than 80% of the 23 isolated *L. monocytogenes* strains were resistant to ampicillin, penicillin G, rifampicin and tetracyline, and none are resistant to chloramphenicol, erythromycin and streptomycin. All strains are susceptible to streptomycin, and more than 50% of the strains are susceptible to chloramphenicol and erythromycin. For SXT, the susceptibility profiles exhibit similar percentage of strains (39%) that fall under the resistance and intermediate categories. However, less than 22 % of the strains are susceptible to the rifampicin, SXT and tetracycline.

TABLE 4 ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF *L. MONOCYTOGENES* STRAINS ISOLATED FROM RAW AND PROCESSED FOODS IN LOCAL MARKETS.

Antibiotics	No. of isolates (%)		
	Resistant	Intermediate	Susceptible
Ampicillin	23 (100)	0 (0)	0 (0)
Chloramphenicol	0 (0)	11 (47.83)	12 (52.17)
Erythromycin	0 (0)	10 (43.48)	13 (56.52)
Penicillin G	23 (100)	0 (0)	0 (0)
Rifampicin	19 (82.61)	2 (8.70)	2 (8.70)
Streptomycin	0 (0)	0 (0)	23 (100)
Sulfametoxazole-trimetophrim	9 (39.13)	9 (39.13)	5 (21.74)
Tetracycline	20(86.96)	0 (0)	3 (13.04)

The results obtained however, are in contrast than the findings by Mauro et al. [10]. Their findings indicate that the isolated *L. monocytogenes* from raw foods and food processing environments were highly sensitive to ampicillin (98.4%), penicillin (100%), tetracycline (98.4%), rifampicin (98.4%) and SXT (98.4%). Comparing our data to that published in Italy [11], an increasing in resistant percentage was observed on the used antibiotics. Pesavento *et al*. [11] reported that the isolated *L. monocytogenes* strains were resistant to ampicillin (20%), penicillin (5%), tetracycline (2.5%), whereas 0% were resistant to SXT. In a study conducted in Germany by Steve *et al*. (2009) [12], every strain isolated from dairy-based food products were resistant to many antibiotics or at least one of the used antibiotics. They found that most *L. monocytogenes* were resistant to ampicillin (90.00%) and penicillin (60.00%), and some were resistant to tetracycline (20.00%) and SXT (16.67%).

TABLE 5 DISTRIBUTION PATTERN OF RESISTANT *L MONOCYTOGENES* STRAINS AGAINST EIGHT ANTIBIOTICS ACCORDING TO THE NUMBER OF ISOLATES.

Isolates	Resistant distribution							
	AMP	C	E	P	RD	S	SXT	TE
LM1	R			R	R		R	R
LM2	R			R	R			R
LM3	R			R	R			R
LM4	R			R	R			R
LM5	R			R	R			R
LM6	R			R	R			R
LM7	R			R	R			R
LM8	R			R	R			R
LM9	R			R	R			
LM10	R			R	R			
LM11	R			R	R			
LM12	R			R	R		R	R
LM13	R			R			R	R
LM14	R			R			R	R
LM15	R			R			R	R
LM16	R			R	R		R	R
LM17	R			R	R		R	R
LM18	R			R	R		R	R
LM19	R			R	R			R
LM20	R			R	R			R
LM21	R			R	R			R
LM22	R			R				R
LM23	R			R	R		R	R

Note: AMP = ampicillin; C = chloramphenicol; E = erythromycin; P = penicillin G; RD = rifampicin; S = streptomycin; SXT = sulfametoxazole-trimetophrim; TE = tetracycline; R = resistant.

As can be seen in Table 5, multiple resistant was observed in all *L. monocytogenes* strains where all strains were found to be resistant towards ampicillin and penicillin G. Most of the strains (56.52%) were resistant to four antibiotics used. At least six (26.09%) and four (17.39%) strains were resistant to five and three antibiotics, respectively.

The high level of resistance in *L. monocytogenes* towards ampicillin and penicillin G may be attributed to their frequent use for most manifestations of listeriosis, which remain as first choice antibiotics [12]. Sulfamethoxazole-trimetophrim (SXT) is considered to be a second-choice therapy for listeriosis infection especially in patients allergic to penicillin [10]. *L. monocytogenes* isolates may acquire or transfer antibiotics resistance gene from mobile genetic elements such as self-transferable and mobilizable plasmids and conjugative transposons, or mutational events in chromosomal genes [12]. The uptake of antibiotic resistance genes from other Gram-positive bacteria takes place either in vitro or in vivo in the intestinal tract, which then the bacteria are not disrupted by antibiotics during a therapy [11].

Based on the findings, estimation can be made where there is an increased in percentage of multi-resistance over the last few years [10, 11, 13]. In 2008, 3.3% of the isolated strains from milk and dairy products were reported to have multiple resistant to antibiotics used [10]. The number of multiple resistant strains was then increased to 27.5% in 2009 [11] and then up to 45.7% in 2010 [13]. The results of the present study therefore provide further evidence of the emergence of multi-resistant strains in nature, thus representing a potential threat to human health.

IV. CONCLUSION

A continued surveillance on its prevalence and on emerging resistances is important. This will identify foods that can represent a risk for the population and ensure effective treatment of human listeriosis. The results obtained from this study provide an important baseline for the contamination status of the foods in local markets with *L. monocytogenes* and preliminary patterns of its resistance to commonly used antimicrobials. The data may be useful for the food producers, and for epidemiological and public health studies concerning the antibiotic susceptibility of the *L. monocytogenes* and other *Listeria* spp.

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