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A review on *Listeria Monocytogenes* Characteristic, prevalence and antimicrobial resistance

Norhadirah Mohd Noor and John Yew Huat Tang*

School of Food Industry, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, **Malaysia**

***Correspondence: jyhtang@unisza.edu.my.** **Revised: 06 Oct. 2019, Accepted: 08 Oct. 2019 e-Published: 11 Nov. 2019**
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Listeria are Gram- positive bacteria, facultative anaerobic and ubiquitously found in environments such as soil, water, fresh produce and within human. There are 17 species within genus *Listeria*, but the species pathogenic to human health and caused mortality is *Listeria monocytogenes.* The disease, or well known as listeriosis is the third main cause of death and ranked number 11 in causing food poisoning outbreak worldwide. This review addresses the prevalence of *L. monocytogenes* in chicken from Asian countries, the antibiotic resistance profile, survivability and biofilm formation of *L. monocytogenes* in recent years. As Malaysian protein source mainly from chicken, it has a potential to be the major carrier for *L. monocytogenes* transmission. Due to the naturally present of *L. monocytogenes* in chicken, thus its prevalence and numbers should be known to ensure it is within the safe level. The characteristic of *L. monocytogenes* such as its survivability on different types of food contact surfaces need to be highlighted as it able to form biofilm over extended period although not sufficient nutrients. *L. monocytogenes* biofilm is resistance not only to processing condition, but also to different types of antibiotics. The increasing resistance towards antibiotic shown by recent studies will become a major threat as antibiotic is the primary means used globally to treat listeriosis. The presence of *L. monocytogenes* from various sources includes chicken is unavoidable, thus strict control measures are very important to prevent listeriosis from occurring. Besides, more further studies are needed to reduce the possibility of biofilm formation.

Keywords: *L. monocytogenes,* prevalence, antibiotic resistance, survivability, biofilm

INTRODUCTION

Chicken based dishes can be found widely across all country regardless the race, culture and religion. Chicken meat taste is acceptable to all generations and it is well accepted by almost all religions in the world. Chicken is packed with protein; 3-ounce serving of cooked chicken breast can supply 140 calories, 3 grams of fat and 25 grams of protein (Juntti, 2018). Consumers prefer eating white meat due to it has lower saturated fat and a good source of protein. Besides, chicken is easy to breed, cheaper cost and the meat can be harvested as early as 30-35 days until 55-60 days (Poultry Hub, 2018).

Chicken can carry many foodborne pathogens such as *Eschericia coli, Listeria monocytogenes, Salmonella* and *Staphylococcus.* These pathogenic bacteria come from surrounding environment and can survive under desired conditions such as high moisture levels, presence of oxygen and optimum temperature, and eventually will contaminate chicken meat during processing. Sources of contamination including improper handling during preparation which lead to cross contamination between raw and cooked chicken or from the bacteria adhered tightly on processing equipment, inadequate time or

temperature for cooking and also from food handler itself. Besides, pathogenic bacteria also can growth at improper holding temperatures (CDC, 2015).

L. monocytogenes, L. seeligeri, L. ivanovii, L. welshimeri, *L. marthii*, *L. innocua*, *L. grayi*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia*, and *L. booriae* are 17 recognized species of the genus *Listeria* (Orsi and Wiedmann, 2016). However, only *L. monocytogenes* can cause serious food borne illness called listeriosis to the consumer and young poultry that acts as the carrier (Crespo et al., 2013). The listeriosis severity can range from mild gastroenteritis to severe disease conditions such as septicemia, encephalitis, meningitis, abortions and stillbirth and even can cause mortality (Zhu et al., 2017).

The previous study showed the prevalence of *L. monocytogenes* in fresh produce although there is no listeriosis outbreak being reported in Malaysia over the past 15 years (Kuan et al., 2014). The published formation of *L. monocytogenes* from meat, milk and milk products from all over the world is very scattered and unsystematic, both in veterinary and public health sectors (Khan, et al., 2013). In this review, the prevalence of *L. monocytogenes* in chicken from Asian countries is being collected from year 2010. It is very critical to understand more about *L. monocytogenes* prevalence as well as other pathogenic bacteria to reduce the risk of contamination. Presence of *L. monocytogenes* even at low quantity would be enough to cause sickness for immunocompromised individual as low as 0.1- 10 million CFU/ml while 10-100 million CFU/ml for healthy individual (Bortulossi, 2008).

Previous studies revealed that *L. monocytogenes* can survive changes in natural environment including frozen temperature, acidic to alkali pH and minimum water activity (FSANZ, 2013). *L. monocytogenes* can grow at temperature up to 45^{o}C and can withstand in high salinity. However, in acidic condition, the bacteria will not be able to survive at high temperature because acidic condition act as synergistic effect to kill it (Lado and Yousef, 2007). *Listeria* is killed by heat treatment more than 65^{o}C such as cooking, pasteurization and sterilization. In addition, food can be contaminated after processing through contact with contaminated hands, equipment and counter tops (Bortulossi, 2008).

Using antimicrobial agent alone can kill *L. monocytogenes* but it has the limitation as more and more pathogens are becoming resistant to it and not all patients can suit to the treatment (Sulakvelidze, et al., 2001). The immense use of antibiotics in agriculture such as added into chicken feed to promote growth and performance in shorter time and to prevent it from enteric diseases may cause *L. monocytogenes* in chicken to become resistant to those antibiotics (Alonso- Hernando et al. 2012; Fallah et al., 2012; Sugiri et al., 2014; Dan et al., 2015). More than 23,000 out of 2 million people in the United States die after becoming ill with antibiotic resistant infections every year. The highest incidence was reported among children below five years old (CDC, 2013). Although there is no illness resulted from antibiotic resistant in *L. monocytogenes,* but due to bacteria capability to adapt and develop resistance towards antibiotics, *L. monocytogenes* too will become resistant (Willing et al., 2018).

Listeriosis outbreak

Listeriosis outbreak were reported in Australia in 2009 where 40 cases resulted from consumption of cooked chicken in pre-packaged wraps and in United States 2008, fatal case of listeriosis caused by eating chicken salad (OzFoodNet, 2009; Marcus et al., 2009). CDC (2016), estimated about 1600 people get sick from *Listeria* spp. each year with 260 deaths. Most recently, in July 2018, nine deaths resulted from listeriosis occurred across Europe. The source of the outbreak is from frozen sweetcorn products and possible contaminated sources such as frozen mixed vegetables also had been recalled from supermarket (Hodgkin, 2018).

The worst listeriosis cases ever documented was from South Africa. The number of deaths had increased from 36 deaths to 164 since December 2017 to February 2018 (NICD, 2018). In March 2018, National Department Health of South Africa had released media statement which confirmed the original source of listeriosis outbreak was come from the enterprise food factory in Polokwane, South Africa.

Table 1; Prevalence of *L. monocytogenes* in chicken and chicken products from Asian countries

Country	Year	Samples	Prevalence (%)	References
Malaysia	2012	Raw chicken	20.0	Goh et al., (2012)
	2012	Chicken and RTE products	13.2	Jamali et al., (2013)
	2013	Chicken offal	26.4	Kuan et al., (2013)
Thailand	2009	Frozen chicken meat	2.5	Kanarat et al., (2011)
	2007	Chicken	13.9	Indrawattana et al., (2011)
Pakistan	2003	Fresh chicken meat	12.5	Mahmood et al,. (2003)
Bangladesh	2016	Fresh chicken	8.3	Islam et al., (2016)
Japan	1998-2003	Minced chicken	15.5	Ochiai et al., (2010)
Indonesia	2013	Fresh chicken carcasses	15.8	Sugiri et al. (2014)
China	2014	Poultry products	12.3	Wu et al., (2015)
Egypt	2010	Frozen chicken leg	8.0	El- Malek et al., (2010)
	2011	Ready- to- eat chicken	17.0	El- Shenawy et al., (2011)
India	2017	Raw chicken meat	23.0	Mahantesh et al., (2017)
Iraq	2017	Raw chicken	8.0	Said Ahmed et al., (2017)
South Korea	2018	Chicken carcass	10.7	Oh et al., (2018)

The infected patients were believed consuming processed foods; ready to eat cold meat (polony) and sausages which indicated that the finished product was cross contaminated with *L. monocytogenes* either before, during or after preparation (Chutel, 2018). The prevalence of *L. monocytogenes* is varied depends on geographic location, weather, different timeline and food samples (Table 1). From the study done by Goh et al., (2012), the result compared the prevalence of *L. monocytogenes* in raw chicken randomly collected from 3 hypermarkets (25.71%) and 3 wet markets in Selangor, Malaysia (14.29%). The total of prevalence from both samples are 20%. The variety in results might be due to the chicken in hypermarket is stored in cold room and displayed on ice which *L. monocytogenes* can highly tolerate to cold stresses (Goh et al., 2012). Not only can survive in refrigeration temperature but it is also can multiply in the refrigerated temperature (Mahmood et al., 2003). Wu et al., (2015) stated that freezing only take effect when more than 3.9 log CFU/ cm^2 of *L. monocytogenes* was presented although the reduction is not significant.

The different case occurred to the study done by Indrawattana et al., (2011), chicken sold in open market (13.9%) is more contaminated than supermarket (0.0%). It is concluded that the contamination was caused by the retailers during distribution and handling process. The prevalence study of *L. monocytogenes* in ready- to- eat (RTE) chicken products and chicken meat done by Jamali et al., (2014) was to compare whether different cooking methods can influence its prevalence. The prevalence of *L. monocytogenes* from chicken gizzard, liver and fillet is 20%, 33.3% and 25%, respectively while 22.2% from fried chicken and 16.7% from chicken satay.

As concluded by Oh et al., (2018), 10.71% prevalence of *L. monocytogenes* in chicken carcasses are not cross- contaminated from feces, instead by the workers and skin to skin contact between carcasses. Frozen and chilled chicken were being tested for the presence of *L. monocytogenes* immediately after slaughtering and processing but the bacteria is absent in the chicken (Kanarat et al., 2011). The source of contamination might come from utensils, processing plant environments and human contact

(El-Malek et al., 2010; Kanarat et al., 2011). Compare to other types of meat sold in retail market, chicken has the most probable as the source of *Listeria* infection and contamination (Ochiai et al., 2010). According to Ochiai et al., (2010), the prevalence of *L. monocytogenes* in retail meat including chicken is nearly identical to other countries.

LISTERIOSIS CLINICAL MANIFESTIONS

Listeria bacteria can infect different sites of the body such as central nervous system, blood circulation system and gastrointestinal tract not only in humans but also to other vertebrates (Vázquez-Boland et al., 2001; Dennis, 2015). *L. monocytogenes* infect people by several transmission routes including consumption of contaminated foods which is the main route of listeriosis infection (WHO, 2018). Next, from pregnant mother to the fetus during or after pregnancy through the placenta. This infection probably will cause the baby to get neonatal listeriosis (Tesini, 2018). Lastly, by direct touching *L. monocytogenes* which can cause lesions on skin (Cutter, 2017; Tesini, 2018; WHO, 2018). The numbers of possible transmission routes and the ability of *L. monocytogenes* to survive under harsh conditions make the spread of listeriosis disease difficult to control (Cutter, 2017).

The most common complications of listeriosis are septicemia which is bacterial pathogens in the blood followed by meningitis and other complications include inflammation of the brain (encephalitis), brain abscess, inflammation of the heart membrane (endocarditis) and localized infection (Dennis, 2015). There are two types of listeriosis which are non- invasive form which is the mild symptoms of listeriosis which typically felt by healthy people and invasive form; the more acute form and affects immunocompromised individuals (WHO, 2018).

The incubation period of listeriosis is between 3 days to 70 days (Goulet et al., 2013). However, for heavily contaminated food, the infection will begin after 20h of consumption (Vázquez-Boland et al., 2001). The symptoms are varied between infected patients. For example, for immunocompromised individuals, symptoms can include fever, muscle aches, headache, stiff neck, confusion, loss of balance and convulsions. While for the pregnant woman typically experience fever, chills and headaches. Listeriosis can spread quickly and harmfully in pregnant woman which resulted in 20% spontaneous abortion or stillbirth (Mestrovic, 2018). Compare to healthy adults, pregnant woman is 20 times more risk to get listeriosis while people with HIV/AIDS are 300 times more risk (WHO, 2018). About two-thirds of babies born from mothers infected with listeriosis got neonatal listeriosis; as early as hours after birth or delayed onset (Mestrovic, 2018). Low-birth weight, obstetric complications, symptoms of sepsis with circulatory or respiratory inadequacy or both are caused by neonates with early- onset. For delayed- onset, the complications are meningitis or sepsis. The risk of death is range from 10- 50% and higher in neonates with early-onset disease (Tesini, 2018).

Healthy people are rarely developed invasive listeriosis, but they might experience with diarrhea, headache, nausea, muscle pain and fever of non-invasive type of *Listeria* infection if they are exposed to a very large dose (WHO, 2018). These symptoms also occurred during febrile gastroenteritis as reported by Ooi and Lorber (2005), healthy persons also had risk to get febrile gastroenteritis caused by *L. monocytogenes.* Gastroenteritis is happened when the lining of stomach, small or large intestines becomes inflamed (Boyce, 2017). To treat gastroenteritis, oral or IV rehydration is enough for mild one or prescription of antidiarrheal agents only if *C. difficile* or *E. coli* O157:H7 infection is not suspected. While antibiotics is only for selected case (Boyce, 2017). Patient with non- invasive type of infection will not have the bacteria spread into bloodstream or other sites and they will recover over time without getting further treatment (CDC, 2015). The duration of symptoms can be at most 1 week or 1-3 days (Ooi and Lorber, 2005).

Listeriosis is a rare disease compared with other common food-borne illnesses such as salmonellosis or botulism. It is a low incidence foodborne disease but a high case fatality rate (Goulet et al., 2013). In facts, in 2015, *L. monocytogenes* ranked number 11 in causing foodborne disease outbreaks (CDC, 2015). Therefore, the public health importance of listeriosis is usually neglected. Listeriosis ranks second after salmonellosis for causing mortal cases related to foodborne illness (Rossi et al., 2008). Angulo et al., (2008) stated that cases of foodborne outbreaks are frequently unrecognized, unreported and not investigated.

There are enough access resources to investigate foodborne outbreaks such as established web- based outbreak surveillance system and food tracing system which is

compulsory to be implemented by every food industry (Wu et al., 2018; Mena and Stevens, 2010). However, these systems are not fully developed yet in some Asia countries caused by economic factor, human resources, laboratory facilities and trained workers (Salleh et al., 2017). Next, people who got food poisoning symptoms usually refused to be treated and diagnosed in hospital and this will lead to under reporting (Institute of Medicine, 2006). The same thing occurred in Malaysia, where most of foodborne cases were underreported due to lot of procedures are required to bring up the cases to the authority (Salleh et al., 2017). For example, during *E. coli* 0104:H4 outbreak in 2011, reporting system done by Germany authority started from detection of pathogen from patient stool samples, notify the detected pathogen to >400 health offices for them to validate the data, then the validation were continued by 16 State Ministries, Federal Health Ministry, and Robert Koch Institute which are responsible to track foodborne illnesses and finally reported to WHO (Soon et al. 2012).

To control foodborne disease, the real causes of disease need to be identified at the first place before investigation can be done (Biggerstaff, 2013). Next, food surveillance system needs to supervise all food chain scopes which include food safety control, laboratory- based surveillance, food inspection, consumer education and quick response system during food emergency cases (Salleh et al., 2017).

Listeria monocytogenes in poultry

Malaysia's livestock industry consists of three industries which are poultry industry (chicken, duck, geese and ostrich), the swine industry (pig), and the ruminant industry (cattle, buffalo, goat and sheep) but the most commercialized industry and advanced production system is poultry industry. In 2012, poultry industry had accounted for 57.5% from the total of livestock sector (Abdurofi et al., 2017).

Poultry has long been recognized as a natural source of nutrients and is the world's primary source of protein. Malaysian is among the highest poultry consumers in the world with an average consumption of 45 kg and 370 eggs per capita annually in 2017. Approximately, 30 million eggs were required a day to meet the demand (AER, 2017). From 100% of the total livestock production in Malaysia during 2010, 53.2% of it came from poultry production (Mohd et al., 2015). Due to Malaysia self- sufficiency is high; 98.4% both in 2013 and 2014 of the local domestic demand for meat, Malaysia managed to export processed poultry products and live broilers business to Singapore and Middle East countries (DOSM, 2015; Abdurofi, Ismail and Md Isa, 2018).

Chicken meat is a type of perishable foods and have high water activity. Meat is the best medium for microorganism growth as it is rich in proteins, lipids and water (Olaoye, 2011). It has to be kept in chilled condition to avoid from spoilage which can occur within a few days. Chicken meat should be handled properly to reduce the risk of cross contamination during processing. The internal temperature of cooked chicken should be more than 73.9^{o}C to ensure all pathogenic bacteria including *L. monocytogenes* being killed (FSIS, 2015). Present study by Rothrock et al., (2017) stated that listeriosis outbreak isolated from poultry specifically from chickens have not been in the previous record. However, Crespo et al., (2013) stated that their study is the first report of *L. monocytogenes* infection in poultry flock in Washington state which mean the risk of listeriosis from poultry is still high especially when cross contaminated or temperature abuse of RTE chicken meal or raw chicken (Rothrock et al., 2017).

From data collected by Ochiai et al., (2010), the highest *L. monocytogenes* prevalence in retailed meat was in chicken (15.5%) compared to beef and pork. The prevalence of *L. monocytogenes* in poultry products and processing plants were detected in high number all around the world. Meanwhile, from study done by Mashak et al., (2015) the highest *L. monocytogenes* number was from beef (8.33%). The same result from the study done by Wu et al., (2015), beef also had the highest number of *L. monocytogenes* compare to other types of meat which is 34.8%. Therefore, although some studies found poultry products have the highest *L. monocytogenes* contamination and some studies were not, still poultry has the potential to act as vehicle transmission of listeriosis

Survivability and biofilm formation of *I. Monocytogenes*

With massive raw food materials production over 64.7 US $ Billion in 2016 (Wahab, 2017), there are possibilities that foodborne illness can be caused by inadequate cleaned and sanitized food equipment and utensils. Food pathogenic bacteria including *Escherichia coli, Salmonella* and *L. monocytogenes* can survive for hours or

days not only on food, but also on hands, sponges, clothes and utensils (Silva et al., 2008). In some cases, *L. monocytogenes* can survive up to 10 years and it was shown that *L. monocytogenes* has greater survivability compare to *S. aureus* and *S.* Typhimurium (Takahashi et al., 2011). Even after disinfection procedures, the pathogenic bacteria may survive on equipment surface thus, when food direct contact with these surfaces, the food will cross contaminate (Silva et al.; Santacruz, 2016; Coyle, 2018).

The survival of *L. monocytogenes* not just because of its capabilities in withstand extreme conditions such as at frozen temperature; -18^{o}C and can grow at temperature above than 45^{O}C, survive in a wide range of pH (4.0- 9.6) and minimum water activity of 0.90, but also favored by accumulation of food residues which lead to biofilm formation (FSANZ, 2013; Dzielciol et al., 2016). *L. monocytogenes* is a Gram- positive bacteria thus it has thicker peptidoglycans to retain the water loss from it. Therefore, it can resist to dryness more than Gram- negative bacteria (Takahashi et al., 2011). However, *L. monocytogenes* can be easily killed by applying high temperature more than 65^{o}C such as pasteurization but there were cases reported the contamination of *L. monocytogenes* occurred when the heat treatment failed to reach inside the food products core (Bortulossi, 2008; Rothrock et al., 2017). Thus, it may allow the bacteria to survive and over extended period, biofilm may develop (Bortulossi, 2008).

L. monocytogenes survived during exposure to high concentration of alkaline cleaning solution but it is killed in sanitizer (Taormina and Beuchat, 2002). This study approved that sanitizing is complementary process of cleaning. This process can reduce *L. monocytogenes* number to a safe level unfortunately not reducing its biofilm formation. Biofilms developed higher tolerance to cleaning and sanitizing agents over time and also tolerate to peracetic acid and QUAT's sanitizer (Fagerlund et al., 2017).

Biofilm is formed as a stress response to increase the bacteria protection against antimicrobial agents, host defenses, nutrient depletion and physical forces such as shear force (Jefferson, 2004). Next, biofilm is form as a mechanism to colonize the bacteria favorable niche. Besides, the bacteria inside biofilm is consist of different species of bacteria which they will form a bacteria community to utilize all the benefits for survival (Jefferson, 2004; Reichhardt et al., 2014).

Biofilm can form at different thickness, biovolume, roughness and range from flat multilayers to honeycomb-like structures. The most observed structure is honeycomb-like structures (Colagiorgi et al., 2017; Guilbaud et al., 2015). The 'honeycomb' like structured composed of *L. monocytogenes* cells stacked together around holes containing dead cells and extracellular DNA (eDNA) (Guilbaud et al., 2015). eDNA acts as an energy and nutrition source as well as structural support (Colagiorgi et al., 2017). The holes act as routes for the exchange of waste, nutrients and water and leathery Extracellular Polymeric Substances (EPS) anchoring the stacked cells in their place (Marsh, Luo and Wang, 2003). EPS are composed of polysaccharides, proteins, nucleic acids, lipids, phospholipids and humic substances and it is form when bacteria make the first contact with the hydrophobic surface and release thin fiber layer to adhere to the surface. EPS acts as glue to bind cells together and other particulates such as food soils and organic material. It also acts as a protective barrier against harsh condition such as during cleaning and sanitizing (Kent, 2010).

Biofilm will form especially when microorganisms adhered tightly on processing equipment and inadequate washing and disinfection process could not remove them from it (Borucki et. al., 2003; Poimenidou et al., 2009). The factors initiate the adhesion of bacteria to surfaces at the first place is still not well understood due to many intrinsic and extrinsic factors (Silva et al., 2008). Studies proved that the worst ability of *L. monocytogenes* to form biofilms is only moderate and the biofilm is not as thick as the other bacteria's biofilm. Yet, depending on the abiotic surface material and *L. monocytogenes* strain, it can adhere strongly to these surfaces which make it difficult to remove (Silva et al., 2008; Reis-Teixeira, Alves and Martinis, 2017). Other than surface hydrophobicity, the formation also dependent on several factors such as bacteria strain, medium and temperature (Colagiorgi et al., 2017).

The contact surfaces or known as abiotic surfaces are highly sensitive for bacterial adhesion eventually will form biofilm (Khelissa et al., 2017). Abiotic surfaces such as polystyrene, glass and stainless-steel act as reservoir for a period of time before *L. monocytogenes* biofilm grown on it (Winkelstroter et. al., 2011). Some parts of

equipment are hard to clean such as at edges, convex surfaces, crevices and small parts which did not allow for direct scrubbing and scraping activities (Poimenidou et. al., 2009). Crack and crevices caused irregularities on surfaces and it will trap the pioneer species of bacteria to survive and form biofilm (Silva et al., 2008; Kent, 2010).

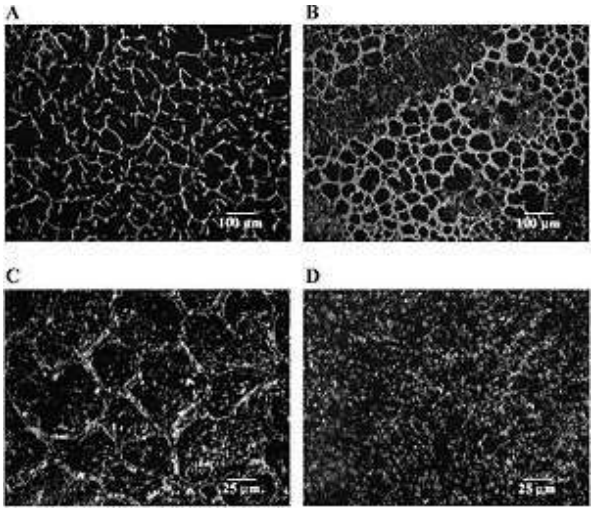

Figure 2; 'Honeycomb' patterns observed under Scanning Electron Microscope after sample processing. A: 3h. B: 6h. C: 24h. D: 72h. (Marsh, Wang and Luo, 2003)

As reported by Di Bonaventura et al. (2008) and Silva et al., (2008), the highest level of biofilm formation is on glass surface compare to stainless steel and polystyrene which are hydrophobic while glass is hydrophilic. The roughness value of contact surface also influenced the rate of bacteria adhesion as it can increase the attractive force between bacteria and the surface to facilitate the rate of initial adhesion (Silva et al., 2008; Rabin et al., 2015). However, at 10-20nm distance of the bacteria from the surface, the repulsive force resulted from the same negative charges produced by the bacteria and surface will reduce the probability of the adhesion but with the help of flagella, the bacteria can swim towards the surface (Rabin et al., 2015).

Overall, biofilm formation has 3 stages; attachment, maturation and dispersion (Crouzet, 2014). Dispersion stage is the most dangerous stage and pose the highest risk to human health as the bacterial cells which released has higher resistance to stressful changes and it has higher growth rate even under refrigeration as compared to bacteria derived from common source (Poimenidou, 2009). *L. monocytogenes* biofilm can grow at temperature as low as 4^{o}C and it is increased with temperature rise (Poimenidou, 2009; Colagiorgi et al., 2017). Even using low temperature during food processing and storage, the risk of biofilm cross contamination is still present (Colagiorgi et al., 2017).

The 'honeycomb' structured of biofilm is hard to remove, able to withstand in extreme conditions and more resistant to disinfection. Thus, if biofilm consists of pathogenic bacteria such as *L. monocytogenes*, it may lead to a serious problem by cross contaminate the food product and becomes threat to the health safety of consumers (Borucki et al., 2003; Khelissa et al., 2017; Kurpas, Wieczorek and Osek, 2018). For instance, although ready-to eat (RTE) food is not categorized in high- risk food group as poultry, (Foodsafety.gov, 2018) but due to no processing treatment needed to be done prior to consume RTE food, so when it was already post-contaminate, it will harbor bacteria into human host.

From EU member states in 2015, 3.5% and 4% of *L. monocytogenes* were detected from 2,847 RTE fish and 2,366 RTE meat samples respectively (Kurpas, Wieczorek and Osek, 2018). In the US state, FDA already applied 'zero tolerance policy' for *L. monocytogenes* in RTE food product which mean the only tolerance level is less than 1 in 25g or less than 0.04 in 1 g (Salas, 2015).

Biofilm requires higher investment for cleaning and maintaining purposes and corrective actions need to be taken to overcome this problem because even at strict hygienic measures, the present of biofilm still being detected in food processing plants (Reis- Teixeira et. al., 2017).

The method to prevent the formation of biofilm is through cultivation of competitive microbiota which also can form biofilm. According to Poimenidou, (2009) some bacterial species may prevent the attachment of *L. monocytogenes* by producing antilisterial metabolites. A study done by Unlu, Nielsen and Ionita, (2015) showed that some of these bacteriocins derived from Lactic Acid Bacteria in Dairy- Based media give both positive and negative result towards *L. monocytogenes* strain. Previous research by Minei et al., (2008) showed that probiotic *Enterococcus faecium* able to reduce the biofilm formation effectively while gives protection from bacteria colonization.

Besides, abiotic surface such as stainless steel is the most preferred surface for bacteria

attachment. This might give problems to the food manufacturer as the usage of stainless steel is abundant in making food processing equipment due to the stability of physical and chemical properties at different processing temperatures (Kent, 2010; Sandmeyer, 2016). Above that, it is also resistant to corrosion and easy to clean. Hence, modify the abiotic surface such as by coating it with antimicrobial surfaces not only will reduce the surface adhesins but also can kill bacteria that attach onto it.

A review done by Shah et al., (2013) mentioned several types of coatings such as poly(ethylene glycol) (PEG), modified chitosan, antibiotic loaded polymer coatings, zwitterionic coatings, formulations of silver, immobilized enzymes and their combinations have been investigated by many studies and already showed positive results in vitro stage. More studies of antibiofilm surface modifications need to be done as now it is at early stages of discovery.

Next, a study done by Feng et al., (2015) suggested the effectiveness of introducing cylindrical nanopores with diameters range from 1 to 100 nm on alumina surface. They found out the most minimal biofilm formation was on surfaces with pore diameters of 1 and 2 nm. These nanopores produced repulsive forces, electrostatic and acid base forces which gave bacteria repelling effects.

Bacterial biofilm formation is unavoidable, better and strict approaches are needed to prevent the formation at the first place rather than finding the solutions to remove the biofilm on abiotic surface.

ANTIBIOTIC RESISTANCE

Antibiotics are commonly used not only as medical treatment, but also widely used in veterinary, agriculture and aquaculture (Economou and Gousia, 2015). Antibiotics acted upon bacteria via 5 mechanisms; to inhibit the bacteria growth by interferes with cell wall, protein or nucleic acid synthesis and antimetabolite activity (Rollins, 2000). Lastly, directly kill the bacteria by disrupting its membrane structure.

Antibiotics are the best therapy to treat invasive *Listeria* infections. If infection occurs during pregnancy, antibiotics (ampicillin and aminoglycosides) will be given promptly to prevent the infectious towards fetus. Before the physicians are certain of the disease, the babies with listeriosis will receive the same antibiotics as their mother although it may harm the babies due to multiple use of antibiotics (Dennis, 2015). The optimal duration for antibiotics therapy is unknown. However, 14- day course is usually sufficient while for meningitis is 21- day (Tesini, 2018).

Over time, bacteria will develop and evolve to bactericidal effect of antibiotics (Ventola, 2015). The first case of *L. monocytogenes* strains become resistant to antibiotic is reported in 1988. Since then, the number of cases whether isolated from foods, animals and humans have also increased (Altuntas, 2012). Infections with antibiotic resistance (AR) strains would incur higher financial cost and more challenging to overcome compare to typical bacteria thus it needs other alternatives to replace the use of antibiotics and also to keep AR bacteria from spreading (CDC, 2015). In the USA, 23 000 deaths which was caused by antibiotic- resistant infections was estimated each year (Chang et al,. 2014).

According to Sugiri et al., (2014), most *L. monocytogenes* strains shown sensitivity towards antibiotics. However, the strains are resistance to penicillin (17.2%), ampicillin (6.9%), erythromycin (6.9%) and combination of ampicillin and penicillin (3.4%). Quinolones, enrofloxacin and tetracycline are antibiotics remarkably used in poultry production in the country which resulted in high resistance towards quinolones (21.05%) and tetracycline (31.57%) (Dan et al., 2015). Ampicillin and penicillin are the most preferred choice to treat listeriosis however, *L. monocytogenes* shown high resistance to ampicillin; 44.9% and penicillin; 41.8% (Table 2). As stated by Fallah et al., (2012), the persistent use of ciprofloxacin and tetracycline in Iranian poultry farm had caused emergence of antibiotic resistance strains towards these antibiotics. Besides, the illegal use of chloramphenicol in the same sector also caused the resistance up to 24.5%.

L. monocytogenes strains were tested at different years (1996 and 2003) to prove the emergence of antibiotic resistance (Alonso-Hernando et al., 2012). Not only the resistance is increased, the strains also showed new resistancy towards streptomycin, gentamicin, neomycin and rifampicin in 2003.

Table 2; Antibiogram profile of *L. monocytogenes* isolates from chicken

Types of antibiotics	Resistance (%)	References
Gentamicin	12.0 10.2	Alonso- Hernando et al. , (2012) Fallah et al., (2012)
Streptomycin	4.0 32.0	Antunes et al., (2002) Alonso- Hernando et al. (2012)
Neomycin	84.0	Alonso- Hernando et al., (2012)
Rifampicin	8.0 8.16	Alonso- Hernando et al., (2012) Fallah et al., (2012)
Enrofloxacin	58.0 23.3, 68.0** 27.5	Antunes et al., (2003) Alonso- Hernando et al., (2012) Fallah et al., (2012)
Ciprofloxacin	25.6, 52.0** 24.5 10.25	Alonso- Hernando et al., (2012) Fallah et al. (2012) Dan et al., (2015)
Nalidixic acid	100.0** 10.52	Alonso- Hernando et al., (2012) Dan et al., (2015)
Furazolidone	52.0	Alonso- Hernando et al., (2012)
Ampicillin	44.9 6.9 10.25	Fallah et al., (2012) Sugiri et al., (2014) Dan et al. ,(2015)
Chloramphenicol	24.5 21.05	Fallah et al., (2012) Dan et al., (2015)
Clindamycin	35.0 8.16	Antunes et al., (2003) Fallah et al., (2012)
Erythromycin	15.3 6.9	Fallah et al., (2012) Sugiri et al., (2014)
Penicillin	41.8 17.2	Fallah et al., (2012) Sugiri et al., (2014)
Tetracycline	34.7 31.57	Fallah et al., (2012) Dan et al., (2015)
Trimethoprim/ sulfamethoxazole	5.26	Dan et al., (2015)
β-lactams	10.52	Dan et al., (2015)
Sulfonamides	5.26	Dan et al., (2015)
Quinolones and fluoroquinolones	21.05	Dan et al., (2015)

**Result compared between years 1996 and 2003

In WHO South East Asia region (Bangladesh, Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand, Timor Leste), AR had caused critical problems to political, social and economy. This region is predicted to pose the highest risk for emergence and spread of AR globally because Asia- Pacific region already covered more than 70% of worldwide population (Kang and Song, 2013). In recent years, the present of AR genes in the main bacterial pathogen in Asian countries had shown rapid increment. The corrective actions taken by the health government also was very limited in this region (Kang and Song, 2013).

Overused and misused of antibiotics and spreading of AR strains from person to person or from animal to human are the factors of the emergence AR strains (CDC, 2015). Based on review done by Chang et al., (2014), there is no precise conclusion to put all the blame on antibiotics usage in the agriculture sector for the increase in resistant strains as commonly described by many studies. The use of antibiotics in animal feed is to promote growth, treat disease and infections of animals thus to prevent them from spreading in husbandry, produce higher quality meat and lastly to improve Feed Conversion Ratio (FCR) (CDC, 2015; Manyi-loh et al., 2018).

Interestingly, indiscriminate administration of antibiotics to patient has caused the increase in AR bacteria strain (CDC, 2015). There are three

possible mechanisms of transferred AR from agriculture to humans which are human comes into direct contact with infected livestock or from consuming contaminated meat or water. Secondly, ongoing transmission in humans of resistant strains originating in livestock and lastly, transfer of resistance genes from human pathogen to human (Chang et al., 2014).

Unlike EU countries, the uses of human antibiotics in animal feed was banned before the enforcement on 1st January 2006 to reduce resistance genes carried by animal. But the antibiotics usage is still allowed only to treat infected animals with prescription by veterinarians (Thanner, Drissnes and Walsh, 2016). The impacts from this action were the decrease in antibiotics sales from 1998 onwards, and total consumptions of feed supplemented with antibiotics are also declined (Cogliani, Goosens and Greko, 2011). The good news was Swedish agricultural sector reported that there is no loss of production after the ban was conducted (Manyi-loh et al., 2018). Thailand is the only country to have declined in the use of antibiotic compare to other Southeast Asia countries. This was due to the strict actions taken by Thailand government including observing the effectiveness of antibiotics and its side effect to the patient and regularly update the medicines list to avoid from misused or overused of antibiotics. Besides, they also enhance the hospital drug other than antibiotics and therapeutic committee skills (Holloway et al., 2017).

Listeria isolates from food and clinical samples have shown resistance towards multiple types of antibiotics such as trimethropim, gentamicin, streptomycin, erythromycin, kanamycin, sulfamethoxazole and rifampin. *L. monocytogenes* multidrug resistance might be caused by self-transferable plasmid within the bacteria (Flachbartova et al., 2016). Wu et al., (2014) reported *L. monocytogenes* isolates from retail ready- to- eat foods in China including chicken (roast and salt- baked) was shown high antibiotic resistance where 17 out of 80 isolates shown multidrug resistant.

Infection by multidrug resistant *L. monocytogenes* particularly for the immunocompromised, children, pregnant woman and the elderly need to be prevented by the continuous study on the incidence of listeriosis and its emerging antibiotics resistance in order to ensure the effectiveness of antibiotics treatment (Pesavento et al. 2010). However, continuous study could not be done without a strengthen system of antibiotic resistance surveillance data done on every country including developed country. Switzerland is the one and only country to have persistent surveillance laboratories and reports (Thanner, Drissnes and Walsh. 2016).

Based on previous the study, antimicrobial susceptibility test against eight common antimicrobials (ampicillin, penicillin, gentamicin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol and cefotaxime) are done on 14 *L. monocytogenes* isolates. *L. monocytogenes* shown 100% of resistance to ampicillin and cefotaxime, while 57% of seafood isolates; 71.4% from clinical isolates resistance towards penicillin. Mostly, all of the isolates were highly susceptible to trimethoprim-sulfamethoxazole, chloramphenicol and tetracycline (Abdollahzadeh et al., 2016).

From risk- assessment done by WHO in the South East Asia region, bacteria with high levels of AR includes *Neisseria gonorrhoeae, E. coli, Klebsiella pneumoniae, Shigella* spp, non-typhoidal *Salmonella,* methicillin resistant *S. aureus* and penicillin resistant *S. pneumoniae*. Although no *L. monocytogenes* strains was recorded in bacteria with high levels of AR, but recent studies proved that *L. monocytogenes* became resistance to many types of antibiotics which indicates that the probability to be in future record is promising (Legendre et al., 2018, Sosnowski et al., 2019).

CONCLUSION

The prevalence of *L. monocytogenes* in chicken is affected by several factors such as bacteria strain, geographic location and weather. This review provided the insight of *L. monocytogenes* prevalence in chicken and chicken-based products from Asian countries and the trend of antibiotic resistance of *L. monocytogenes* isolates from chicken in recent years. As *L. monocytogenes* can survive and multiply in stress condition and also able to form biofilm over extended period, so the presence of *L. monocytogenes* need to be prevented and controlled at early stage to avoid from additional cost to remove the biofilm especially for food manufacturer. Although antibiotic is the only certified and approved way to treat listeriosis, more studies need to be done to reduce the increasing trend of antibiotic resistant strain. Besides, some patient was allergic to certain types antibiotics. There is still inadequate data on risk of *L. monocytogenes* contamination whether in its planktonic stage or biofilm stage in Asian countries. Hence, future studies are suggested to fill in the research gap. Moreover, Asian countries also need to enhance their

surveillance system in order to control and reduce the risk of food- borne cases.

AUTHOR CONTRIBUTIONS

NMN and JYHT designed and prepared the review paper. All authors read and approved the final version.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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