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Review

Listeria monocytogenes – An examination of food chain factors potentially contributing to antimicrobial resistance

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ABSTRACT

When compared to common bacterial agents associated with foodborne disease, *Listeria monocytogenes* is an infrequent but pernicious pathogen linked to disproportionally high levels of morbidity and mortality in susceptible individuals. To improve clinical outcomes, invasive infections require prudent administration of antimicrobial agents. While *L. monocytogenes* has historically been susceptible to antimicrobials effective against Gram positive bacteria, there have been reports of antimicrobial resistance (AMR) in this organism, including in isolates originating from food-producing animals, food processing environments (FPE), and food (i.e. the food chain). Considering human listeriosis is acquired predominantly through foodborne transmission, this is concerning. In particular, food chain factors may influence *L. monocytogenes* AMR through i. horizontal exchange of AMR genes, ii. induction of AMR-related stress responses through sub-lethal exposure to factors controlling microbial growth in food, and iii. recurring exposure to disinfectants in the FPE. In light of the significant technical challenges of fully understanding the influence of the food chain on AMR, this review examines how environments and conditions spanning the food continuum may influence current AMR trends through genetic, physiological, and selection pressure-based perspectives. When combined with effective surveillance, such knowledge will be useful in prolonging the efficacy of the currently available repertoire of therapeutic treatment options.

1. Introduction

Listeria monocytogenes is a ubiquitous Gram positive, facultatively anaerobic, non-spore forming bacterium commonly occurring in natural environments (Farber and Peterkin, 1991; Sauders et al., 2006, 2012). It was first recognized as a human foodborne pathogen in 1981 following a Canadian outbreak linked to contaminated coleslaw (Schlech et al., 1983). It is estimated that more than 99% of human listeriosis results from consumption of contaminated food, particularly ready-to-eat (RTE) foods, such as deli meats, dairy products (i.e. soft cheeses), smoked fish, and seafood (Mead et al., 1999; Vázquez-Boland et al., 2001; Swaminathan and Gerner-Smidt, 2007). Potential outcomes following ingestion of infectious doses of *L. monocytogenes* tend to reflect host status. In general, non-invasive gastrointestinal illness is observed in immunocompetent individuals while invasive listeriosis infections leading to septicemia, meningitis, and abortion typically occur in immunocompromised individuals or pregnant women (Drevets and Bronze, 2008). In recent years, the incidence of sporadic listeriosis has increased in several European countries,

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including Belgium, Denmark, Finland, France, and the United Kingdom (Gillespie et al., 2006; Goulet et al., 2008). Elsewhere, recurring outbreaks highlight the need for improved control and effective treatment options (Vit et al., 2007; Weatherill, 2009; Allerberger and Wagner, 2010; Fretz et al., 2010; Koch et al., 2010; CDC, 2011).

There is high morbidity and mortality (i.e. 20–40%) associated with invasive listeriosis infections (Drevets and Bronze, 2008), and positive clinical outcomes are associated with effective detection and subsequent administration of antimicrobial therapy (McLauchlin et al., 1991). Therefore, it is critical that we obtain an accurate understanding of antimicrobial resistance (AMR) in this organism. While this topic has been extensively investigated (reviewed by Charpentier and Courvalin, 1999; Lungu et al., 2011), a number of technical challenges may limit our ability to understand changing trends in AMR for *L. monocytogenes*. Currently there exists a lack of standardized methodology and few established resistance breakpoints. Both the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have issued breakpoints for ampicillin, penicillin, and trimethoprim/sulfamethoxazole, with EUCAST detailing additional breakpoints for meropenem and erythromycin (CLSI, 2010; EUCAST, 2014). Furthermore, given the widespread use of selective enrichments required to isolate listeriae from complex biological samples (e.g., environmental and food samples), unintentional bias may be introduced in terms of subtypes and species recovered from foods and their respective processing environments due to well-known competition issues (Petran and Swanson, 1993; Curiale and Lewus, 1994; MacDonald and Sutherland, 1994; Sheridan et al., 1994; Walsh et al., 1998; Bruhn et al., 2005). Additionally, acriflavin, a plasmid curing agent (Axelsson et al., 1988; Mesas et al., 2004), is used in selective enrichment broths to suppress the growth of other Gram positive organisms (Bruhn et al., 2005) but may lead to under-estimations of resistance phenotypes conferred by plasmid-mediated genes in *L. monocytogenes* (Orsi et al., 2008a). Collectively, such challenges hinder accurate estimates of AMR in *L. monocytogenes*, thereby limiting our ability to gain accurate information about changing temporal or spatial trends in AMR.

In light of these challenges, we will avoid an exhaustive review of epidemiological data regarding AMR in *L. monocytogenes*, and focus on food chain phenomena that contribute to or potentially are involved in the development of AMR in this organism. As it stands, the majority of *L. monocytogenes* remain susceptible to most clinically relevant options and those isolates that possess increased tolerance do not approach current levels describing resistance (Charpentier and Courvalin, 1999; Lungu et al., 2011). However, molecular determinants affording increased tolerance are well described for the genus *Listeria* and there is growing evidence that exposure of *L. monocytogenes* to stressful conditions present in FPE and food (i.e. the food chain) may influence resistance to clinically important antimicrobials (Lungu et al., 2011). Therefore, in order to preserve treatment options for this organism it is crucial that we understand factors that contribute to the development of AMR in this organism, especially at the food and FPE level.

2. Influence of the food chain on acquisition of horizontally transferred antimicrobial resistance

2.1. *L. monocytogenes* niches and antimicrobial resistance gene reservoirs: opportunities for genetic exchange?

It is well established that AMR genes are frequently encoded on mobile genetic elements (White et al., 2002). Therefore, factors that influence horizontal gene transfer may play a fundamental role in the acquisition of AMR genes in *L. monocytogenes*. Members of the genus *Listeria* frequently demonstrate a high level of genome synteny where gene loss and gain events are rare, yet not undocumented (den Bakker et al., 2010). Specifically, evidence for homologous recombination (Orsi et al., 2008b; Dunn et al., 2009), paired with the observation of plasmids (Poyart-Salmeron et al., 1990; Nelson et al., 2004; Gilmour et al., 2010), conjugative transposons (Poyart-Salmeron et al., 1992; Bertsch et al., 2013), and prophages (Zink et al., 1995; Loessner et al., 2000) in *L. monocytogenes* suggests that horizontal gene transfer plays an important role in the acquisition of new genetic information within this species. Given that RTE foods, which become contaminated after a lethality step, pose the greatest risk to the public (FDA/FSIS/CDC, 2003), there are two key environments that may influence introduction of AMR in *L. monocytogenes*: i. the food processing environment (FPE) itself, and ii. contaminated food.

Regarding the former, post-processing contamination of minimally processed, RTE food is a recurring problem and a primary concern for food processors, and has been linked to strains that are persistently established within FPE (Kathariou, 2002; Gandhi and Chikindas, 2007; Carpentier and Cerf, 2011; Ferreira et al., 2014). Thus, it is possible that niches and harbourage sites favourable to the growth of *L. monocytogenes* within the FPE may also create conditions favourable for the exchange of genetic material, particularly for persistently established strains that survive in FPE for long periods of time. A recent study highlighted the bacteriological diversity of food processing facility drains associated with the presence or absence of *Listeria* spp., and identified other genera that were associated with the presence of *Listeria*. (Fox et al., 2014). More specifically, *Arthrobacteria*, *Bacillus*, *Clostridium*, *Dysgonomonas*, *Enterobacter*, *Microbacterium*, *Pedobacter*, *Psychrobacter*, *Rhodococcus*, *Tanerella*, and *Vibrio* were found at higher levels in the presence of *Listeria*. This study also revealed that the both *Listeria* positive drains examined in the study harboured more than one species of *Listeria* and often multiple strains of a given species (as determined by PFGE analysis). In summary, these results indicate that bacterially diverse niches are present in the FPE that provide exposure of *L. monocytogenes* strains to diverse genera of other bacteria as well as other listeriae. Additional research will be necessary to determine if such niches and harbourage sites are conducive to horizontal gene transfer either among different strains of *Listeria* or between *Listeria* spp. and other genera of bacteria.

Contaminated food may also create conditions in which diverse bacterial species are afforded contact and time to potentially exchange genetic material, particularly for products with extended shelf-life. Recently functional metagenomics analysis has identified various food products as potential reservoirs for AMR genes. Two separate analyses found that the spinach metagenome possessed varied AMR determinants encoding for resistance to several different classes of antibiotics. Berman and Riley identified novel molecular elements encoding resistance to fluoroquinolones, cephalosporins, and trimethoprim (Berman and Riley, 2013), while Carter et al. (2012) identified genes encoding multidrug transporters, a beta-lactamase enzyme, and a tetracycline resistance gene amongst genetic material deriving from the spinach microflora. The significance of RTE food as a reservoir of AMR genes was recently demonstrated in a study examining the occurrence of tetracycline resistance genes in ready-to-serve foods (Li and Wang, 2010). *Tet*(*M*), the predominant determinant encoding resistance in *L. monocytogenes*, was the most frequently detected gene conferring tetracycline resistance amongst 900 tet^R isolates from RTE foods, which encompassed a wide variety of commensal genera, including *Brochothrix*, *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Pseudomonas*, *Sphingobacterium*, and *Stenotrophomonas* (Li and