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Relative effectiveness of selected preenrichment media for the detection of *Salmonella* from leafy green produce and herbs

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ABSTRACT

Four buffered preenrichment media (BAX® System MP Media (BAX)), Universal Preenrichment Broth (UPB), modified Buffered Peptone Water (mBPW), and Buffered Peptone Water (BPW)) were compared with lactose broth (LB) in the *Bacteriological Analytical Manual's* (BAM) *Salmonella* culture method for the analysis of 9 leafy green produce and herb types. Artificially contaminated test portions were preenriched in each medium and the results were analyzed statistically using Fisher's Exact 2-tailed F test ($p < 0.05$) with pairwise comparisons. There was no difference in recovery of *Salmonella* from curly parsley and basil among the five media ($p > 0.05$). UPB was consistently among the most effective media for recovery of *Salmonella* from the nine produce types; however, *S.* Typhimurium and *S.* Newport were isolated from cabbage more frequently with mBPW than with UPB ($p < 0.05$). Comparisons of the results among the preenrichment media from all experimental trials, with leafy green produce and herbs, demonstrate that *Salmonella* is more effectively detected and isolated using buffered enrichments than with the currently recommended LB ($p < 0.05$). There were no significant differences among the buffered preenrichments for the detection of *Salmonella*-positive test portions of the produce tested (BAX (160 *Salmonella*-positive test portions/480 test portions), UPB (176/480), mBPW (184/480), BPW (169/480), LB (128/480))($p > 0.05$).

1. Introduction

1.1. The need for improved methods for detection of Salmonella contamination in leafy green produce and herbs

Recent estimates on the percentage of foodborne illnesses in the U.S. associated with the consumption of contaminated produce, particularly with leafy greens such as spinach and lettuce, range from approximately 12–14% (Lynch et al., 2009; Jablasone et al., 2012; Boore et al., 2010; Stopforth et al., 2008; Doyle and Erickson, 2008). Compared to estimates prior to 1970s, which were <1%, these figures represent significant increases in the number of produce-related outbreaks during the last several decades (Buck et al., 2003; Lynch et al., 2009). Although these rates may reflect increased consumption of produce, changes in agricultural practices, processing, and packaging of produce may also be contributing factors (Lynch et al., 2009; Sivapalasingam et al., 2004; Olaimat and Holley, 2012; Gil et al., 2015). Contamination of leafy green produce can occur at any phase during the movement of produce within the farm to fork continuum. These include those of production, harvesting, transport, packaging, and preparation for consumption (Olaimat and Holley, 2012). When grown in open fields, leafy greens are exposed to many types of wildlife, including mammalian and avian species, which commonly harbor *Salmonella* in their gastrointestinal tracts and therefore excrete *Salmonella* into the environment (Hanning et al., 2009; Quiroz-Santiago et al., 2009; Liu et al., 2013). Leafy greens can also come into contact with *Salmonella* through contaminated surface water. Heavy rain or flooding may facilitate both the contamination of surface water with pathogenic bacteria and transfer of that contaminated water

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onto leafy green produce in the field (Liu et al., 2013). Additionally, leafy green produce can become contaminated if, at any point in the farm to fork continuum, it is handled by employees or food preparers who are infected with these pathogens (Gil et al., 2015).

Over the last several decades, produce consumption in the U.S has risen significantly, most likely due to increased consumer awareness and interest in the health benefits associated with raw produce consumption (Sivapalasingam et al., 2004), although ethnic diversification, cultural influences, changing dietary habits, and internationalization of the produce market may also have contributed to these changes (Hoelzer et al., 2012; Sewell and Farber, 2001). Other social changes have increased consumers' reliance on easy-to-prepare meals, salad bars, and ready-to-eat vegetable products, which require less time and effort than traditional home cooking, while still appearing to provide convenient and healthy dietary options (Collins, 1997; Phillips and Harrison, 2005). Increased consumption of produce is possibly associated with the higher frequency of produce-related foodborne infections that have occurred; leafy green produce and herbs, have in particular, been associated with multiple illnesses and outbreaks (Sivapalasingam et al., 2004; Johnston et al., 2005; Liu et al., 2013; Doyle and Erickson, 2008). Multiple causative agents have been responsible for these illnesses, including viruses, protozoa, and bacterial pathogens, although the most frequently identified bacterial pathogens have been *E. coli* O157:H7 and *Salmonella* spp. (Abadias et al., 2008; Quiroz-Santiago et al., 2009; Tauxe et al., 1997).

An important part of reducing the risk of foodborne illnesses associated with leafy greens and herbs is to reliably identify sources of contamination (Buck et al., 2003; Johnston et al., 2005). Once identified, practices associated with or contributory to contamination can be eliminated, reduced, or modified in order to prevent future occurrences (Buck et al., 2003). To reduce the time of preparation for fresh produce, procedures such as cleaning, slicing, and cutting might be performed in fields immediately following harvest rather than transported to an off-field site designed for such purpose (Kozak et al., 2013). This practice may increase the likelihood for internal contamination of produce to occur as natural barriers of produce are disrupted in an environment where the presence of bacterial pathogens is more likely than that of a site that is hygienically maintained (Kozak et al., 2013; Tauxe et al., 1997; Duffy et al., 2005). Similarly, some innovative packaging methods, such as modified atmosphere packaging to extend the shelf life of freshly cut bagged produce, may also increase the risks for outbreaks and infections by creating microenvironments that are favorable to the growth of some bacterial pathogens (Fröder et al., 2007; Sewell and Farber, 2001). Amending such practices can help minimize risks of illnesses related to fresh produce, but surveillance and traceback efforts are built on a foundation of reliable detection/isolation assays.

1.2. The existing culture method for Salmonella on leafy greens

The reference culture method for the detection and isolation of *Salmonella* from foods that is used by the United States Food and Drug Administration (FDA) is described in the *Bacteriological Analytical Manual* (BAM) (FDA, 2015). The BAM *Salmonella* culture method requires preenrichment of food samples in a nonselective medium for 24 ± 2 h. The purpose of preenrichment is to resuscitate low levels of injured *Salmonella* and to allow them to proliferate to detectable levels (Budu-Amoako et al., 1992). By bringing injured *Salmonella* to a healthy physiological state during preenrichment, the *Salmonella* are conditioned for competitive growth during the selective enrichment procedures that follow (Budu-Amoako et al., 1992). Several factors may influence the effectiveness of preenrichment, such as the biological and chemical characteristics of the initial food matrix (e.g., plant associated antimicrobial compounds including polyphenols such as flavonoids and tannins), length of incubation time, preenrichment temperature, composition and level of matrix-associated bacterial competitors, and the formulation of the preenrichment media itself (Coppo and Marchese, 2014; Nam et al., 2004; Worcman-Barninka et al., 2001; D'Aoust et al., 1992). In this study, we have evaluated the relative effectiveness of five preenrichment media for the detection and isolation of *Salmonella* from leafy green produce and herbs.

The leafy green produce types selected for this study were those that had been previously involved with produce-related outbreaks. The leafy green most frequently associated with outbreaks has been Romaine lettuce, although this may be a consequence of the amount of this lettuce produced and the fact that it is consumed at relatively high levels in comparison to other leafy green produce types (Liu et al., 2013; Himathongkham et al., 2007; Delaquis et al., 2007). Spinach was the source of an *E. coli* O157:H7 outbreak in 2006 (Abadias et al., 2008; Neal et al., 2008). Culantro (*Eryngium foetidum*), cilantro (*Coriandrum sativum*), parsley (*Petroselinum crispum*), and basil (*Ocimum basilicum*) are leafy green herbs that have been sources of foodborne bacterial infections (Duffy et al., 2005; Hsu et al., 2006; Golberg et al., 2011). We therefore included these items in our evaluations of the preenrichment media. However, with the exception of basil in pesto, herbs are typically consumed in lower quantities than other leafy green produce types, as their use is primarily to enhance the flavor of main dishes rather than be the principle component (Hsu et al., 2006).

All the preenrichment media selected for this study have been used to isolate *Salmonella* and other common foodborne pathogens from foods. The presence of nutrients and buffering capacity of BAX® System MP Media (DuPont Qualicon, Wilmington, DE) is suited for the growth of *Salmonella* and pathogenic *E. coli* and has been shown to be an effective preenrichment medium for the analysis of environmental samples and some foods (Peng et al., 2011; Wallace et al., 2013). Universal Preenrichment Broth (UPB) was developed for use with foods to isolate both *Salmonella* and *Listeria monocytogenes* and is effective with a variety of food matrices (Hammack et al., 2001; Kanki et al., 2009). Buffered Peptone Water (BPW) and modified BPW (mBPW) have previously been evaluated as preenrichment media with the BAM *Salmonella* culture method, and effective for use with some foods (Wang et al., 2015; Hammack et al., 2006). Lactose broth is the default preenrichment medium recommended by the BAM *Salmonella* culture for the detection of *Salmonella* in foods unless otherwise specified (FDA, 2015a,b). The formulations of the selected preenrichment media are presented in Table 1.

2. Materials and Methods

2.1. Leafy green produce

Fresh leafy green produce (iceberg lettuce, Romaine lettuce, baby spinach, Italian parsley, curly parsley, basil, cabbage, culantro, and cilantro) was purchased from local retail outlets or produce wholesalers. Each produce type (approximately 2500 g) was artificially contaminated with a single *Salmonella* serotype prepared from pure culture. Inoculation and mixing procedures were performed in a biological safety cabinet (Nuaire Inc., Plymouth, MN). The bulk inoculated leafy green produce was stored for approximately 72 h (inoculated on a Friday and evaluated the following Monday) and refrigerated at 2–8° C.