Microbial occurrence and antibiotic resistance in ready-to-go food items

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Abstract Foodborne pathogens, such as *Escherichia coli*, and *Salmonella*, are commonly prevalent in contaminated food products seen through annual food recalls. Excessive use of antibiotics through the past few decades has led to a multitude of antibiotic resistant bacteria, including foodborne pathogens. We investigated microbial occurrence and their antibiotics resistances in ready-to-go food items, i.e. canned food, bagged food, and baby food. A total of 112 isolates were isolated from varying food items, and 21 of these isolates were identified through 16S rRNA sequencing revealing *Bacillus* sp., *Staphylococcus* sp. and *Micrococcus* sp. Bagged food items showed the most microbial diversity as well as the largest colony forming unit (log 20–25 CFU/g). Isolates showed antibiotic resistance to ampicillin, streptomycin, chloramphenicol, and kanamycin at concentrations of 100, 500, and 1000 µg/mL. 57% isolates were ampicillin resistance followed by kanamycin (26%). A variety of microorganisms present in ready-to-go food items may not be pathogenic, however their occurrence and multiple antibiotic resistance (MAR) poses risk of transferring their genes to foodborne pathogens.

Keywords Microorganisms · Ready-to-go food · Foodborne pathogens · Antibiotic resistance · Multiple antibiotic resistance

Introduction

The adulterated, or contaminated food by the presence of microorganisms has the ability to cause illnesses in humans and animals (i.e. gastroenteritis). Major foodborne pathogenic microorganisms include *Salmonella enterica*, *Escherichia coli*, *Listeria*, *Campylobacter*, *Clostridium perfringens*, *Staphylococcus aureus* among others (CDC 2015; Foodsafety.gov 2016). In efforts to limit the amount of pathogenic microorganisms in ready-to-go food, bagged, and canned food, a variety of food preservation methods, i.e. heat/cold, pressurizing, chemical, bio-preservation, and irradiation, have been created. However, some microorganisms can grow in extreme environments including high pressures, extreme radiation, aerobic and anaerobic conditions, high heat, antibiotics, and low temperatures (Gabani et al. 2012; Woappi et al. 2016). The Food and Drug Administration (FDA) recommends a variety of techniques to detect the presence of pathogenic microorganisms. Bacteriological analytical methods (BAM) involve sampling specific amounts of food, based on three factors: consumer group, contamination during manufacturing, and the food history (FDA 2016).

The antibiotic acts through a unique mechanism of action, but with the same end result to deactivate the pathogenic activity or killing of pathogenic bacteria (Franco et al. 2009; Allen et al. 2014; Sharma et al. 2013). However, the overuse and misuse of antibiotics has led to the emerging evolution of antibiotic-resistant bacteria (Bhunia 2008; Gabani et al. 2012; Woappi et al. 2016; Berman and Riley 2013; Wellington et al. 2013). The multiple antibiotic resistance (MAR) bacteria in ready-to-eat baby spinach have reported in gut microflora and other bacterial pathogens (Walia et al. 2013). In the current study, we investigated that microbial occurrence in food

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products, i.e. canned food, bagged food, and baby food, from local grocery stores would reveal antibiotic resistance. Therefore we aimed to isolate and identify the microbial occurrence in a variety of bagged, canned, baby food items, and investigated antibiotic resistance among isolates.

Materials and methods

Food samples acquisition

A variety of food samples were obtained from local grocery stores. Among these food samples a total of 26 samples were canned food items (i.e. Cut sweet potatoes, candied yams, black beans, pinto beans, garbanzas chick peas, turnip greens, mustard greens, extra long tender asparagus spears, sweet peas, cut green beans, cut leaf spinach, mini O's & meatballs, pink salmon, potted meat beef and chicken, chunk light tuna in vegetable oil, vienna sausages, ground and formed sliced dried beef, chopped clam in clam juice, premium lunch meat: pork, premium chunk white chicken in water, sliced carrots, whole super sweet kernel corn, corned beef hash, mushroom pieces and stems, seasoned southern style county cabbage, sliced beets) from 7 different companies.

A total of 9 samples were bagged food items (i.e. green beans, broccoli, spinach, mushrooms, green onion, baby bell peppers (assorted), carrots, shredded lettuce, and rad-ish) from 6 different companies.

A total of 17 samples were baby food items packaged in glass jar (i.e. applesauce, sweet potatoes and applesauce, turkey rice dinner, pear apricot, whole wheat vegetable, rolled oats with apples, vegetables and chicken, sweet potatoes, apples and bananas, pears, squash and apples, peas and carrots, carrots, apple mango carrot, fruit and yogurt very berry, yellow diced peaches, mac and cheese with chicken and vegetables) from two different companies.

Each food sample was designated with letters and numbers for identification purposes. Canned food items were designated as C1–C26, bagged food items were designated as V1–V9, and baby food items were designated as F1–F17. Food was purchased from local grocery store and stored at 4 °C under sterile conditions until analyzed. Each isolated microorganism was designated with the type of sample followed by chronological letters A, B, C, and so forth (Figs. 1 and 2).

Isolation of microorganisms from food items

The surfaces of all food packages were sterilized by 70% ethanol before samples were taken out. Microbial

occurrences in selected food items were observed using a growth enrichment method. The growth enrichment method was performed on bagged, canned and baby food items by suspending respective food items (1.0 g) in 50 mL sterile nutrient broth (NB) medium (readymade purchased from Fisher Scientific, USA) in 250 mL Erlenmayer flask at 32 °C for 72 h. The enriched medium was serially diluted and plated on to Nutrient Agar (NA; readymade purchased from Fisher Scientific, USA) plates for single cell observation to determine the colony-forming unit (CFU/g). Agar plates, which contained greater than 200 colonies were designated as too numerous to count (TNTC). The observed colonies were visually separated and isolated on NA slants, and stored at 4 °C until further studies.

16S rRNA sequence and phylogenetic tree analyses

The microorganisms were isolated and extracted for total genomics DNA using PureLink™ Genomic DNA MiniKit K1820-01 (Invitrogen, USA). The 16S rRNA gene sequences from all isolates were then amplified using universal primer (F-518: CCAGCAGCCGCGGTAA-TACG, R-800: TACCAGGGTATCTAATCC) and sequenced at MacroGen Service Center (Rockville, MD, USA). All sequences were compared with closest relatives from GenBank and Ribosomal Database Project (RDP) release 11 (<https://rdp.cme.msu.edu/index.jsp>). Phylogenetic relationships among food isolates with varying foodborne pathogens, i.e. *Listeria monocytogenes*, *Salmonella enterica*, *Serratia liquefaciens*, *Campylobacter jejuni*, *Escherichia/Shigella coli*, and *Klebsiella pneumonia*, were constructed by neighbor-joining method (NJ) with pairwise deletion of gaps in RDP database.

Antibiotic resistance assay

Each identified microbial isolate from all food types were screened for antibiotic resistance on NA plates supplemented with varying amounts, i.e. 100, 500, and 1000 µg/mL, of ampicillin, streptomycin, chloramphenicol, and kanamycin antibiotics (purchased from Sigma-Aldrich, MO, USA). The NA plates were divided into 16 sections to streak isolated colonies and labeled appropriately. Antibiotic sensitive strain, i.e. *E. coli*, was used as a control and single streaked on the first section of every plate. Based on the studies performed by Bengtsson-Palme and Larsson (2016) for concentrations of antibiotics predicated to select for resistant bacteria, in current studies any isolate showed growth at 100 µg/mL will be considered as antibiotic resistance. The plates were incubated at 32 °C for 72 h and results in terms of R (resistance) and S (Sensitive) were recorded at time frames of 24, 48, and 72 h (Table 1).

Fig. 1 Microbial occurrence in canned food items [i.e. Cut sweet potatoes (C1); Candied yams (C2); Black beans (C3); Pinto beans (C4); Garbanzas chick peas (C5); Turnip greens (C6); Mustard greens (C7); Extra long tender Asparagus spears (C8); Sweet peas (C9); Cut green beans (C10); Cut leaf spinach (C11); Mini O's and meatballs (C12); Pink Salmon (C13); Potted meat beef and Chicken (C14); Chunk light tuna in vegetable oil (C15); Vienna sausages (C16);

Grounded and formed sliced dried beef (C17); Chopped clam in clam juice (C18); Premium lunch meat: pork (C19); Premium chunk white chicken in water (C20); Sliced carrots (C21); Whole (super sweet) corn (C22); Corned beef hash (C23); Mushroom pieces and stems (C24); Seasoned southern style county cabbage (C25); Sliced beets (C26)]. The isolates were identified from pure culture on NA plates using 16S rRNA sequences

Results

Microbial occurrence in food samples

Varieties of microorganisms were observed from screened food items. *Micrococcus yunnanensis* presented the highest CFU (log 18.4 CFU/g) in seasoned southern style cabbage and in Vienna sausages (log 18.2 CFU/g) (Fig. 1). There were no observable differences noticed between CFU from different companies. 16S rRNA sequence of isolated colonies revealed a diverse variety of microorganisms, i.e. *Bacillus atrophaeus* (log 16 CFU/g), *B. andresenii* (log 10 CFU/g), *Methylobacterium fujisawaense* (log 6 CFU/g), and *Micrococcus yunnanensis* (log 16 CFU/g), were observed from mustard green cane (Fig. 1). Cut green beans and mustard green revealed highest colony forming units (log 7.6–24 CFU/g) among identified microorganisms. Among identified microorganisms, 84% belonged to *Bacillus* sp., and 15% revealed similarity with *Micrococcus* sp. *M. fujisawaense* and *Staphylococcus xylosum* was found

in mustered green and chopped clam in clam juice, respectively (Fig. 1).

Bagged food items presented the greatest diversity of microorganisms, as well as high CFU. Most abundant included *Bacillus aerophilus* (log 21 CFU/g) in green beans, and *Staphylococcus* sp. (log 20.2 CFU/g) in spinach (Fig. 2). 16S rRNA sequences revealed close proximity with *Bacillus* sp., *Pseudomonas* sp., and *Acinetobacter* sp. among the isolates from bagged food items, i.e. green beans, broccoli, spinach, green onions, baby bell peppers, carrots, shredded lettuce and radish. Uniquely, baby bell pepper food item showed occurrence of all three microbial types. Mushrooms revealed occurrence of *Kluyvera* sp. (Figure 2).

Among 17 baby food items examined for microbial occurrence (Fig. 2), only three food items, i.e. apple sauce, squash and apples, and mac and cheese revealed occurrence of *Staphylococcus hominis* (log 1.6 CFU/g), *Bacillus methylotrophicus* (log 15.6 CFU/g), and *Paenibacillus vulneris* (log 0.3 CFU/mL), respectively (Fig. 2). Most

Fig. 2 Microbial occurrence in bagged (V1–V9) and baby (F1–F17) food items [i.e. Green beans (V1); Broccoli (V2); Spinach (V3); Mushrooms (V4); Green Onion (V5); Baby bell peppers (assorted) (V6); Carrots (V7); Shredded lettuce (V8); Radish (V9); Apple sauce (F1); Sweet potatoes and Apples (F2); Turkey Rice dinner (F3); Pear apricot (F4); Whole wheat vegetable (F5); Rolled oats with apples

(F6); Veges and chicken (F7); Sweet potatoes (F8); Apples and banana (F9); Pears (F10); Squash and apples (F11); Peas and carrots (F12); Carrots (F13); Apple mango carrot (F14); Fruit and yogurt: very berry (F15); Yellow diced peaches (F16); Mac and cheese with vegetables (F17)]. The isolates were identified from pure culture on NA plates using 16S rRNA sequences

food-items did not reveal any microbial growth in nutrient broth.

Phylogenetic relationships among identified Microorganisms with foodborne pathogens

16S rRNA sequencing through amplified genomic DNA identified the microbial isolates acquired from the food products. 16S rRNA sequencing revealed 954 base pairs (bp), 959, 948, 955, 918, 952, 943, 950, 933, 949, 703, 944, 957, 132, 1032, 957, 955, 945, 934, 952, 950, 950, 950, 935, 953, 935, 939, 962, 953, 834, and 932 bp from amplified genomic DNA using F518 primer, and revealed significant levels of similarity in ribosomal sequence database to *Bacillus atrophaceus*, *Staphylococcus xylosus*, *Delftia lacustris*, *Staphylococcus petrasii* subsp. *jettensis*, *Serratia proteamaculans*, *Paenarthrobacter nicotinovorans*, *Acinetobacter oleivorans*, *Pseudomonas humanensis*,

Sphingobacterium anhuiense, *Exiguobacterium antarcticum*, *Exiguobacterium alkaliphilum*, *Pseudomonas proszekii*, *Xanthomonas campestris*, *Buttiauxella ferrugutiae*, *Pseudomonas chlororaphis*, *Micrococcus yunnanensis*, *Bacillus subtilis*, *Bacillus velezensis*, *Bacillus atrophaceus*, *Bacillus altitudinis*, *Paenibacillus vulneris*, *Bacillus subtilis* subsp. *inaquosorum*, *Bacillus atrophaceus*, *Bacillus subtilis* subsp. *inaquosorum*, *Bacillus atrophaceus*, *Ewingella americana*, *Sphingobacterium anhuiense*, *Micrococcus yunnanensis*, *Bacillus velezensis*, *Brevibacterium frigoritolerans*, *Methylobacterium fujisawaense*, respectively. The identified microorganisms were designated with their respective designations, i.e. MC1–MC40.

The sequences of all isolates were deposited in the GenBank database and accession numbers were obtained: KX225428: *Bacillus atrophaceus*—MC3; KX225429: *Staphylococcus xylosus*—MC5; KX225430: *Delftia lacustris*—MC6; KX225431: *Staphylococcus petrasii* subsp.

Table 1 Antibiotic resistance among 16S rRNA identified microorganisms from variety of food isolates

Organism	Ampicillin (μg/mL)			Streptomycin (μg/mL)			Chloramphenicol (μg/mL)			Kanamycin (μg/mL)		
	100	500	1000	100	500	1000	100	500	1000	100	500	1000
BAGGED FOOD												
<i>Bacillus atrophaeus</i> JCM 9070	R*	S*	S	R	R	R	R	R	R	S	S	S
<i>Delftia lacustris</i> DSM 4543	R	R	R	R	R	R	S	S	S	S	S	S
<i>Serratia proteamaculans</i> DSM 4543	R	R	R	R	R	R	S	S	S	R	R	R
<i>Arthrobacter nicotinovorans</i> DSM 420	S	S	S	S	S	S	S	S	S	S	S	S
<i>Acinetobacter oleivorans</i> DR1	S	S	S	S	S	S	R	S	S	S	S	S
<i>Pseudomonas parafulva</i> AJ2129	S	S	S	R	S	S	R	S	S	R	S	S
<i>Sphingobacterium kitahiroshimense</i> 10C	S	S	S	S	S	S	S	S	S	S	S	S
<i>Exiguobacterium undae</i> DSM 14481	S	S	S	S	S	S	S	S	S	S	S	S
<i>Pseudomonas umsongensis</i> PS 3-10	R	S	S	S	S	S	R	S	S	R	S	S
<i>Xanthomonas gardneri</i> ATCC 19865	S	S	S	S	S	S	S	S	S	S	S	S
<i>Kluyvera cryocrescens</i> ATCC 33435	S	S	S	S	S	S	S	S	S	S	S	S
<i>Exiguobacterium antarcticum</i>	S	S	S	S	S	S	R	S	S	R	S	S
<i>Bacillus methylotrophicus</i> CBMB 205	S	S	S	S	S	S	S	S	S	S	S	S
<i>Bacillus aerophilus</i> 28 K	S	S	S	S	S	S	S	S	S	S	S	S
<i>Ewingella americana</i> GTC 1277	S	S	S	R	R	S	S	S	S	S	S	S
<i>Sphingobacterium anhuiense</i> CW 186	R	S	S	S	S	S	S	S	S	S	S	S
CANNED FOOD												
<i>Staphylococcus xylosus</i> ATCC 29971 (T)	S	S	S	S	S	S	S	S	S	S	S	S
<i>Bacillus andreesei</i> 8-4-E13 (T)	S	S	S	S	S	S	S	S	S	S	S	S
<i>Micrococcus yunnanensis</i> YIM 65004 (T)	R	S	S	S	S	S	S	S	S	R	R	S
<i>Bacillus tequilensis</i> KCTC 13622 (T)	S	S	S	S	S	S	S	S	S	S	S	S
<i>Bacillus vietnamensis</i> 15-1 (T)	S	S	S	S	S	S	S	S	S	S	S	S
<i>Methylobacterium fujisanaense</i> DSM 5686 (T)	S	S	S	S	S	S	S	S	S	S	S	S
<i>Bacillus methylotrophicus</i> CBMB 205 (T)	S	S	S	S	S	S	S	S	S	S	S	S
BABY FOOD												
<i>Staphylococcus hominis</i> subsp. <i>Novobiosepticus</i> GTC 1228 (T)	S	S	S	S	S	S	S	S	S	S	S	S
<i>Bacillus methylotrophicus</i> CBMB 205 (T)	S	S	S	S	S	S	S	S	S	S	S	S
<i>Paenibacillus vulneris</i> CCUG 53270 (T)	S	S	S	S	S	S	S	S	S	S	S	S

*R resistance, *S sensitivity

iettensis—MC7; KX225432: *Serratia proteamaculans*—MC9; KX225433: *Arthrobacter nicotinovorans*—MC10; KX225434: *Acinetobacter oleivorans*—MC12; KX225435: *Pseudomonas humanensis*—MC13; KX225436: *Sphingobacterium anhuiense*—MC14; KX225437: *Exiguobacterium antarcticum*—MC15; KX225438: *Pseudomonas pros-ekii*—MC18; KX225439: *Xanthomonas campestris*—MC20; KX225440: *Buttiauxella ferrugutiae*—MC21; KX225441: *Micrococcus yunnanensis*—MC23; KX225442: *Bacillus subtilis*—MC26; KX225443: *Bacillus methylotrophicus*—MC27; KX225444: *Bacillus atrophaeus*—MC28; KX225445: *Bacillus invictae*—MC29; KX225446: *Paenibacillus vulneris*—MC30; KX225447: *Bacillus subtilis* subsp. *inaquosorum*—MC31; KX225448:

Bacillus atrophaeus—MC32; KX225449: *Bacillus subtilis* subsp. *inaquosorum*—MC34; KX225450: *Bacillus atrophaeus*—MC35; KX225451: *Ewingella Americana*—MC36; KX225452: *Sphingobacterium anhuiense*—MC37; KX225453: *Micrococcus yunnanensis*—MC38; KX225454: *Bacillus methylotrophicus*—MC39; KX225455: *Methylobacterium fujisawaense*—MC41. Microorganisms *Exiguobacterium alkaliphilum*, *Pseudomonas chlororaphis*, and *Brevibacterium frigoritolerans* designated to MC17, MC22, and MC40, respectively, revealed less than 90% BLAST match score of their total length and did not assigned with accession numbers.

The phylogenetic relationships among food isolates with varying foodborne pathogens, i.e. *Listeria monocytogenes*,

Salmonella enterica, *Serratia liquefacians*, *Campylobacter jejuni*, *Escherichia/Shigella coli*, and *Klebsiella pneumonia*, provides approximate distances in neighbor-joining tree representing their relationship among themselves and foodborne pathogens in database (Fig. 3a–c). Microorganisms *Exiguobacterium antarcticum* (MC22) and *Bacillus vietnamensis* (MC40) appears to be at the distal end when comparing to foodborne pathogens (Fig. 3a–c). Microorganism *Staphylococcus xylosus* (MC5) revealed close association with *Listeria monocytogenes* (Fig. 3a). As expected, *Serratia proteamaculans* (MC9) shows close proximity to *Salmonella enterica* and *Serratia liquefacians* (Fig. 3a, b). Both isolates MC9 and MC36 revealed close relationship with foodborne pathogens *Escherichia/Shigella coli* and *Klebsiella pneumonia* (Fig. 3c).

Antibiotic resistance among foodborne isolates

The antibiotic resistance among 112 isolates was examined against commonly used antibiotics, i.e. ampicillin, streptomycin, chloramphenicol, and kanamycin at varying concentrations (100, 500 and 1000 µg/mL) (Table 1). Ampicillin revealed 64 isolates being resistant between 100 µg/mL and 1000 µg/mL attributing to 57%. Total 18 isolates revealed antibiotic resistance against chloramphenicol, giving 16% overall resistance. Individually, among the canned food items, microorganism *Micrococcus yunnanensis* isolated from garbanzas chick peas, mustered green, chunk light tuna, and vienna sausages, and seasoned southern style country cabbage revealed poly-resistance against antibiotics ampicillin (100 µg/mL) and kanamycin (100 and 500 µg/mL) (Table 1).

The bagged food isolates showed greater resistance compared to canned and baby food isolates. *Bacillus atrophaeus*, *Delftia lacustris*, and *Serratia proteamaculans* showed wide spectrum of antibiotic resistance against ampicillin, streptomycin and kanamycin (100–1000 µg/mL) (Table 1). *Pseudomonas parafulva* revealed antibiotic resistance again streptomycin, chloramphenicol, and kanamycin, however at lower concentration (100 µg/mL). Similarly, *P. umsongensis* revealed antibiotic resistance against ampicillin, chloramphenicol, and kanamycin at lower concentration (100 µg/mL) (Table 1).

Discussion

Ready-to-go food is being practiced to limit with microbial occurrence using varying preservation techniques including heat methods (pasteurization, boiling, canning), cold preservation (refrigeration at 32°–38°F or freezing at < 32°F/0 °C), bio-preservatives (fermentation), reduction of moisture availability (drying, smoking, osmotic pressure,

freeze drying), chemical preservation (smoking, sodium benzoate, nitrates, sulfites), oxygen exclusion (replace oxygen with nitrogen), ultra-high pressure (111,000 psi), and irradiation which is limited to chicken, fruits, vegetables and ground beef products (Ananou et al. 2007; Dave and Ghaly 2011; Aneja et al. 2014).

Microbial occurrence among canned food items

Variety of food items packaged cans do not allow oxygen to enter; therefore, it is not optimal for the growth of aerobic microorganisms. However, there are multiple pathogens that can withstand anaerobic conditions, including *Bacteroides* (causes intra—abdominal infections), *Clostridium* (known for food poisoning and botulism pathogen), and *Actinomyces* (causes variety of infections). Canned food showed significant diversity and CFU among microorganisms. Occurrence of Actinobacteria (*Micrococcus yunnanensis*) revealed CFU log 18.4 CFU/g (Fig. 1). 16S rRNA sequence of isolated colonies showed a diverse variety of microorganisms included in the phyla Firmicutes (i.e. *Bacillus atrophaeus*, *B. andreesenii*, *Staphylococcus xylosus*), Proteobacteria (*M. fujisawaense*) and Actinobacteria (Fig. 3a–c).

Regardless the benefits of *Bacillus* sp. for human health, some *Bacillus* spp. are associated to a wide variety of foods including meats, milk, vegetables, and fish etc., causing diarrheal type food poisoning. The occurrence of *Bacillus* spp. in extra long tender asparagus spears in this study coincides with earlier studies that *Bacillus* spp. is also among most prevalent pathogen in green-leafy vegetables (Elhariry 2011; Tango et al. 2014). Genus *Methylobacterium* are ubiquitous in nature and can be detected in soil and freshwater environments including drinking water (Gallego et al. 2005). Our observation is in agreement that staphylococci species is the most commonly found microorganism. *Staphylococcus xylosus* (Micrococcaceae) is the most prominent species reported in many Italian and Spanish sausages, in Slovak meat, and in Tunisian traditional salted meat (Essid et al. 2007; Simonová et al. 2006).

Microbial occurrence among bagged food items

In our study, pathogenic bacteria were not found; however, microbial occurrence and MAR poses greater risk of transferring their genes to foodborne pathogens. We observed food spoilage microorganisms from all bagged food-items (Fig. 2). The relationship between isolates and pathogenic microorganisms are shown in neighbor joining phylogenetic trees (Fig. 3a–c). Bagged vegetables, including ready to eat salads, spinach, broccoli, mushrooms, carrots, green beans and others, are becoming more ideal for consumers due to their convenience, as well as,

Fig. 3 a–c 16S rRNA gene sequence-based, neighbor-joining phylogenetic tree showing relationship among microbial isolates from canned, bagged and baby food items showing close proximity with respective homologues. The isolates were compared for their genetic distance against foodborne pathogens, **a** *Listeria monocytogenes*; *Salmonella enterica*; **b** *Serratia liquefaciens*; *Campylobacter jejuni*; **c** *Escherichia/Shigella coli*; *Klebsiella pneumonia*. Numbers at the nodes indicate levels of bootstrap support based on neighbor-joining analysis of 1000 resampled data sets. Bar, five substitutions for 1000 nucleotide positions

their health benefits including vitamins, minerals and antioxidants; However, a threat comes with this convenience; foodborne pathogens. The growth of pathogens such as *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* can be restricted at ≤ 4 °C. However, studies on frozen produce showed the survival of these pathogens for at up to 24 days after storage (Jung et al. 2014).

The fresh-bagged vegetables are consumers' favorite due to their affordability, effortless and tasty adventure as they are an integral part of a healthy diet, providing vitamins, minerals, antioxidants and other health-promoting compounds to consumer (State of the plate 2017). However, the fresh can become contaminated with human pathogens causing life-threatening diseases. Ever-ending outbreaks of foodborne pathogens (reviewed by Jung et al

2014; Cole and Singh 2016; Josephs-Spaulding et al. 2016) have enforced to explore microbial occurrence in fresh vegetables. Jung et al. (2014) reviewed effect of the food production chain from farm practices to vegetables processing on outbreak incidences. Jeddi et al. (2014) revealed microbial occurrence in fresh-cut vegetables, ready-to-eat salads, and mung beans.

Microbial occurrence in baby food items

We examined total 17 baby food items packaged in glass jar for microbial occurrence (Fig. 2), and most did not reveal any microbial growth. Due to undeveloped immune system, infants and young children are always at risk associated with foodborne bacterial infections. Powdered baby food in different forms is widely used for convenience and economic reasons. A joint FAO/WHO consultation group (2004–2006) identified the specific microorganisms which are with Powdered Infant Formula (PIF) contamination as *Cronobacter* sp., *Enterobacter agglomerans*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Salmonella enteritidis*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Escherichia coli*, *Citrobacter koseri*, *Acinetobacter* sp., *Bacillus cereus*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium botulinum*,

Fig. 3 continued

Serratia sp. and *Staphylococcus* sp. *Listeria monocytogenes*. Among them microorganisms *Cronobacter* sp. and *Salmonella enterica* were identified as the most common pathogens in PIF. US Food and Drug Administration issued health professionals letter on *Enterobacter sakazakii* infections associated with use of PIF in neonatal intensive care units (FDA 2002).

Phylogenetic relationships of isolates

A near-relationship occurred among food isolates and foodborne pathogen *Campylobacter jejuni* (Fig. 3b). Champion et al. (2005) reported comparative phylogenomics of the foodborne pathogen *Campylobacter jejuni*, which could provide a robust methodological prototype and may be applicable to other microbes. Our studies provide genetic resemblances of *Serratia proteamaculans* with *Campylobacter jejuni* revealing genetic markers predictive of infections sources microorganisms *Serratia proteamaculans* (MC9) and *Ewingella americana* (MC36). Spröer et al. (1999) reported phylogenetic relationship of 38 species with Enterobacteriaceae by comparative 16S rDNA analysis. In agreement, we have observed 16S rRNA sequence similarity greater than 95% among varying genera of family Enterobacteriaceae with isolates (Fig. 3a–c).

Antibiotic resistance in food isolates

Antibiotics have been overused in human and animal societies. Consequently, bacteria have developed antibiotic resistance (Verraes et al. 2013). The usages of antibiotics in agriculture allow microorganisms to acquire antibiotic resistance genes (Durso and Cook 2014). Some foodborne microorganisms have been identified as antibiotic resistance reservoirs (lactic acid bacteria) as well as antibiotic resistance phenotypes in fermented food products (*S. thermophiles* and *L. helveticus*) (Devirgiliis et al. 2014). Marder et al. 2014, showed outbreaks of STEC O157 associated with spinach and lettuce contained antibiotic resistance DNA sequences (Berman and Riley 2013).

The use of antibiotics has been an important component of the public and animal health system for treating infectious diseases. Occurrence of antibiotics residues in aquatic and food of animal and plant origin represent a major concern, as consistent exposure to antibiotics is a serious health hazard in both humans and environment. Food acts as a transport vessel for antibiotic resistant bacteria and resistance genes to humans through agricultural products (beef, chicken, pork, and plant products), agricultural runoff into irrigation water (used on vegetation and soil) and the food-packaging process (Verraes et al. 2013; Durso and Cook 2014). In our studies we tested the susceptibility of bacterial isolates to four different commonly used

antibiotics, and observed MAR among few isolates (Table 1) from bagged food. We did not observe the level of antibiotic resistance due to varying time interval.

Conclusion

A wide variety of microorganisms were observed in ready-to-go food items, may not be pathogenic, however are resistant to antibiotics. Among 112 isolates from ready-to-go food items, total 21 isolates were identified through 16S rRNA sequencing revealing *Bacillus* sp., *Staphylococcus* sp. and *Micrococcus* sp. A broader microbial diversity along with highest colony forming unit (log 20–25 CFU/g) was obtained from bagged food items, revealing antibiotic resistance against ampicillin (57%) followed by kanamycin (26%). The isolates showed antibiotic resistance to ampicillin, streptomycin, chloramphenicol, and kanamycin at concentrations of 100, 500, and 1000 µg/mL MAR being noticed in *B. atrophaeus*, *D. Lacustris*, *S. proteamaculans*, *P. parafulva*, and *P. umsongensis* posing intense risk of gene transfer into other pathogens in nature. The bacteria survival under harsh food processing or preservation techniques may be due to their ability to sustain (i.e. spore formation and/or undamaged cell wall) under harsh environmental conditions. The excessive use of antibiotics in agriculture has produced bacteria that can thrive in a wide array of antibiotics and emerging into antibiotic-resistant extremophiles.

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References

- Allen KH, Trachsel J, Looft T, Casey AT (2014) Finding alternatives to antibiotics. *Ann N Y Acad Sci* 1323:91–100
- Ananou S, Maqueda M, Martinez-Bueno M, Valdivia E (2007) Biopreservation, an ecological to improve the safety and shelf-life of foods. In: Mendez-Vilas A (ed) *Communicating current education and educational topics and trends in microbiology*, vol 1(2). Formatex. RJCES, Badajoz, pp 475–486
- Aneja KR, Dhiman R, Aggarwal NK, Aneja A (2014) Emerging preservation techniques for controlling spoilage and pathogenic microorganisms in fruit juices. *Int J Microbiol* 2014:758942. <https://doi.org/10.1155/2014/758942>
- Bengtsson-Palme J, Larsson DGJ (2016) Concentrations of antibiotics predicted to select for resistance bacteria: proposed limits for environmental regulation. *Environ Int* 86:140–149
- Berman HF, Riley WL (2013) Identification of novel antimicrobial resistance genes from microbiota on retail spinach. *BMC Microbiol* 13:272. <https://doi.org/10.1186/1471-2180-13-272>
- Centers for Disease Control and Prevention. CDC (2015) Estimates of foodborne illnesses in the United States. <https://www.cdc.gov/foodborneburden/>. Accessed 15 June 2016
- Champion OL, Gaunt MW, Gundogdu O, Elmi A, Witney AA, Hinds J, Dorrell N, Wren BW (2005) Comparative phylogenomics of

- the foodborne pathogen *Campylobacter jejuni* reveals genetic markers predictive of infection sources. *Proc Natl Acad Sci USA* 102:16043–16048
- Cole M, Singh OV (2016) Foodborne pathogens and their apparent linkage with antibiotic resistance. In: Singh OV (ed) Foodborne pathogens and antibiotic resistance. Wiley Blackwell, Hoboken
- Dave D, Ghaly AE (2011) Meat spoilage mechanisms and preservation techniques: a critical review. *Am J Agric Biol Sci* 6:486–510
- Devirgiliis C, Zinno P, Stirpe M, Barile S, Perozzi G (2014) Functional screening of antibiotic resistance genes from a representative metagenomic library of food fermenting microbiota. *BioMed Res Int*, Article ID 290967
- Durso ML, Cook LK (2014) Impacts of antibiotic use in agriculture: What are the benefits and risks? *Curr Opin Microbiol* 19:37–44
- Elhariry HM (2011) Attachment strength and biofilm forming ability of *Bacillus cereus* on green-leafy vegetables: cabbage and lettuce. *Food Microbiol* 28:1266–1274
- Essid I, Ismail HB, Ahmed SBH, Ghedamsi R, Hassouna M (2007) Characterization and technological properties of *Staphylococcus xylosus* strains isolated from a Tunisian traditional salted meat. *Meat Sci* 77:204–212
- Foodsafety.gov (2016) Symptoms of food poisoning. Bacteria and viruses, <http://www.foodsafety.gov/poisoning/symptoms/symptoms.html>; <https://www.foodsafety.gov/poisoning/causes/bacteriaiviruses/>. Accessed 07 Jan 2016
- Franco BE, Martinez AM, Rodriguez MAS, Al Wertheimer (2009) The determinants of the antibiotic resistance process. *Infect Drug Resist* 2:1–11
- Gabani P, Prakash D, Singh OV (2012) Emergence of antibiotic-resistant extremophiles (AREs). *Extremophiles* 16:697–713
- Gallego V, García MT, Ventosa A (2005) *Methylobacterium hispanicum* sp. nov. and *Methylobacterium aquaticum* sp. nov., isolated from drinking water. *Int J Syst Evol Microbiol* 55:281–287
- Jeddi MZ, Yunesian M, Gorji ME, Noori N, Pourmand MR, Khaniki GRJ (2014) Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. *J Health Popul Nutr* 32:391–399
- Josephs-Spaulding J, Beeler E, Singh OV (2016) Human microbiome versus foodborne pathogens: friend or foe. *Appl Microbiol Biotechnol* 100:4845–4863
- Jung Y, Jang H, Matthews KR (2014) Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. *Microb Biotechnol* 7:517–527
- Marder PE, Garman NK, Ingram AL, Dunn RJ (2014) Multistate outbreak of *Escherichia coli* O157:H7 associated with bagged salad. *Foodborne Pathog Dis* 11:593–595
- Sharma SK, Singh L, Singh S (2013) Comparative study between penicillin and ampicillin. *Sch J Appl Med Sci* 1:291–294
- Simonová M, Stropfová V, Marcináková M, Lauková A, Vesterlund S, Moratalla ML, Bover-Cid S, Vidal-Carou C (2006) Characterization of *Staphylococcus xylosus* and *Staphylococcus carnosus* isolated from Slovak meat products. *Meat Sci* 73:559–564
- Spröer C, Mendrock U, Swiderski J, Lang E, Stackebrandt E (1999) The phylogenetic position of *Serratia*, *Buttiauxella* and some other genera of the family Enterobacteriaceae. *Int J Syst Bacteriol* 49:1433–1438
- State of the plates (2017) 2015 Study on America's consumption of fruit and vegetables. Available at http://www.pbhfoundation.org/pdfs/about/res/pbh_res/State_of_the_Plate_2015_WEB_Book_marked.pdf. Accessed 20 Jan 2017
- Tango CN, Choi NJ, Chung MS, Oh DH (2014) Bacteriological quality of vegetables from organic and conventional production in different areas of Korea. *J Food Prot* 77:1411–1417
- US Food and Drug Administration (FDA) (2002) Health professionals letter on *Enterobacter sakazakii* infections associated with use of powdered (dry) infant formulas in neonatal intensive care units. <http://www.fda.gov/Food/RecallsOutbreaksEmergencies/SafetyAlertsAdvisories/ucm111299.htm>. Accessed 14 May 2016
- U.S. Food and Drug Administration (FDA) (2016) Bacteriological analytical manual (BAM). <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>. Accessed 14 May 2016
- Verraes C, Van Boxtael S, Van Meervenne E, Van Coillie E, Butaye P, Catry B, de Schaetzen MA, Van Huffel X, Imberechts H, Dierick K, Daube G, Saegerman C, De Block J, Dewulf J, Herman L (2013) Antimicrobial resistance in the food chain: a review. *Int J Environ Res Public Health* 10:2643–2669
- Walia S, Rana SW, Maue D, Rana J, Kumar A, Walia SK (2013) Prevalence of multiple antibiotic-resistant gram-negative bacteria on bagged, ready-to-eat baby spinach. *Int J Environ Health Res* 23:108–118
- Wellington EMH, Boxall ABA, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM, Williams P (2013) The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect Dis* 13:155–165
- Woappi Y, Jangiti R, Singh OV (2016) Antibiotrophs: the complexity of antibiotic-subsisting and antibiotic-resistant microorganisms. *Crit Rev Microbiol* 42:17–30