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Varying occurrence of extended-spectrum beta-lactamase bacteria among three produce types

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Abstract

A monitoring effort that spanned across 1.5 years was conducted to examine three types of produce-associated microbiota. The average amount of antibiotic-resistant bacteria recovered from lettuce, tomato, and cucumber was 1.02×10^{10}, 2.05×10^{7}, and 4.78×10^{9} cells per 50 g of each produce, respectively. A total of 480 bacterial isolates were obtained and identified from their 16S rRNA genes, revealing isolates that were ubiquitously recovered from all three types of produce. However, sporadic presence of *Klebsiella pneumoniae* and *Acinetobacter baumannii* was detected on lettuce and cucumbers but not tomatoes. End-point PCR revealed that the *K. pneumoniae* and *A. baumannii* isolates were positive for genes encoding extended spectrum beta-lactamase. Whole genome sequencing of two of the *K. pneumoniae* isolates further suggested the presence of the $bla_{\text{CTX-M-15}}$ gene in a conjugative plasmid, as well as other antibiotic resistance genes and virulence-associated traits in either conjugative plasmids or the chromosomal genome. Quantitative microbial risk assessment indicated varying levels of ingestion risk associated with different types of produce. In particular, the risk arising from ESBL-positive *K. pneumoniae* in lettuce, but not in cucumbers or tomatoes, was higher than the acceptable annual risk of 10^{-4}.

Practical applications

Three types of vegetables were sampled and evaluated over 1.5 years to determine differences in their associated bacterial isolates. Particular emphasis was placed on identifying pathogenic strains that were positive for extended spectrum beta-lactamase (ESBL). Quantitative estimates of the microbial risk associated with the ESBL-positive pathogens showed that different produce types may incur varying levels of ingestion risk. Most of the currently reported ESBL-positive bacterial isolates have been identified in nosocomial environments. However, the carriage of such drug-resistant bacteria in vegetables suggests a possible connection between our daily diet and human health.

HIGHLIGHTS

- *Klebsiella pneumoniae* and *Acinetobacter baumannii* were isolated from lettuce and cucumbers
- Both types of isolated pathogenic species were positive for extended spectrum beta-lactamase (ESBL)
- Quantitative microbial risk assessment denoted varying levels of ingestion risk among produce
- Carriage of ESBL-positive pathogens in food suggests a possible link between daily diet and health

1 | INTRODUCTION

Unsafe food can cause over 200 diseases, and the risk of incurring illness from raw food is particularly high (WHO, 2015). Consumers of raw food are particularly vulnerable to the most common foodborne pathogens such as *Salmonella*, *Campylobacter*, *Listeria*, and enterohemorrhagic strains of *Escherichia coli* since the lack of cooking would mean a higher probability of these pathogens remaining viable at the point of ingestion. The problem with produce-associated pathogens is further compounded by the presence of drug resistance among the

pathogenic strains, particularly those that possess extended spectrum beta-lactamase (ESBL) or carbapenemase genes (Rahal, 2000). Antibiotics within the beta-lactam family are commonly used to treat infected hosts against gram-negative bacterial pathogens. However, ESBL and carbapenemase confer resistance against a wide spectrum of beta-lactams, and bacterial pathogens positive for such drug-resistance enzymes may result in increased mortality or morbidity upon host infection.

Prior studies reported the presence of ESBL-producing Enterobacteriaceae in retailed vegetables (Raphael, Wong, & Riley, 2011; Ruimy et al., 2010; Zurfluh et al., 2015). Similarly, viable antibiotic-resistant bacteria can be recovered from produce sampled at the point-of-harvest (Alsalah, Al-Jassim, Timraz, & Hong, 2015; Schwaiger, Helmke, Holzel, & Bauer, 2011). However, it remains unknown if the extent of ingestion risk differs for different produce, since most studies did not attempt to examine the different occurrence frequency of foodborne pathogens among produce types. A combination of cultivation-based and molecular-based methods to examine produce-associated microbiota would facilitate subsequent assessment of the different risks associated with ingesting different produce types.

In this study, we performed a comparative analysis of the produce-associated microbiota in three types of produce, namely, lettuce, cucumbers, and tomatoes, which were collected over a period of 1.5 years. Particular attention was paid to determine the occurrence of antibiotic-resistant opportunistic pathogens that may be present in the sampled fruits and vegetables. Quantitative microbial risk assessment (QMRA) was used to estimate the risk arising from the ingestion of these produce types and to determine if different produce would result in differing risks upon ingestion.

2 | EXPERIMENTAL SECTION

2.1 | Food sampling

Sampling trips to local vegetable markets in Taif, Thuwal, and Jeddah were made once in January 2014, twice in February 2015, once in March 2015, and once in June 2015. Each sampled set of produce included approximately 1 kg each of tomatoes, lettuce, and cucumbers. The food samples were produced locally in the northwest (Tabuk), central (Hail, Al Qassim), southwest (Najran), east (Al Dwaser), and west (Al Madinah, Taif, Asir) municipalities of Saudi Arabia. The produce was shipped to the vegetable markets in Taif, Thuwal, and Jeddah through local distributors. All fruits and vegetables were placed in plastic bags provided by the stall owners and placed in a cooler to be transported immediately to the laboratory. All samples were stored at 4 °C and processed for microbiological analysis within 1–2 days.

2.2 | Preparation of produce and bacterial cultivation

In the laboratory, all samples were processed with gloved hands to prevent cross-contamination of samples with the handler's skin microbiota. The lettuce leaves were peeled off from the stalks to obtain 50 g of leaves prior to washing. Tomatoes and cucumbers were washed as a whole and then aseptically peeled to obtain 50 g of their skin. The leaves or fruit peels were then further processed based on procedures described previously (Alsalah et al., 2015). Briefly, 50 g of each sample was individually blended with 250 ml of deionized water to approximate typical household food preparation conditions. A total of 50 ml of the blend was mixed with 50 ml of Miller LB broth and incubated for 24 hr at 37 °C to resuscitate injured and to minimize the occurrence of viable but nonculturable bacterial isolates. After incubation, the suspension was left to stand for approximately 15 min, diluted accordingly in 1X PBS and spread onto MacConkey or Brilliant Green Bile Lactose agar. Agar plates were supplemented with either 8 μg/ml meropenem (Sigma-Aldrich, Buchs, Switzerland) or 8 μg/ml ceftazidime (Sigma-Aldrich). Meropenem was used because it is a carbapenem within the new class of beta-lactam antibiotics and is typically used as a last line of defense for the treatment of many gram-negative bacterial infections (McKenna, 2013). Ceftazidime is a third-generation cephalosporin, and bacterial isolates resistant to ceftazidime typically encode an extended-spectrum beta-lactamase. Opportunistic bacterial pathogens isolated from meropenem- and ceftazidime-supplemented agar plates are hence likely to be those associated with nosocomial infections (Zowawi, Balkhy, Walsh, & Paterson, 2013).

2.3 | Characterization of bacterial isolates

Colonies growing on the media plates were randomly selected and restreaked twice to acquire pure cultures. All colonies were then extracted for DNA by the heat-lysis method or using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) based on the manufacturer's protocol. The 16S rRNA genes of bacterial isolates were amplified and sent in for Sanger sequencing at the KAUST Genomics core lab based on a procedure described earlier (Ansari, Harb, Jones, & Hong, 2015). The sequencing results were searched against the National Center for Biotechnology Information (NCBI) 16S rRNA gene database with BLASTN (Basic Local Alignment Tool). Isolates that were identified to be *Klebsiella pneumoniae* at the 16S rRNA gene level ($n = 21$) were further tested for the presence of beta-lactamase genes (e.g., $bla_{\text{CTX-M-15}}$, bla_{IMP}, bla_{KPC}, bla_{NDM}, $bla_{\text{OXA-48}}$, and bla_{VIM}) using primer sets and protocols detailed previously (Zowawi et al., 2014). Gram-negative *K. pneumoniae* that were determined to be positive for at least one of the beta-lactamase genes were further identified by multi-locus sequence-typing (MLST). Seven housekeeping genes were used for the *K. pneumoniae* MLST analysis (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB*). The thermal cycling conditions correspond to initial denaturation at 95 °C for 2 min, followed by 35 cycles of 94 °C for 20 s, 50 °C (or 60 °C for *gapA* and 45 °C for *tonA*) for 30 s and 72 °C for 30 s, and a final elongation step at 72 °C for 5 min (Diancourt, Passet, Verhoef, Grimont, & Brisse, 2005). Isolates that were identified to be *Acinetobacter baumannii* at the 16S rRNA gene level ($n = 2$) were also tested for the presence of beta-lactamase (e.g., bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{VIM}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-40}}$, $bla_{\text{OXA-51}}$, and $bla_{\text{OXA-58}}$) using primer sets and protocols detailed previously (Zowawi et al., 2015). Seven housekeeping genes were used for the *A. baumannii* MLST analysis (*rpoD*, *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, and *gpi*). The thermal cycling conditions

correspond to initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 60 s, 55°C for 60 s and 72°C for 2 min, and a final elongation step at 72°C for 2 min (Bartual et al., 2005). Positive polymerase chain reaction (PCR) amplicons of the anticipated sizes for both antibiotic resistance genes and housekeeping genes were purified and verified for identity using Sanger sequencing. Antibiotic resistance genes were identified using BLASTN against the nucleotide database on NCBI. MLSTs were identified using *K. pneumoniae* and *A. baumannii* MLST databases from Institut Pasteur and Oxford Scheme, respectively.

2.4 | Whole genome sequencing

Genomic DNA obtained from two *K. pneumoniae* isolates was submitted to the KAUST Genomics core lab for sequencing on the Illumina MiSeq platform. Raw sequences were preprocessed and checked for quality using Trimmomatic v.0.3.2. Assembly was performed using Spades v.3.7.1 with kmer sizes 21, 33, 55 and 77. The *K. pneumoniae* genome and plasmids were binned using guanine-cytosine content, average contig coverage and contig tetranucleotide frequency to differentiate between the assembled chromosomal genome and plasmidic genome (Haroon et al., 2013). Genome contigs were then annotated for resistance genes and other virulence traits using the RAST service (Aziz et al., 2008; Overbeek et al., 2014) and as described previously (Mantilla-Calderon et al., 2016).

2.5 | Quantitative microbial risk assessment

The microbial risk arising from the presence of *K. pneumoniae* and *A. baumannii* in the produce was further evaluated by QMRA. Phylogenetic identification of isolates denoted the presence of *K. pneumoniae* at individual fractions of 0.03, 0.01, and 0.004, respectively, relative to the average plate counts of viable antibiotic-resistant isolates obtained from plates spread with enrichment culture from lettuce, tomato and cucumber. The presence of *A. baumannii* was only detected in plates spread with enrichment culture from cucumber and accounted for a fraction of 0.004. The probability of bacteria remaining on the surface of the fruits and vegetables after preparation was estimated to be 1×10^{-6} (Karapinar & Gonul, 1992). The risk from opportunistic pathogenic species was characterized using an exponential distribution model for daily risk; see Equation 1:

$$P\ (\text{response}) = 1 - e^{(-k \times \text{dose})} \quad (1)$$

where k is a numerical constant that denotes the probability of an organism surviving to reach and infect a host, and *P* is the probability of infection or death. The k constant for *K. pneumoniae* was estimated at 7.68×10^{-7} with a lower-bound value of 3.84×10^{-7} and higher-bound value of 1.54×10^{-6} (Struve & Krogfelt, 2003). The k constant for *A. baumannii* was estimated at 6.93×10^{-6} with a lower-bound value of 3.47×10^{-6} and higher-bound value of 1.39×10^{-5} (Ketter et al., 2014).

Annual risk was calculated based on Equation 2, below:

$$P_{\text{annual}} = 1 - (1 - P_{\text{daily}})^{\text{number of exposure days per year}} \quad (2)$$

The main exposure route considered for QMRA was through ingestion. Two questionnaire-based surveys were sent out to a total of 200 individuals residing in Saudi Arabia to assess the local consumption rates of fruits and vegetables. The average body weight of the surveyed cohort was 67 kg per person. The number of exposure days per year was determined to be 327 days and calculated based on the survey results in which 45% of the respondents consumed fruits or vegetables at least once per day, with 50–100 g per serving. The remaining portion of the surveyed cohort (42%) consumed at a weekly frequency or less, while 13% reported consumption rates of every meal per day (Supporting Information Data set S1). For cucumbers and tomatoes, it was assumed that 2% of the consumed mass was derived from the fruit peels. The majority of the individuals reported tomatoes, lettuce, and cucumbers as three types of produce that were eaten raw on a regular basis. Several other produce types were also listed but on a less frequent basis. There is currently no legislation or guideline to denote the permissible level of risk incurred from consuming fruits and vegetables. To provide perspectives on the microbial risk outcomes, the annual risk evaluated in this study was therefore compared against a microbial risk of 10^{-4} (Smeets, Medema, & van Dijk, 2009).

2.6 | Nucleotide accession number

All Sanger-based sequences associated with *K. pneumoniae* and *A. baumannii* are listed in Supporting Information Data set S2.

3 | RESULTS

3.1 | Profile of cultivated bacterial isolates among different produce

The average number of antibiotic-resistant bacteria that grew on MacConkey plates supplemented with antibiotics was 1.02×10^{10}, 2.05×10^{7} and 4.78×10^{9} cells per 50 g of lettuce, tomato, and cucumber, respectively. A total of 480 isolates ($n_{\text{lettuce}} = 187$, $n_{\text{tomatoes}} = 10$, $n_{\text{cucumber}} = 192$) was further obtained and identified at the species level based on 16S rRNA gene sequencing. The majority of the isolates that were ubiquitously recovered from the three types of produce across all five sampled sets were *Stenotrophomonas* spp., *Pseudomonas* spp., *Enterococcus* spp., *Klebsiella* spp., *Enterobacter* spp., *Ochrobactrum* spp., *Acinetobacter* spp., and *Staphylococcus* spp. (Figure 1). In particular, *Stenotrophomonas*, *Pseudomonas*, *Enterococcus*, and *Klebsiella* were four genera that accounted for the predominant percentage of isolates that were recovered, albeit at different frequencies among the produce. For example, *Stenotrophomonas* spp. accounted for 10.4 and 18.8% of isolates recovered from cucumber and lettuce, respectively, but accounted for 1.7% of isolates recovered from tomatoes. Likewise, *Klebsiella* spp. accounted for 3.1% of total isolates recovered from lettuce, but accounted for 1.3% and 0.6% of isolates recovered from tomatoes and cucumbers, respectively (Figure 1).

A further evaluation at the species level revealed the presence of several types of opportunistic pathogenic species. To illustrate, *Enterococcus faecalis* and *E. faecium*, both of which are commonly used as

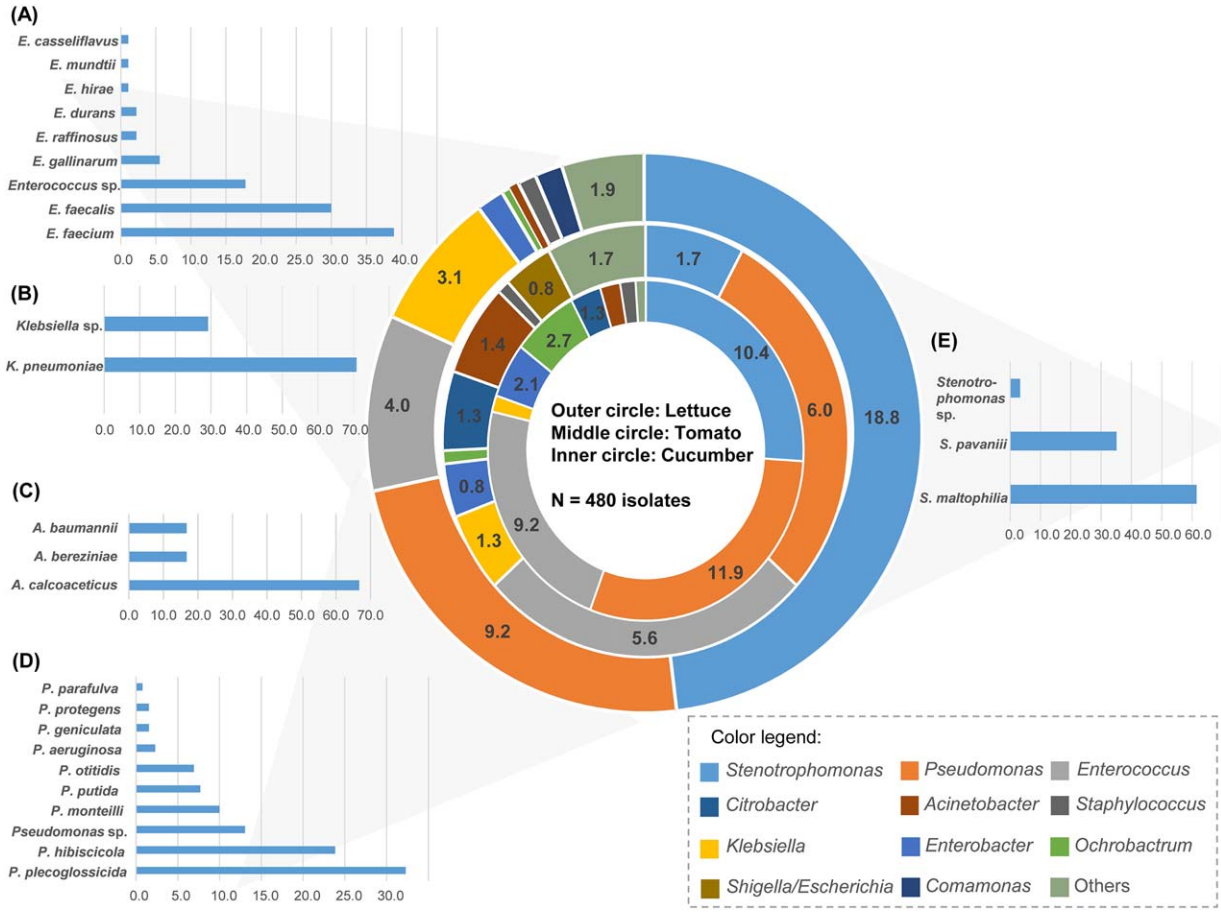

FIGURE 1 Profile of the heterotrophic bacterial isolates recovered from lettuce (outer circle), tomato (middle circle) and cucumbers (inner circle). Numbers shown in the individual segments of the circles are the percentage of isolates identified as affiliated with that genus, determined after normalizing against the total isolates recovered from that particular produce. Percentages that are $< 0.8\%$ are not shown. Bacterial species classified within genera (a) *Enterococcus*, (b) *Acinetobacter*, (c) *Pseudomonas*, and (d) *Stenotrophomonas* are further shown at the species level

fecal indicators, were the predominant *Enterococcus* spp. isolated from all three types of produce (Figure 1a). *K. pneumoniae* accounted for up to 70% of 24 isolates identified to be *Klebsiella* spp. (Figure 1b), and the majority of the *K. pneumoniae* isolates were recovered from lettuce, followed by tomato and cucumber.

In addition to *K. pneumoniae*, two *A. baumannii* were also recovered from cucumber along with *A. bereziniae* and *A. calcoaceticus* from the other types of produce (Figure 1c). *P. aeruginosa* were recovered less frequently relative to other *Pseudomonas* spp. (Figure 1d), but *S. maltophilia* accounted for up 61.5% of the 148 isolates identified as *Stenotrophomonas* (Figure 1e). Unlike *K. pneumoniae* and *A. baumannii*, neither *P. aeruginosa* nor *S. maltophilia* was particularly associated with either type of produce.

3.2 | MLST and presence of beta-lactamase genes among *K. pneumoniae* and *A. baumannii* isolates

MLST of the $bla_{\text{CTX-M-15}}$-positive *K. pneumoniae* revealed different serotypes namely, ST661 (3 out of 13 isolates), ST37 (3 out of 13), ST789 (2 out of 13), and ST101 (2 out of 13), and the remaining isolates were individually classified under ST1593, ST43, ST889 (Supporting Information Data set S2). Positive PCR amplification of the correct anticipated size was observed for the $bla_{\text{CTX-M-15}}$ gene among 13 out of the 21 *K. pneumoniae* isolates. All 13 *K. pneumoniae* that tested positive for the $bla_{\text{CTX-M-15}}$ gene were isolated from lettuce.

Both *A. baumannii* were isolated from cucumbers and were positive for the $bla_{\text{OXA-51}}$ gene. MLST of both *A. baumannii* isolates suggested high sequence similarity of their housekeeping genes and that neither isolate was classified into any of the existing STs that are present in the database.

3.3 | Whole genome sequencing of two *K. pneumoniae* isolates

Given that all *K. pneumoniae* isolates retrieved from lettuce were positive for the $bla_{\text{CTX-M-15}}$ gene, draft genome sequencing of two of the isolates, named accordingly isolates L7 and L14, was further performed to identify the full spectrum of plasmid-encoded antibiotic resistance genes and virulence-associated traits. The $bla_{\text{CTX-M-15}}$ gene in L7 was encoded on an IncF plasmid type that shared more than 99% identity

TABLE 1 List of plasmid-encoded genes conferring antibiotic resistance that were detected in *K. pneumoniae* isolates recovered from lettuce

	K. pneumoniae L7	*K. pneumoniae* L14
Beta-lactam	$bla_{CTX\text{-}M\text{-}15}$ $bla_{TEM\text{-}1B}$ $bla_{OXA\text{-}1}$	$bla_{CTX\text{-}M\text{-}15}$
Fluoroquinolone	*aac*(6′)Ib-cr	
Quinolone		*qnr*S1
Aminoglycoside	*aac*(6′)Ib-cr *aad*A1 *str*A *str*B	*aad*A1 *str*A
Tetracycline	*tet*(B)	*tet*(A)
Phenicol	*cat*B3	*cm1*A1
Macrolide	*mph*(B)	*mph*(A)
Sulfonamide	*sul*1 *sul*2	*sul*1 *sul*2 *sul*3
Trimethoprim	*dfr*A14 *dfr*A1	

similarity with pKpN6 plasmid from *K. pneumoniae* subsp. *pneumoniae* MGH 78578 (GenBank accession number CP000651). In addition, isolate L7 was positive for the $bla_{TEM\text{-}1B}$ and $bla_{OXA\text{-}1}$ genes. The $bla_{CTX\text{-}M\text{-}15}$ gene in L14 was encoded on an IncI1 plasmid type. Both isolates also contained other genes coding for resistance against quinolone, aminoglycoside, tetracycline, phenicol, macrolide, sulfonamide, and trimethoprim on their respective plasmids (Table 1). To maintain the stability of the plasmid in progeny cells, both plasmids contain the CcdB toxin and CcdA antitoxin system.

RAST individually identified a total of 95 and 104 genes potentially involved in the virulence, disease and defense category for isolates L7 and L14, respectively. Specifically, core genes in this category shared by both isolates include those encoding 16 kDa heat shock proteins A and B, as well as outer membrane lipoprotein YidQ required for hyperadherence. In addition, mediator of hyperadherence YidE and an uncharacterized protein YidR were also present. Multiple antibiotic resistance (Mar) loci were also present alongside those conferring resistance against heavy metals (e.g., zinc, copper, arsenic, cobalt). In addition to virulence-associated traits, both isolates possess the HipA and HipB proteins, which are proteins facilitating the formation of dormancy during stressful environmental events (Supporting Information Data set S3).

3.4 | QMRA for the presence of *K. pneumoniae* and *A. baumannii* in produce

QMRA was performed to assess the risks associated with *K. pneumoniae* and *A. baumannii* because these two microbial agents have been reported to be common causative agents in nosocomial and non-nosocomial infections in Saudi Arabia (Sonnevend et al., 2015; Uz Zaman et al., 2014; Yezli, Shibl, Livermore, & Memish, 2014). The two bacteria were isolated at different frequencies in the three types of examined produce. The majority of *K. pneumoniae* isolated were recovered from lettuce, while both of the *A. baumannii* were recovered from cucumbers.

Point and annum estimates of the microbial risk arising from both bacteria were compared against the acceptable limit of 10^{-4} (i.e., the risk of infecting 1 individual per 10,000 persons). It was determined that the high-bound and low-bound point risks arising from *K. pneumoniae* ranged from 2.22×10^{-4} to 3.97×10^{-10}, with the high-bound point risk estimation slightly above 10^{-4} risk due to ingestion of *K. pneumoniae* in lettuce (Table 2). Based on the surveyed consumption rates per annum, the annual risk arising from *K. pneumoniae* was of concern when consuming lettuce as the annual risk ranged from 9.05×10^{-3} to 7.01×10^{-2} (Table 2), but the annual risks were within acceptable limits when consuming tomatoes and cucumbers.

In comparison, the point estimates of risk from *A. baumannii* in cucumbers ranged from 3.35×10^{-7} to 2.68×10^{-6}, considerably lower than the acceptable risk of 10^{-4}. However, the presence of *A. baumannii* in cucumbers can result in an annual risk that ranged from 1.10×10^{-4} to 8.76×10^{-4}, which is slightly higher than the acceptable limit of 10^{-4} (Table 2).

4 | DISCUSSION

Similar to the findings reported by an earlier study (Erlacher, Cardinale, Grube, & Berg, 2015; Leff & Fierer, 2013), this study demonstrated variations in produce-associated microbiota among three different types of fruits and vegetables. To illustrate, $bla_{CTX\text{-}M\text{-}15}$-positive *K. pneumoniae* was found only in lettuce, while bla_{OXA51}-positive *A. baumannii* was only found in cucumbers. The presence of ESBL or carbapenem-resistant bacterial pathogens in a specific type of produce has been hypothesized to arise as a culmination of various factors. First, irrigation water quality and farming practices may be different across different farms and hence predispose the produce to different frequencies of microbial contaminants. Lettuce positive for ESBL-resistant *K. pneumoniae* mainly originated from Taif, while lettuce negative for the same ESBL-resistant pathogen was from other geographical locations (i.e., Al Madinah and Asir). Second, the leafy arrangement of lettuce heads may have shielded the bacterial pathogens from solar irradiation and/or other harsh environmental conditions and hence facilitated their longer persistence. Third, cucumbers were observed during handling to have rougher apparent surface topography than tomatoes, and it was previously found that the adhesion of microorganisms onto surfaces is positively correlated with rougher surface topography (Pang, Hong, Guo, & Liu, 2005). Lettuce was also previously found to have a higher contamination index than tomatoes and that once a bacterial contaminant is attached onto lettuce, higher populations may be retained in the phyllosphere of lettuce compared to tomatoes (Barak, Liang, & Narm, 2008).

The implications of isolating drug-resistant bacterial isolates from produce can be of concern. Whole genome sequencing of two of the

TABLE 2 Quantitative microbial assessment of *Klebsiella pneumoniae* and *Acinetobacter baumannii* present in the produce

Parameters	Assumed value	Source
Average weight per person among surveyed cohort (kg)	67	Supporting Information Data set S1
Average meals per day that included raw produce	0.97	
Weight of produce consumed per meal (g/meal)	50–100	
Total amount of produce consumed per day (g/day)	48.5–97	
Actual weight of lettuce consumed per body weight per day (g/kg/day)	0.72–1.45	
Actual weight of cucumber or tomato skins consumed per body weight per day, assuming that skins account for 2% of total weight (g/kg/day)	0.01–0.03	
Average total antibiotic resistant bacteria from respective produce enrichment culture per 50 g	Lettuce: 1.02×10^{10} Tomato: 2.05×10^{7} Cucumber: 4.78×10^{9}	
Assumed range of probability of bacteria remaining after handling	High bound: 1.0×10^{-3} Low bound: 1.0×10^{-6}	(Karapinar & Gonul, 1992)
Fraction of *K. pneumoniae* isolates	Lettuce: 0.03 Tomato: 0.01 Cucumber: 0.004	Figure 1b
Range of *K. pneumoniae* dose ingested from lettuce	7.23×10^{1} to 1.45×10^{2}	
Range of *K. pneumoniae* dose ingested from tomato	1.04×10^{-3} to 2.07×10^{-3}	
Range of *K. pneumoniae* dose ingested from cucumber	9.67×10^{-2} to 1.93×10^{-1}	
k of *K. pneumoniae*	Mean: 7.68×10^{-7} High bound: 1.54×10^{-6} Low bound: 3.84×10^{-7}	(Struve & Krogfelt, 2003)
Point estimate of risk arising from *K. pneumoniae*	Lettuce: 2.78×10^{-5} to 2.22×10^{-4} Tomato: 3.97×10^{-10} to 3.18×10^{-9} Cucumber: 3.71×10^{-8} to 2.97×10^{-7}	
Annual risk arising from *K. pneumoniae*	Lettuce: 9.05×10^{-3} to 7.01×10^{-2} Tomato: 1.30×10^{-7} to 1.04×10^{-6} Cucumber: 1.21×10^{-5} to 9.71×10^{-5}	
Fraction of *A. baumannii* isolates	Lettuce: 0.00 Tomato: 0.00 Cucumber: 0.004	Figure 1c
Range of *A. baumannii* dose ingested from cucumber	9.67×10^{-2} to 1.93×10^{-1}	
k of *A. baumannii*	Mean: 6.93×10^{-6} High bound: 1.39×10^{-5} Low bound: 3.47×10^{-6}	(Ketter et al., 2014)
Point estimate of risk arising from *A. baumannii*	Cucumber: 3.35×10^{-7} to 2.68×10^{-6}	
Annual risk arising from *A. baumannii*	Cucumber: 1.10×10^{-4} to 8.76×10^{-4}	

K. pneumoniae isolates retrieved from lettuce further revealed a wide spectrum of antibiotic and heavy metal resistance genes, as well as virulence associated traits, suggesting that they are opportunistic pathogens. Both *K. pneumoniae* and *A. baumannii* had been listed as common pathogens in the Arabian Peninsula region (Zowawi et al., 2013). However, documented outbreaks tend to originate from nosocomial environments, and there had not been any other reports of $bla_{\text{CTX-M-15}}$-positive *K. pneumoniae* or bla_{OXA51}-positive *A. baumannii* isolated from local produce in community settings.

To assess whether the detected abundance of *K. pneumoniae* and *A. baumannii* would constitute a significant risk to public health, QMRA was conducted based on the surveyed consumption frequencies and dietary habits (Supporting Information Data S1). QMRA analyses suggest an annual risk arising from $bla_{\text{CTX-M-15}}$-positive *K. pneumoniae* in lettuce ranging between 9.05×10^{-3} and 7.01×10^{-2} (Table 2), which is higher than the acceptable probability of 10^{-4}. In contrast, microbial risk arising from *K. pneumoniae* in cucumbers and tomatoes was negligible. The annual risk of consuming bla_{OXA51}-positive *A. baumannii* in cucumber ranged from 2.1×10^{-4} to 8.39×10^{-4}. Although this estimated risk was slightly higher than the acceptable probability of 10^{-4}, the quantified risk may be higher than the actual risk since both *A. baumannii* isolates recovered from cucumbers were likely to be of clonal origin given that the ST profiles and antimicrobial resistance patterns were similar.

In both instances, there was no direct evidence to suggest there had been any dissemination of *K. pneumoniae* or *A. baumannii* from the community via ingestion of produce to infect any hospitalized patients. However, MLST of the *K. pneumoniae* isolates grouped them into ST661, ST1593, ST37, ST43, ST101, ST889, and ST789. Isolates belonging to the ST37 and ST661 types were also isolated from vegetables imported to Switzerland from Dominican Republic and other Asian countries (Zurfluh et al., 2015), and they were also found to be ESBL producers. Among these STs, *K. pneumoniae* belonging to ST661, ST37, and ST101 have been reported to cause urologic infections in Italy, United States and in some Mediterranean countries such as Spain and Tunisia (Cubero et al., 2015; Little, Qin, Zerr, & Weissman, 2014; Mammina et al., 2012). Coincidentally, $bla_{\text{CTX-M-15}}$-positive *K. pneumoniae* have also been identified as one of the main mechanisms for ESBL resistance among nosocomial *K. pneumoniae* isolates studied in Saudi Arabia (Zowawi et al., 2013).

Unlike *K. pneumoniae*, the *A. baumannii* isolated in this study was not assigned to any of the known STs in MLST, and it was not possible to establish a link to nosocomial strains based on the current experimental design. A local survey for carbapenem resistance mechanisms among *A. baumannii* isolated between 2006 and 2011 at various Saudi medical centers identified genes for bla_{VIM}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-40}}$, $bla_{\text{OXA-89}}$, $bla_{\text{OXA-66}}$, and novel chromosomal $bla_{\text{OXA-51}}$-like among these isolates (Alsultan et al., 2013; Alsultan, Hamouda, Evans, & Amyes, 2009). A more recent study that looked into 117 *Acinetobacter* spp. collected from hospitals between 2011 and 2013 also found all isolates to be positive for $bla_{\text{OXA-51}}$-type (Zowawi et al., 2015). Along with these studies, our findings suggest the frequent occurrence of $bla_{\text{OXA-51}}$ genes in *A. baumannii* isolated from both nosocomial and community settings.

5 | CONCLUSIONS

In summary, through a 1.5-year monitoring effort on produce-associated microbiota, we have demonstrated sporadic presence of drug-resistant bacterial pathogens on certain types of produce. Our findings suggest that different produce types have varying extents of predisposition toward adherence and subsequent occurrence of pathogens. Although this study mainly emphasized the local occurrences of ESBL and the produce-associated microbial communities in Saudi Arabia, our findings revealed several causes for concern that can be applied to food safety in a global context. This study, along with others reported from other geographical locations, has highlighted the presence of drug-resistant bacteria associated with certain types of produce. Most of the current reports of ESBL or carbapenem-resistant bacterial isolates were identified in nosocomial samples. However, the carriage of such drug-resistant bacteria in the food that we consume daily suggests a possible connection between our daily diet and our health.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Data S1 Demographic evaluation and response results from two batches of surveyed cohort.

Data S2 Sequences of 16S rRNA genes, multi-locus sequence typing and antibiotic resistance genes.

Data S3 Virulence traits identified in genomes of *Klebsiella pneumoniae* L7 and L14.

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