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Fresh produce as a potential vehicle for transmission of *Acinetobacter baumannii*

Qutaiba Ababneh*, Ekhlas Al-Rousan and Ziad Jaradat

Abstract

Acinetobacter baumannii is a Gram-negative bacterium that has gained a stronghold inside healthcare settings. Due to the ability of *A. baumannii* to acquire antibiotic resistance easily, its presence in food products could pose a major threat to the public health. The aim of this study therefore, was to investigate the prevalence of *A. baumannii* in fresh produce and study their genetic diversity. A total of 234 samples of vegetables and fruits were collected. *A. baumannii* isolates were identified using CHROMagar and two different PCR assays. Also, the isolates were tested for their ability to resist antibiotics and form biofilms. The genetic diversity of the isolates was determined using multi-locus sequence typing (MLST). Of the 234 samples collected, 10 (6.5%) and 7 (8.75%) *A. baumannii* isolates were recovered from vegetables and fruits, respectively. Antibiotic susceptibility testing revealed that 4 of these isolates were extensively drug-resistant (XDR). All isolates were able to form biofilms and MLST analysis revealed 6 novel strains. This study demonstrated that fresh produce constitutes a reservoir for *A. baumannii*, including strong biofilm formers and XDR strains. This represents a significant concern to public health because vegetables and fruits may serve as a vehicle for the spread of *A. baumannii* and antibiotic resistance into the community and healthcare settings.

Keywords: *Acinetobacter baumannii*, Fresh produce, MLST, Biofilm formation, Antibiotic resistance

Introduction

Acinetobacter baumannii is a Gram-negative bacterium that has become an immensely dangerous pathogen inside healthcare settings due to its ability to resist multiple groups of antimicrobial agents (Nasr 2020). This pathogen can cause a wide range of diseases, including urinary tract infections, skin and soft tissue infections, bacteremia, pneumonia, osteomyelitis, and meningitis (Williams et al. 2020). Several factors contributed to the success of *A. baumannii* as a nosocomial pathogen, including its capacity to adapt to adverse environmental conditions, desiccation resistance, antibiotic resistance and genome plasticity. Besides, *A. baumannii* can survive exposure to regularly used disinfectants such as phenols and chlorhexidine, and it can tolerate the dry environment for months (Gallego 2016).

Although *A. baumannii* is commonly known as a nosocomial pathogen, it has also been isolated from diverse sources such as food, water, soil and animals (Lupo et al. 2014; Rafei et al. 2015; Al Atrouni et al. 2016; Karumathil et al. 2016; Carvalheira et al. 2017a; Carvalheira et al. 2017b). The presence of *A. baumannii* in food is considered a serious problem, as contamination of the food chain with this bacterium might enable it to find its way into healthcare settings, and thus exacerbate the burden of nosocomial infections caused by this pathogen (Lupo et al. 2014). In recent years, consumption of the fresh produce (fruits and vegetables) has increased due to modernization of the agriculture methods and the surge in production (Carvalheira et al. 2017a). Consumption of raw or minimally processed fresh produce can serve as a source for the spread of this pathogen, both in communities and hospital environments (Berlau et al. 1999). Vegetables and fruits may

*Correspondence: qoababneh@just.edu.jo

Department of Biotechnology and Genetic Engineering, Faculty of Science and Arts, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan

acquire *A. baumannii* while growing in the soil, during harvesting, from organic fertilizers, from contaminated irrigation water, as well as during transportation and handling (Machado-Moreira et al. 2019). Moreover, vegetables and fruits have high-water activity, which helps in the growth of microorganisms, including *Acinetobacter* species.

Few studies reported the isolation of *A. baumannii* from fresh produce. For example, this pathogen have been isolated from apple, melon, bean, carrot, potato, and radish (Berlau et al. 1999), while a Japanese group have isolated it only from leek (Oie et al. 2008). In addition, two studies reported the contamination of lettuce samples with *A. baumannii* (Karumathil et al. 2016; Carvalheira et al. 2017b). However, none of these studies attempted to elucidate the link of this pathogen with the clinical context, as the *A. baumannii* clonality of strains investigated was not determined. Information about the clonality of *A. baumannii* isolated from fresh produce will increase our understanding of any potential exchange of *A. baumannii* clones between food and healthcare settings. Therefore, this study aimed to investigate the prevalence clonality, antibiotic resistance and biofilm formation of *A. baumannii* in fresh produce collected from retail markets in the city of Irbid, Jordan.

Materials and methods

Samples collection and isolation of *A. baumannii*

A total of 234 samples (154 vegetables and 80 fruits) were collected between October 2018 and February 2020 from different hypermarkets and retail markets in Jordan (Table 1). *A. baumannii* was isolated from all samples following the procedure described previously (Rafei et al. 2015). Briefly, all samples were processed within 24 h of collection in a UV-sterilized laminar flow. Ten grams from each sample were weighted inside the laminar flow on a sterile aluminum sheet and suspended in 90 mL sterile distilled water (10% w/v). The suspension was homogenized in an orbital shaker water bath for 15 min, then decanted for 30 min. Five milliliters of the suspension were added to 20 mL of Dijkshoorn enrichment media and mixed in an orbital shaker water bath at 150 rpm for 48 h at 37°C (Carvalheira et al. 2017b). CHROMagar *Acinetobacter* plates (CHROMagar, France) were used for samples culturing and incubated for 24–48 h at 37°C. Red colonies with white halo were regarded as presumptive *A. baumannii* and were selected for further analysis.

Molecular identification of *A. baumannii*

A. baumannii was identified at the molecular level by partial polymerase chain reaction (PCR) amplification of the *hyp* (Hamouda 2017) and $bla_{OXA\text{-}51}$ genes (Turton et al. 2006). In addition, a multiplex PCR assay was used to differentiate between *A. baumannii, A. nosocomialis,* and *A. pittii* (Chen et al. 2014). Genomic DNA was extracted using Wizard Genomic DNA Purification Kit (Promega/USA) as per the manufacturer's instructions. All PCR products were purified from agarose gels using GeneJet Gel Extraction Kit (ThermoFisher, USA) and subjected to DNA sequencing (Macrogen, South Korea). The obtained DNA sequences were analyzed by BLAST search. DNA isolated from the reference strain *A. baumannii* ATCC 19606 was included as a positive control for all PCR assays.

Antibiotic sensitivity testing

The disk diffusion method was used to perform the antibiotic sensitivity testing against the following antibiotics: doripenem (10 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ceftriaxone (30 µg), cefepime (30 µg), ceftazidime (30 µg), amikacin (30 µg), tobramycin (10 µg), gentamicin (10 µg), ampicillin-sulbactam (10/10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), piperacillin (100 µg), and piperacillin-tazobactam (100/10 µg). All antibiotics were purchased from Oxoid, UK. The minimal inhibitory concentrations (MICs) of colistin (Sigma, Germany), polymyxin B (Duchefa Biochemie, Netherlands) and tigecycline (Cayman, USA) antibiotics were determined by the broth micro-dilution method as described previously (Wiegand et al. 2008). The Clinical and Laboratory Standards Institute (CLSI) antibiotic susceptibility breakpoints (CLSI 2018) were used to classify the isolates into susceptible, intermediate, or resistant. The reference strain *A. baumannii* ATCC19606 was included in all antibiotic susceptibility tests as controls.

Biofilm formation

Biofilm formation was assayed by the semi-quantitative method in a sterile 96-well microtiter plates as described previously (Hu et al. 2016). For each isolate, the biofilm assay was performed in triplicate per 96-well plate, and the optical densities (ODs) of 3 independent plates were compared with the cut-off OD (ODc) to determine the biofilm phenotype as follows; non-biofilm producer: $OD \leq ODc$; weak biofilm producer: $ODc < OD \leq 2 \times ODc$; moderate biofilm producer: $2 \times ODc < OD \leq 4 \times ODc$; or strong biofilm producer: $OD > 4 \times ODc$.

Multi-locus sequence typing (MLST)

MLST was performed on all isolates following the Pasteur scheme by PCR amplification of internal fragments of 7 housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB),* and subsequent DNA sequencing of the PCR amplicons (Macrogen, South Korea). The PCR conditions for MLST are described on the PubMLST

Table 1 Prevalence of *A. baumannii* in the collected fresh produce samples

Sample Source	No. of samples with confirmed *A. baumannii* / No. of samples collected	No. of samples with presumptive *A. baumannii* colonies	Molecular identification of *A. baumannii* by PCR			No. of samples with confirmed *A. pittii* isolates
			A	B	C	
Vegetables						
Tomato	0/10	4	0	0	0	1
Cucumber varieties [d]	3/12	11	3	3	3	3
Carrot	0/10	10	0	0	0	3
Lettuce	0/10	10	0	0	0	8
Lemon	0/10	4	0	0	0	0
Sweet Green Pepper	0/10	8	0	0	0	0
Sweet Yellow Pepper	1/10	8	0	1	1	2
Sweet Red Pepper	1/10	7	0	1	1	2
Arugula	2/10	10	0	2	2	7
Mint	2/10	10	1	2	2	6
Parsley	0/10	10	0	0	0	8
Red Radish	1/10	9	1	1	1	6
Coriander	0/10	10	0	0	0	6
Chili Green Pepper	0/10	6	0	0	0	0
Chili Red Pepper	0/4	1	0	0	0	0
Cherry Tomato	0/8	1	0	0	0	0
Fruits						
Apple varieties [e]	2/19	7	1	2	2	0
Pear	2/10	4	1	2	2	0
Grape	1/11	3	1	1	1	0
Strawberry	1/10	5	0	1	1	0
Peach	0/12	6	0	0	0	2
Guava	1/8	2	0	1	1	0
Mango	0/10	4	0	0	0	0
Total	**17/234**	**150**	**8**	**17**	**17**	**54**

A: No. of *hyp* gene positive isolates.

B: No. of *A. baumannii* confirmed by the Multiplex PCR assay.

C: No. of $bla_{oxa\text{-}51}$ gene positive isolates.

[d] British cucumber, snake cucumber and *Cucumis melo*

[e] Red, yellow and green apples

website (https://pubmlst.org/abaumannii/). Amplification reactions for the MLST PCR consisted of 20 ng/µl of extracted DNA, 0.4 µM of each primer, 1X PCR ready mix (iNtRON, South Korea). Alleles and sequence types were identified using the tools of the PubMLST database (Jolley et al. 2018). goeBURST analysis was performed using the PHYLOViZ tool (version 2.0) as described previously (Francisco et al. 2012).

Results

Isolation and identification of *A. baumannii*

The fresh produce samples analyzed in this study included imported (42/234; 18%) and domestic (192/234; 82%) products. All mango and the majority of the apple samples (18/19) were imported. Other types of samples were imported include, carrot (6/10), lemon (1/10), pears (3/10), grapes (1/11), peach (2/12) and chili green pepper (1/10). All other fresh produce were grown locally.

CHROMagar was used to identify the presumptive *A. baumannii* colonies, which were isolated from 150 (64.1%) samples. Most of the tested cucumber, carrot, lettuce, arugula, mint, parsley, red radish samples contained presumptive *A. baumannii* isolates. However, not all the presumptive isolates were identified by PCR as *A. baumannii*, instead several isolates were identified as *A. pittii* by the multiplex PCR assay and DNA sequencing (Table 1). *A. pittii* colonies were detected in 54 (23%) of samples collected.

Approximately 64% of the samples harbored presumptive *A. baumannii*, but only 17 isolates (7.3%) were recovered from the 234 collected samples. Table 1 summarizes the number of presumptive colonies and the confirmed *A. baumannii* isolates for each sample. When tested for the presence of the *hyp*-gene by uniplex PCR, more than half of the multiplex PCR-confirmed *A. baumannii* isolates tested positive for *hyp* gene. However, all the multiplex PCR positive *A. baumannii* isolates harbored the $bla_{OXA\text{-}51}$ gene. In the multiplex PCR assay, four genes were amplified; the *recA* gene that exists in all *Acinetobacter* species, the *gyrB* gene that is present only in *A. baumannii* and *A. nosocomialis*, the internal transcribed spacer (ITS) region of *A. baumannii*, and the ITS region of *A. pittii*. It is important to mention that the multiplex PCR results were confirmed by DNA sequencing. With respect to the source of the samples, 4 of the 17 confirmed *A. baumannii* isolates were recovered from imported products, while the remaining isolates were from domestic samples. Isolates AP4 and AP8 were recovered from red apple samples from Italy and the USA, respectively. In addition, we isolated A. *baummannii* (GP1) from a green grape sample from Egypt, and one isolate (PR1) from Spanish pears.

Antibiotic sensitivity testing

The majority of the isolates were sensitive to most of the tested antibiotics except for ceftriaxone, for which all the isolates displayed resistance or intermediate phenotypes. Based on their resistance profiles, the isolates were grouped into 11 resistance patterns (A to K) (Table 2). Six isolates exhibited the same resistance pattern A, while 2 isolates had the same resistance pattern B. Each of the remaining 9 isolates had different resistance patterns.

Four isolates recovered from red radish, red apple, green grape, and guava were classified as XDR, however, all of these isolates were sensitive to ampicillin-sulbactam antibiotic except the isolate that was recovered from guava. This isolate was sensitive to trimethoprim-sulfamethoxazole, tobramycin, colistin and polymyxin B, while exhibited resistance to tigecycline. The other 3 XDR isolates were sensitive to colistin, polymyxin B and tigecycline.

Biofilm formation

Thirteen of the 17 isolates (76.5%) displayed strong ability to form biofilms in vitro. Among the 4 XDR isolates, two were strong biofilm formers, while the other two isolates were weak and moderate biofilm formers (Table 3). The isolates from cucumber, arugula, red apple, pears, strawberry and sweet pepper were all strong biofilm formers. Two isolates recovered from mint and green grapes were classified as moderate biofilm, and the weak biofilm formers were recovered from arugula and guava.

Multi locus sequence typing (MLST)

MLST analysis showed that 11 isolates belonged to six known sequence types (STs), while the other six isolates were novel strains (Table 3). Five isolates belonged to ST40, two isolates had ST2 and four isolates belonged to ST481, ST602, ST724 and ST897. Two of the XDR isolates (AP8 and GP1) recovered in this study belonged to ST2, one belonged to ST724 while the XDR isolate from guava had a new ST. Data from the PubMLST *Acinetobacter* database and the literature indicated that strains belonging to ST481 and ST897 have been previously isolated from animal sources. ST40 strains have been isolated from clinical and food sources, while isolates

Table 2 Antibiotic resistance profiles for the *A. baumannii* isolates

Resistance pattern	No. of isolates	Antibiotic resistance phenotypes																		
		DOR	IPM	MEM	CIP	LEV	CRO	FEP	CAZ	AK	TOB	CN	SAM	TE	SXT	TZP	PRL	CT	POL B	TGC
A	6	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	ND	ND	ND
B	2	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	S	S
C	1	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	S	S	S
D	1	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	R	S	S	R
E	1	S	I	S	S	S	R	S	I	S	S	S	S	S	S	S	S	ND	ND	ND
F	1	S	S	S	S	S	I	S	S	S	S	S	R	S	S	S	S	ND	ND	ND
G	1	S	S	S	S	S	I	S	S	R	S	I	I	S	S	S	S	ND	ND	ND
H	1	S	S	S	R	R	R	I	I	S	S	S	S	S	S	S	S	ND	ND	ND
I	1	S	S	S	S	S	I	S	I	S	S	S	S	S	S	S	I	ND	ND	ND
J	1	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	I	ND	ND	ND
K	1	S	S	S	S	S	I	S	S	S	S	S	S	S	I	S	S	ND	ND	ND

DOR Doripenem, *IPM* Imipenem, *MEM* Meropenem, *CIP* Ciprofloxacin, *LEV* Levofloxacin, *CRO* Ceftriaxone, *FEP* Cefepime, *CAZ* Ceftazidime, *AK* Amikacin, *TOB* Tobramycin, *CN* Gentamicin, *SAM* Ampicillin-sulbactam, *TE* Tetracycline, *SXT* Trimethoprim-sulfamethoxazole, *TZP* Piperacillin-tazobactam, *PRL*, Piperacillin, *CT* Colistin, *POL B* Polymyxin B, *TGC* Tigecycline, *ND* Not determined, *S* Sensitive, *R* Resistant, *I* Intermediate

Table 3 Characteristics of all recovered *A. baumannii* isolates

Isolate	Source	Antibiotic Resistance	Antibiotic resistance pattern	Biofilm Formation	Sequence Type	PubMLST source
CUC6	Cucumber	Non-MDR	A	Strong	897	Animal
RK1	Arugula	Non-MDR	A	Weak	40	Sputum, urine, upper respiratory tract, wound, blood, and food
RD2	Red Radish	XDR	B	Strong	724	Blood
MT1	Mint	Non-MDR	E	Moderate	40	Same as isolate RK1
SYP1	Sweet Yellow Pepper	Non-MDR	A	Strong	40	Same as isolate RK1
SRP1	Sweet Red Pepper	Non-MDR	G	Strong	1854	New Type
SKCUC1	Snake Cucumber	Non-MDR	I	Strong	1857	New Type
CUCML1	*Cucumis Melo*	Non-MDR	A	Strong	1856	New Type
MT13	Mint	Non-MDR	K	Strong	602	Upper respiratory tract and environment
RK7	Arugula	Non-MDR	A	Strong	1862	New Type
AP4	Red Apple	Non-MDR	J	Strong	481	Animal (Dog mouth)
PR1	Pear	Non-MDR	A	Strong	40	Same as isolate RK1
STY1	Strawberry	Non-MDR	F	Strong	40	Same as isolate RK1
AP8	Red Apple	XDR	B	Strong	2	Sputum, urine, upper respiratory tract, wound, blood, and medical environment
PR2	Pear	Non-MDR	H	Strong	1855	New Type
GP1	Green Grape	XDR	C	Moderate	2	Same as isolate AP8
GV1	Guava	XDR	D	Weak	1863	New Type

belonging to ST602 have been previously isolated from environmental and upper respiratory tract samples. The PubMLST database contains data about only one ST724 strain isolated from blood, whereas hundreds of ST2 isolates have been isolated from clinical, animal and environmental sources (Brahmi et al. 2016; Khurshid et al. 2020; Shelenkov et al. 2021).

Six isolates had new allelic profiles and were assigned the new sequence types ST1854-ST1857 and ST1862–ST1863 (Table 4). The data related to these 6 isolates were deposited in the PubMLST database. Isolate SKCUC1 harbored a new allele sequence of the *rpoB* gene, which was assigned the allele number 318. The sequence of the *rpoB*-318 allele shares 99.78% identity with alleles 5 and 48. The other five isolates carried new allelic combinations. eBURST analysis revealed that ST1854, ST1855, ST1856 and ST1863 were single locus variants (SLV) for ST40, ST316, ST1036 and ST610, respectively (Fig. 1). Also, ST1862 is a double locus variant (DLV) to ST764 and ST1354, while ST1857 is a DLV to ST1228.

Discussion

Fresh fruits and vegetables are an integral part of healthy and balanced diets; providing us with carbohydrates, fibers, minerals, vitamins and many other micronutrients, as well as protecting us from many diseases such as obesity,

Table 4 Characteristics of the new STs found in the study

Isolate ID	Isolate PubMLST ID	MLST genes							Assigned ST	Comment
		Cpn60	*fusA*	*gltA*	*pyrG*	*recA*	*rplB*	*rpoB*		
SRP1	6923	69	2	2	2	5	1	14	1854	New allelic combination
PR2	6924	3	8	6	2	4	1	5	1855	New allelic combination
CUCML1	6925	3	16	2	2	5	1	4	1856	New allelic combination
SKCUC1	6926	25	3	2	2	156	4	318	1857	New *rpoB* allele
RK7	6939	12	3	2	2	4	1	14	1862	New allelic combination
GV1	6940	40	2	7	2	40	4	4	1863	New allelic combination

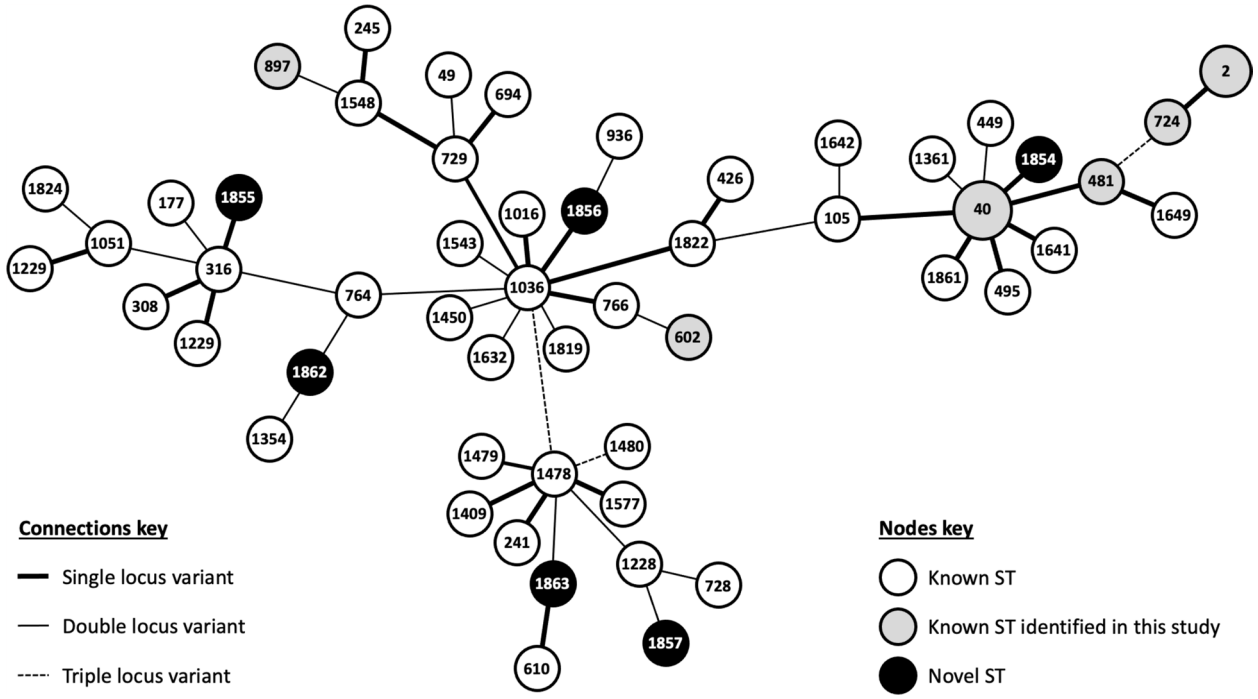

Fig. 1 Minimum spanning tree of MLST data generated by PHYLOViZ. Circles correspond to STs. The ST chosen for analysis shared at least 5 alleles with the novel STs detected in this study

cancer and cardiovascular diseases (Iwu and Okoh 2019). Therefore, the worldwide demand for fresh produce has been increasing in recent years. At the same time, foodborne illness and disease outbreaks associated with the consumption of fresh produce have also increased globally (Machado-Moreira et al. 2019). Contamination of fresh produce with different types of foodborne pathogens has been widely demonstrated. Although not regarded as a foodborne pathogen, *A. baumannii* have been isolated from a variety of foods, such as fish, dairy products, meat and fresh produce. Thus, food products contaminated with *A. baumannii* could be a potential source of infection for humans, especially if similar *A. baumannii* strains were isolated from food and clinical samples. Currently, there is still a large gap in our knowledge regarding the prevalence of *A. baumannii* in fresh produce, and whether the presence of *A. baumannii* in this type of food might lead to infections in humans. Therefore, the aim of this study was to investigate the prevalence of *A. baumannii* in fresh produce collected from local markets in Jordan, and to determine pathogen characteristics, including genetic diversity, antibiotic resistance phenotypes and biofilm formation capability.

Fresh produce is considered one of the major contributors to foodborne illnesses when compared to other dry and non-fresh food products. The Center for Disease Control and Prevention (CDC) reported that contaminated fresh produce is the cause of almost 46% of foodborne illnesses in the US through the period 1998 to 2008 (Karumathil et al. 2016). Fresh produce can be contaminated with *A. baumannii* from the soil in the field due to the use of natural fertilizers or contaminated irrigation water. Al Atrouni et al. (2016) isolated *A. baumannii* from soil samples near agricultural zones, which might have been irrigated with wastewater or reclaimed water (Al Atrouni et al. 2016). In addition, agriculture soil might be contaminated from other sources such as domestic or wild animal feces that graze in the same agricultural area. Indeed, *A. baumannii* was isolated from animal's fecal samples by several research groups (Beuchat 1996; Brandl 2006; Rafei et al. 2015; Al Atrouni et al. 2016; Carvalheira et al. 2017a). Additionally, fresh produce can be contaminated during the harvesting, handling, or transportation processes, or in retail markets (Brandl 2006; Carvalheira et al. 2017b).

In the present study, 7.3% of the 243 fresh produce samples examined were found to be contaminated with *A. baumannii*, such as cucumber, mint, arugula, red radish and peppers, which is a major food safety concern, since these vegetables are frequently used in preparing raw salads. Moreover, 4 of the 17 *A. baumannii* isolates were recovered from imported products. This finding requires further investigation to elucidate whether these strains originated from exporting countries or they are

local strains. Nowadays, international importing allows a continuous supply of fresh produce throughout the year, and it has been growing significantly due to globalization of trade and the increased demand (Carstens et al. 2019). At the same time, imported fresh produce has been implicated in several multi-national outbreaks and contributed to the introduction of new types of antimicrobial resistant determinants and pathogenic bacteria to the importing countries (Vital et al. 2017; Carstens et al. 2019). Therefore, the continuous surveillance of microbial contamination of imported and domestic produce could be proven critical to prevent and reduce the number of illnesses caused by these food products.

Ten out of the 16 vegetable types investigated were free of *A. baumannii,* such as lettuce and carrots, which is in agreement with the findings of a study by Karumathil et al. (2016). In this study, only one *A. baumannii* isolate was recovered from 100 lettuce samples and no isolates were found in the carrot samples investigated (Karumathil et al. 2016). A common practice among local retailers is to wash carrots and lettuce to remove soil traces, as well as to keep the fresh look on these vegetables. Also, retailers tend to remove the outer most leaves of lettuce to make them more appealing to consumers. These practices might explain why we were unable to isolate *A. baumannii* from the carrot and lettuce samples investigated in this study. *A. baumannii* was sporadically isolated from vegetables and fruits in a number of studies, in contrast to many other studies that reported the isolation of this opportunistic pathogen. For example, several studies reported the detection of *A. baumannii* in raw vegetable salads, vegetables and fruits (Berlau et al. 1999; Houang et al. 2001; Bezanson et al. 2008; Oie et al. 2008; Dahiru and Enabulele 2015; Karumathil et al. 2016; Carvalheira et al. 2017b). Furthermore, *A. baumannii* strains were rarely isolated from fruits, as only a few studies reported a low prevalence of this pathogen in fruits. In our study however, 8.75% of fruit samples harbored *A. baumannii*, with 7 isolates recovered from 5 types of fruits; apples, pears, grapes, guava and strawberries. Variations in the types of samples tested and the detection methods may explain the differences in the prevalence of *A. baumannii* between the current study and previous ones. The aforementioned types of fruits are hand-picked, and typically are not washed before being sold to consumer, thus cross-contamination from handlers at different stages of farming, packaging and retailing may have contributed to the presence of *A. baumannii* on such types of fruits.

Besides *A. baumannii*, 52 *A. pittii* isolates were recovered from vegetables and 2 isolates from fruits. Previous studies reported the isolation of this species from fresh produce (Berlau et al. 1999; Rafei et al. 2015; Carvalheira et al. 2017a). However, higher prevalence of *A. pittii* was observed in the fresh produce samples investigated in the current study. In addition, *A. pittii* was recovered from food sources other than fresh produce, such as meat, cheese and milk (Rafei et al. 2015; Al Atrouni et al. 2016; Carvalheira et al. 2017a; Cho et al. 2018). In recent years, multi-drug resistant *A. pittii* has become dominant in various countries, causing nosocomial infections at a high rate, especially in intensive care units (Pailhoriès et al. 2018). Therefore, the presence of *A. pittii* in fresh produce is alarming and may lead to the spread of this emerging pathogen into healthcare settings. Continuous monitoring with molecular epidemiological techniques is warranted to reduce the spread of the pathogen into the healthcare systems.

The majority of the recovered isolates were susceptible to clinically relevant antibiotics. However, 4 isolates from red radish, red apple, green grape, and guava displayed resistance to 16 antibiotics, including carbapenems. Many of these antibiotics are still among the drugs of choice to treat *A. baumannii* infections in humans. The introduction of these XDR isolates via the food chain is a public health concern because not only limits the therapeutic options available to treat infections caused by such strains, but it may contribute to transferring antibiotic resistance determinants to the gut microbiota in humans. Furthermore, the presence of antibiotic-resistant pathogens on fresh produce might contribute to the distribution of resistance between different strains, species and even genera. Horizonal gene transfer via mobile genetic elements such as plasmids, may enhance the rapid spread of antibiotic resistance determinants among pathogenic bacteria, and *A. baumannii* is no exception. Therefore, continuous monitoring of the presence of antibiotic-resistant bacteria on fresh produce is important for risk assessment and implementing food safety interventions. The other isolates that were sensitive to the drugs might have been originated from the soil and contaminated the product during harvesting or handling. Thus, they were not in contact with humans, and consequently not in contact with antibiotics.

Biofilms formed on the surface of fresh produce can cause serious risks for fresh product quality and public health, as bacterial biofilms may not be easily removed by simple washing with water (Bae et al. 2014). Also, certain types of biofilms are resistant to the cleaning and disinfection procedure commonly practiced in fresh produce retail markets and the food industry (Joseph et al. 2001; Lapidot et al. 2006). Many foodborne pathogenic bacteria can form biofilm, such as *Listeria monocytogenes, Staphylococcus* spp., *Clostridium* spp., *Salmonella enterica, Bacillus* spp., *Escherichia coli, Serratia* spp., *Campylobacter* spp. *and Pseudomonas* spp. (Bai et al. 2021). Furthermore, biofilm formation is considered an important

virulence factor in *A. baumannii*. To the extent of our knowledge, this is the first study to investigate the biofilm formation capacity of *A. baumannii* isolated from fresh produce. All isolates investigated in this study were able to form biofilms, with the majority classified as strong formers. This suggests that if these isolates spread into food preparation facilitates, they may form biofilms on surfaces within these facilities, and thus become a persistent source of contamination in the food chain.

To the best of our knowledge, this is the first study to determine the clonality of *A. baumannii* isolated from fresh produce. The two XDR isolates belonging to ST2 were recovered from two fruit samples (GP1 and AP8) collected from the same hypermarket. It is known that infections caused by carbapenem-resistant *A. baumannii* belonging to ST2 are widespread in many countries, Thus, the presence of ST2 strains in fruits or vegetables suggests a possible route of transmission that involves individuals infected with or carriers for this pathogen. Furthermore, a number of isolates investigated in this study belonged to ST40, ST2, and ST602. *A. baumannii* strains belonging to these STs were previously isolated in Jordan from clinical samples (Ababneh et al. 2021b) and intensive care unit environmental surfaces (Ababneh et al. 2021a). This suggests a possible transmission of these isolates from the hospital sewage to the vegetable and fruit samples through irrigation water, or by cross-contamination from handling personnel who might be infected or colonized with *A. baumannii*. Two isolates recovered from cucumber (CUC6) and apple (AP4) samples belonged to ST481 and ST897, respectively. We found two records in the *A. baumannii* Pubmlst database for two strains belonging to ST897; one of which has been isolated from animals. A strain with ST481 have been also isolated from an animal source (Pailhoriès et al. 2015). The isolation of ST481 and ST897 strains from animals might suggest a possible route of transmission of CUC6 and AP4 isolates to fresh produce through the use of animal manure as natural fertilizer, wildlife animals or water runoff containing animal feces.

Six isolates investigated in this study are novel strains, as they didn't belong to any previously known sequence types of *A. baumannii*. Five of these isolates were non-MDR and displayed strong ability to form biofilms in vitro, which suggest that these are environmental strains that have not been detected in clinical settings or exposed to antibiotics. New clones of *A. baumannii* are frequently introduced into the community and clinical settings. Due to the high plasticity of *A. baumannii* genome, these new clones can eventually develop or acquire antibiotic resistance if gained entry into clinical settings, which may represent an additional concern. The ubiquitous distribution of *A. baumannii* in nature may allow new strains to be introduced through many routes into the food processing environments with various fresh produce types or raw foodstuff. Furthermore, if these strains are able to form biofilms, they will become a recurrent source of contamination, resistant to disinfection and potential source of human infections. Therefore, uncovering the food reservoirs of *A. baumannii* and their transmission routes within the food chain is important for preventing the transmission of this pathogen.

Conclusions

To conclude, this study demonstrated that fresh produce constitutes a reservoir for *A. baumannii*, including strong biofilm formers and XDR strains. The presence of *A. baumannii* in fresh produce represents a significant concern to public health because vegetables and fruits may serve as a vehicle for *A. baumannii*, increasing their dissemination into the community and healthcare settings. Therefore, continuous monitoring and clonal typing of *A. baumannii* strains detected outside clinical settings may increase our understanding of the population evolution of this pathogen, and help predict new possible routes of entry into clinical settings. Unlike animal food products, fresh produce is generally consumed with no terminal microbial kill step, thus the potential risk for human exposure to fresh produce associated pathogens is greater. Fresh produce retailers, distributors and farmers must ensure that their products meet all food safety requirements to prevent *A. baumannii* and other pathogens from reaching consumers. On the other hand, consumer should also do their part in protecting themselves by ensuring that their fresh produce is washed and cooked thoroughly before eating.

Abbreviations

XDR: Extensively Drug-Resistant; non-MDR: Non-Multi-Drug Resistant; PCR: Polymerase Chain Reaction; BLAST: Basic Local Alignment Search Tool; CLSI: Clinical and Laboratory Standards Institute; OD: Optical Density; MLST: Multi-locus sequence typing; MIC: Minimal Inhibitory Concentrations; ITS: Internal Transcribed Spacer; ST: Sequence Type; SLV: Single Locus Variant; DLV: Double Locus Variant; DOR: Doripenem; IPM: Imipenem; MEM: Meropenem; CIP: Ciprofloxacin; LEV: Levofloxacin; CRO: Ceftriaxone; FEP: Cefepime; CAZ: Ceftazidime; AK: Amikacin; TOB: Tobramycin; CN: Gentamicin; SAM: Ampicillin-sulbactam; TE: Tetracycline; SXT: Trimethoprim-sulfamethoxazole; TZP: Piperacillin-tazobactam; PRL: Piperacillin; CT: Colistin; POL B: Polymyxin B; TGC: Tigecycline; S: Sensitive; R: Resistant; I: Intermediate; ND: Not Determined.

Authors' contributions

QA: Conceptualization; Funding acquisition; Supervision; Writing - review & editing. EA: Data curation; Formal analysis; Methodology; Writing - original draft. ZJ: Supervision, Formal analysis; Writing - review & editing. The author(s) read and approved the final manuscript.

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Declarations

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Not applicable.

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The authors of the confirm that the manuscript has been read and approve the final article and that there are no other persons who satisfied the criteria for authorship but are not listed.

Competing interests
None to declare.

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