Prevalence of β -Lactam Drug-Resistance Genes in *Escherichia coli* Contaminating Ready-to-Eat Lettuce

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Abstract

Thirty-four *Escherichia coli* isolates from 91 ready-to-eat lettuce packages, obtained from local supermarkets in Northern California, were genotyped by multilocus sequence typing, tested for susceptibility to antimicrobial agents, and screened for β -lactamase genes. We found 15 distinct sequence types (STs). Six of these genotypes (ST1198, ST2625, ST2432, ST2819, ST4600, and ST5143) have been reported as pathogens found in human samples. Twenty-six (76%) *E. coli* isolates were resistant to ampicillin, 17 (50%) to ampicillin/sulbactam, 8 (23%) to cefoxitin, and 7 (20%) to cefuroxime. *bla*_{CTX-M} was the most prevalent β -lactamase gene, identified in eight (23%) isolates. We identified a class A broad-spectrum β -lactamase SED-1 gene, *bla*_{SED}, reported by others in *Citrobacter sedlakii* isolated from bile of a patient. This study found that fresh lettuce carries β -lactam drug-resistant *E. coli*, which might serve as a reservoir for drug-resistance genes that could potentially be transmitted to pathogens that cause human infections.

Keywords: antimicrobial resistance, β -lactamase genes, *Escherichia coli*, ready-to-eat lettuce

Introduction

THE INCREASE AND spread of antimicrobial drug-resistant bacterial strains has become a problem worldwide, and food may contribute as an important source of antibiotic resistance genes (ARGs) found in the human intestine. In particular, fresh vegetables carry saprophytic bacteria that harbor drug-resistance genes, which can enter the human gut when it is eaten uncooked (Raphael *et al.*, 2011; Berman and Riley, 2013; Richter *et al.*, 2019). *Escherichia coli* have been regarded as an indicator of contamination and health risk in foods (Paruch and Mæhlum, 2012). Some groups of *E. coli* can cause gastrointestinal infections as well as extraintestinal infections. In the United States, multistate outbreaks of gastroenteric illness caused by lettuce contaminated with *E. coli* O157:H7 have been widely reported (Glowacki *et al.*, 2019). More recent studies have shown sequence types (STs) of extraintestinal pathogenic *E. coli* (ExPEC) shared by *E. coli* isolates from retail meat products and patients with community-acquired urinary tract infections (Yamaji *et al.*, 2018; Borges *et al.*, 2019).

In this study, we wished to discover if ExPEC and drug-resistant strains of ExPEC can be found on green leafy veg-

etables. Therefore, the purpose of this study was to determine the STs and ARG carriage of *E. coli* isolated from packaged ready-to-eat lettuce.

Materials and Methods

Ninety-one ready-to-eat lettuce packages were purchased from chain supermarkets located within a 11 kilometers vicinity of a university community in Northern California from October 2018 to July 2019. These lettuce products represented 12 brands and the main types included Boston (butterhead lettuce), iceberg (crisphead lettuce), and green/red leaf lettuce, which were distributed by four different California producers.

For each package, 25 g of lettuce were placed in a UV-pretreated polyethylene bag containing 50 mL of sterile phosphate-buffered saline (PBS; pH 7.4). After brief kneading by hand, the lettuce was incubated in PBS for 45 min at room temperature and washed three times. The combined rinsate (50 mL $\times$ 3) was then centrifuged at 12,000 g for 5 min at room temperature. The pellet was resuspended in 2 mL of PBS and preincubated in double strength MacConkey broth (Difco) at a 1:1 dilution at 35°C for 24 h. A separate 10 μ L

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aliquot was then spread-plated on MacConkey agar (Difco) to isolate Gram-negative bacteria. *E. coli* was presumptively identified by lactose fermentation and indole (Sigma-Aldrich) tests.

Single colonies from tryptic soy agar plates were selected and inoculated into 2 mL tryptic soy broth and incubated in a shaking incubator for 15 h at 37°C. The aliquots of grown cultures were used to isolate DNA as described previously (Yamaji *et al.*, 2018). The allelic number and the corresponding genotype number were designated by the curator of the multilocus sequence typing (MLST) based on the seven-gene scheme described at website https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search

All isolates were assessed for susceptibility to ampicillin (AMP), cefoxitin (FOX), cefotaxime (CTX), cefuroxime (CXM), ceftriaxone (CRO), ampicillin/sulbactam (SAM), ceftazidime (CAZ), fosfomycin (FOS), gentamicin (CN), nitrofurantoin (NIT), trimethoprim-sulfamethoxazole (TMP-

SMX), and nalidixic acid (NAL) by the standard disk diffusion assay, according to the Clinical and Laboratory Standards Institute-CLSI (CLSI, 2015). *E. coli* ATCC 25922 was used as reference strain in all tests.

The *E. coli* isolates were tested for the presence of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, and *bla*_{OXA} β -lactamase genes by polymerase chain reaction (Dallenne *et al.*, 2010). DNA sequences were visually inspected, aligned, and compared against sequences in GenBank with BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Statistical analysis was performed by Fisher's exact test (two-tailed) with significance set at $p \leq 0.05$.

Results and Discussion

Of the 91 lettuce packages, 47 (52%) contained organic and 44 (48%) contained conventionally grown (nonorganic) lettuce. Thirty-four *E. coli* isolates were recovered from 18

TABLE 1. SEQUENCE TYPES, ANTIMICROBIAL RESISTANCE PROFILES, AND β -LACTAMASE (*BLA*) GENES OF THE 34 *ESCHERICHIA COLI* ISOLATED FROM READY-TO-EAT LETTUCE^a

Strain ^b	ST	Antimicrobial resistance profile	β -lactamase gene ^c
Organic lettuce			
1	8951	AMP+	Other
2A	UNK	AMP+SAM+NA+	Other
2B	8951	Pan-susceptible ^d	None
3	2625	AMP+	Other
4	5154	AMP+FOS+	Other
5A	8951	AMP+SAM+	<i>bla</i> _{SHV} type
5B	UNK	AMP+SAM+	Other
5C	9285	AMP+SAM+	<i>bla</i> _{TEM} type
6	5154	AMP+FOX+CXM+SAM+	<i>bla</i> _{TEM} type+ <i>bla</i> _{SHV} type
7A	UNK	AMP+SAM+	<i>bla</i> _{TEM} type+ <i>bla</i> _{SHV} type
7B	UNK	AMP+SAM+	Other
8	6374	Pan-susceptible	None
9	4600	AMP+FOX+CXM+	<i>bla</i> _{CTX-M} type
10	8951	AMP+SAM+	Other
11	UNK	AMP+FOX+CXM+SAM+	<i>bla</i> _{CTX-M} type
12A	6374	AMP+	Other
12B	3222	AMP+CXM+SAM+	Other
13A	1198	AMP+SAM+	Other
13B	2432	AMP+FOX+CXM+SAM+	<i>bla</i> _{SHV} type
14A	UNK	AMP+SAM+	Other
14B	4277	AMP+SAM+	<i>bla</i> _{TEM} type+ <i>bla</i> _{SHV} type
15A	2819	AMP+CXM+	<i>bla</i> _{CTX-M} type
15B	2819	AMP+CXM+	<i>bla</i> _{CTX-M} type
16	2432	AMP+FOX+	<i>bla</i> _{SHV} type+ <i>bla</i> _{CTX-M} type
17	UNK	AMP+FOX+	<i>bla</i> _{CTX-M} type
18	UNK	AMP+SAM+	<i>bla</i> _{TEM} type
Nonorganic lettuce			
19	5351	Pan-susceptible	None
20	3567	Pan-susceptible	None
21	6376	AMP+SAM+	Other
22	UNK	Pan-susceptible	None
23	8951	Pan-susceptible	None
24A	UNK	AMP+SAM+	Other
24B	5143	FOX+	<i>bla</i> _{CTX-M} type
25	5143	FOX+	<i>bla</i> _{CTX-M} type

^aIsolates with intermediate susceptibility were classified as nonsusceptible.

^bDifferent strains isolated from the same package have the same number followed by a different letter.

^cOther: ampicillin-resistant isolates that did not have any *bla*_{TEM}-type, *bla*_{CTX-M} type, *bla*_{OXA}-type and *bla*_{SHV}-type.

^dPan-susceptible is defined as susceptible to the 12 antibiotics tested this study.

AMP, ampicillin; CXM, cefuroxime; FOS, fosfomycin; FOX, cefoxitin; NAL, nalidixic acid; SAM, ampicillin/sulbactam; ST, sequence type; UNK, unknown.

(38%) organic and 7 (16%) conventionally grown lettuce ($p=0.01$). The isolates were classified into 15 distinct STs. Ten isolates could not be assigned to a known ST (Table 1). The most common genotypes were ST8951 (16%) and ST5154 (12%). ST8951 has been previously identified in deciduous forest soil and turkey, including ground meat as well as clinical samples (<https://enterobase.warwick.ac.uk/species/index/ecoli>). The type of clinical sample is not described in the database. ST5154 has been reported from cattle, but not from any human infections (Ribeiro *et al.*, 2016; <https://enterobase.warwick.ac.uk/species/index/ecoli>). Other genotypes, including ST1198, ST2625, ST2432, ST2819, ST4600, and ST5143, have been described as pathogens in human samples from Japan (Fukuoka), USA (Nashville and Tennessee), or Canada, China (Hangzhou and Shanghai), and Korea, as noted in the MLST database and previous studies (<https://enterobase.warwick.ac.uk/species/index/ecoli>).

ST1198 has been implicated in urinary tract infection and is a member of clonal complex 131 (Lee *et al.*, 2010), which is one of the pandemic lineages of ExPEC (Riley, 2014). Foster *et al.* (2015) reported ST4600 as an enteropathogenic *E. coli* strain, isolated from a patient with diarrhea. The clinical sources of the other genotypes are not described.

Among 34 *E. coli* isolates, 26 (76%) were resistant to AMP, 17 (50%) to SAM, 8 (23%) to FOX, 7 (20%) to CXM, 1 (3%) to FOS, and 1 (3%) to NAL. Of the β -lactamase genes examined, *bla*_{CTX-M} was the most prevalent, found in 8 (23%) isolates, followed by *bla*_{SHV}, detected in 5 (15%) isolates, and *bla*_{TEM} detected in 4 (12%) isolates. The high frequency of *E. coli* strains carrying *bla*_{CTX-M} is surprising, considering that the most common β -lactamase genes found in clinical strains of *E. coli* is *bla*_{TEM} and/or *bla*_{SHV} (Yamaji *et al.*, 2018; Borges *et al.*, 2019; Tarlton *et al.*, 2019). The high frequency of CTX-M β -lactamase genes in ready-to-eat lettuce poses a public health concern, since it is the most common extended-spectrum β -lactamases (ESBL) expressed by clinical strains of *E. coli*, which is becoming increasingly prevalent in extraintestinal infections worldwide (Yamaji *et al.*, 2018; Borges *et al.*, 2019; Richter *et al.*, 2019; Tarlton *et al.*, 2019).

To our surprise, in one strain (ST3222), we found a class A broad-spectrum β -lactamase SED-1 gene, *bla*_{SED}, reported by others to have been found in *Citrobacter sedlakii* in the bile fluid of a patient (Petrella *et al.*, 2001). It suggests that some of these β -lactamase genes are horizontally transferred among saprophytic Gram-negative bacteria found in the environment, and that commensal *E. coli* from mammalian intestine contaminating lettuce could acquire them. Such genes can then enter the human intestine through ingestion of contaminated food product eaten uncooked.

Conclusions

We found that retail packaged lettuce purchased in Northern California frequently contains *E. coli* strains belonging to recognized ExPEC group, and that many of them carry ESBL genes. The magnitude of lettuce contributing to the spread of ExPECs and resistance genes into the human intestine may be underestimated.

Disclosure Statement

No competing financial interests exist.

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