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Review

# Vegetables and Fruit as a Reservoir of $\beta$ -Lactam and Colistin-Resistant Gram-Negative Bacteria: A Review

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**Abstract:** Antibacterial resistance is one of the 2019 World Health Organization's top ten threats to public health worldwide. Hence, the emergence of  $\beta$ -lactam and colistin resistance among Gram-negative bacteria has become a serious concern. The reservoirs for such bacteria are increasing not only in hospital settings but in several other sources, including vegetables and fruit. In recent years, fresh produce gained important attention due to its consumption in healthy diets combined with a low energy density. However, since fresh produce is often consumed raw, it may also be a source of foodborne disease and a reservoir for antibiotic resistant Gram-negative bacteria including those producing extended-spectrum  $\beta$ -lactamase, cephalosporinase and carbapenemase enzymes, as well as those harboring the plasmid-mediated colistin resistance (*mcr*) gene. This review aims to provide an overview of the currently available scientific literature on the presence of extended-spectrum  $\beta$ -lactamases, cephalosporinase, carbapenemase and *mcr* genes in Gram-negative bacteria in vegetables and fruit with a focus on the possible contamination pathways in fresh produce.

**Keywords:**  $\beta$ -lactamases; mobile colistin resistance; Gram-negative bacteria; vegetables; fruit



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## 1. Introduction

Fresh produce is considered a good source of minerals, vitamins, phytonutrients and dietary fiber. Accordingly, there is a consensus that a diet rich in vegetables and fruit may decrease the risk of heart diseases and protect against some types of cancer [1]. In 2003, the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) started an initiative worldwide to promote fruit and vegetable intake for health, with a recommended minimum consumption of 400 g of vegetables and fruit per day [2]. Following this recommendation, the intake of fresh produce as ingredients in healthy diets has been increasing and has gained popularity globally [3]. Consequently, the consumption of contaminated fresh produce, such as vegetables and fruit eaten raw, has been associated with an increasing number of outbreaks of foodborne disease [1]. In addition, fresh produce represents a route of human exposure to antibiotic-resistant bacteria and has often served as a reservoir of antibiotic resistance genes, representing a major public health threat [3,4].

In this context, one of the main public health preoccupations worldwide is the emergence of Gram-negative bacteria displaying resistance to oxyimino-cephalosporins (3GCs), carbapenem and colistin [3].  $\beta$ -lactams, essentially extended-spectrum cephalosporins and

carbapenems, are the main therapeutic choices to treat infections caused by resistant Gram-negative bacteria [5–7]. However, resistance to these antibiotic drugs has been increasing in recent years mostly through  $\beta$ -lactamase production. Various  $\beta$ -lactamases have been identified worldwide, including penicillinases, extended-spectrum  $\beta$ -lactamases (ESBLs), cephalosporinases (AmpC), and carbapenemases [7]. Given these circumstances, the approved alternative is colistin, but its re-use in clinical practice has led to the appearance of colistin-resistant bacteria, particularly through horizontal transfer (*mcr*) [8].

The transfer of these multidrug-resistant Gram-negative bacteria to fresh produce may occur during production via animal manure, through the use of contaminated irrigation water, or be linked to humans during the post-harvest stage, as well as during transport, conservation and processing by handlers [9]. The ingestion of antibiotic-resistant bacteria poses a potential public health concern since they are able to colonize the gut and exchange resistance genes with intestinal bacteria during their passage through the intestines which facilitates their further dissemination in the environment [3]. Extended-spectrum  $\beta$ -lactamase, cephalosporinase and carbapenemase producers as well as *mcr* gene-producing Gram-negative bacteria isolated from fresh vegetables and fruit have been reported in several countries around the world [3,4,10,11].

Thus, the aim of this review is to highlight the current situation of the worldwide dissemination of ESBL, cephalosporinase, carbapenemase and *mcr* gene-producing Gram-negative bacteria from fresh vegetables and fruit, their genetic characteristics, and possible contamination pathways.

## 2. $\beta$ -Lactam Resistance and Gram-Negative Bacteria

$\beta$ -lactam resistance in Gram-negative bacteria can be attributed to two main mechanisms, these include the acquisition of  $\beta$ -lactamase genes, as well as the modification of the target (penicillin-binding proteins) [12].  $\beta$ -lactamase enzymes have played an important clinical role and have served as the principal resistance mechanism detected for  $\beta$ -lactam drugs [13,14].

The first enzyme detected presenting  $\beta$ -lactamase activity originated from *Bacillus coli* in 1940, currently supposed to be the class C, AmpC chromosomal cephalosporinase from *Escherichia coli* [14]. Given this, various extended-spectrum cephalosporins were introduced in the 1980s, which were stable against penicillinase hydrolysis, such as TEM-1 (TEMoniera) and SHV-1 (sulfhydryl variable). A few years later, Enterobacteriaceae species developed several derivatives of TEM-1, TEM-2 and SHV-1; these variants extended their hydrolysis spectrum to include oxyimino-cephalosporins, hence the term ‘extended-spectrum’  $\beta$ -lactamases (ESBL) [14,15]. Afterwards, a novel variant of the ESBL family named Cefotaximase-Munchen (CTX-M) was described, which became the predominant ESBL in enterobacterial species worldwide [14], as well as the family of Guyana extended-spectrum  $\beta$ -lactamases (GES) reported as ESBL variants in 2000 [16]. The  $\beta$ -lactamases belonging to Ambler class C, called cephalosporinases, are derived from the *ampC* gene in the chromosome of various Enterobacteriaceae species [17,18]. In the early 1990s, plasmid-encoded AmpC cephalosporinases were described in species lacking an inducible AmpC enzyme. Afterwards, plasmid-mediated AmpC, such as Dharhan hospital (DHA), cephamycinase (CMY), cefoxitinase (FOX), moxalactamase (MOX) and Ambler Class C (ACC), were reported worldwide [19].

In this worrisome situation, carbapenems were introduced to clinics in the late 1980s and showed significant activity in the treatment of infections caused by AmpC and ESBL-producing Gram-negative bacteria [16,20]. The first carbapenemase reported in Enterobacteriaceae was the *Serratia marcescens* enzyme (SME-1) in London in 1982. Since then, various carbapenemase enzymes belonging to the Ambler class A  $\beta$ -lactamases have been reported, including imipenemase (IMI-1) and non-metallo-carbapenemase class A (NmcA); however, the *K. pneumoniae*-carbapenemase (KPC) type was the most commonly found [5,16,21]. On the other hand, the first MBL variant was discovered in *Bacillus cereus* in 1966 and was called the BCII enzyme. Until 1989, only four MBL enzymes had been identified

and were all chromosomally encoded, therefore they were deemed clinically negligible. Afterwards, various plasmid-encoding class B carbapenemases were described, such as Imipenem-resistant *Pseudomonas*-type carbapenemases (IMP), Verona integron-encoded MBL (VIM), and recently, New Delhi MBL (NDM) [22,23]. In class D  $\beta$ -lactamases, several variants with relatively weak carbapenemase activity have also been reported as carbapenemase enzymes, including OXA-48, OXA-58, OXA-24/40 and OXA-23 [22]. In Enterobacteriaceae, class D carbapenemases are mainly represented by the OXA-48-like enzymes [24].

### 3. Colistin Resistance in Gram-Negative Bacteria

Colistin is a cationic polypeptide antibiotic belonging to the polymyxin family [25]. It was described initially in 1947 in *Paenibacillus polymyxa*, and it is commonly used in human and veterinary medicines, plant cultivation and animal husbandry [25–27]. Although in the 1970s its use was discontinued due to its neuro- and renal toxicity, it was reintroduced in the mid-2000s as a last line therapeutic option for the treatment of extensively drug-resistant (XDR) Gram-negative infections, such as those caused by carbapenem-resistant GNB [26,28].

The initial target site of colistin is lipopolysaccharide (LPS), more exactly lipid A, located in the outer membrane, which plays a major role in cell permeability. The electrostatic interaction between the cationic region of colistin, which is from the diamino-butyric acid (Dab) residues, and the negatively charged phosphate groups of lipid A, replace the magnesium and calcium ions previously united with the phosphate group. This destabilizes the lipid A and increases the permeability of the outer membrane, leading to the entry of colistin by a self-promoted uptake mechanism and eventual bacterial death [26,29]. Another antibacterial mechanism is the inhibition of a crucial respiratory enzyme, the type II NADH-quinone oxidoreductase (NDH-2) in the bacterial cell membrane [29]. The increased use of colistin has led to the emergence of colistin-resistant strains worldwide [25]. Colistin resistance is mainly achieved by modification of LPS, and consequently the reduced or absent affinity for colistin. This mechanism, although universal in Gram-negative bacteria, may differ between species. The lipid A of LPS undergoes changes, essentially due to the addition of positively charged residues such as phosphoethanolamine (PEtn) and/or 4-amino-4-deoxy-L-arabinose (L-Ara4N). These molecules decrease the overall negative charge of LPS, leading to a smaller electrostatic interaction with the positive charges of colistin that inhibits cell lysis [26,30].

Previously, the genes responsible for most of these additions were thought to be due to chromosomal mutations in genes of a two-component regulatory system, such as *pmrAB*, *PhoPQ*, and *mgrB*, which are not transferable [30]. In late 2015, Liu et al. described the mobilized colistin resistance (*mcr-1*) gene in an *E. coli* isolate recovered from livestock in China [8,31]. MCR-1 confers resistance by modifying the colistin target through the action of phosphoethanolamine transferase, which ensures the transfer of phosphoethanolamine (PEA) onto the glucosamine saccharide of lipid A, contributing as in chromosomal resistance to reduce the net negative charge of lipid A and consequently, colistin binding [32]. After the discovery of the *mcr-1* gene, nine other *mcr* gene types (*mcr-2* to *mcr-10*) were identified. The second mobile colistin resistance gene, *mcr-2*, was found initially in *E. coli* strains isolated from pigs and calves in Belgium [33]. The gene *mcr-3* was identified in *E. coli* from pigs in China [34], and *mcr-4* was reported in *Salmonella enterica* serovar *Typhimurium* strains isolated from pigs in Italy [35]. In 2017, a novel transposon-associated phosphoethanolamine transferase gene (*mcr-5*) was described in d-tartrate-fermenting *Salmonella enterica* subsp. *enterica* serovar *paratyphi B* in Germany [36]. In 2018, further variants were described; *mcr-6* was identified in *Moraxella* spp. isolated from pigs in Great Britain [37], while *mcr-7* and *mcr-8* were described in *K. pneumoniae* strains isolated from animals (chickens and pigs) in China [38,39]. In 2019, a novel variant *mcr-9* was reported in a *Salmonella enterica* serovar *Typhimurium* strain isolated from a human in

Washington State in 2010 [40] and more recently, Wang et al. reported the detection of an *mcr-10* variant in an *Enterobacter roggenkampii* clinical strain in China [41].

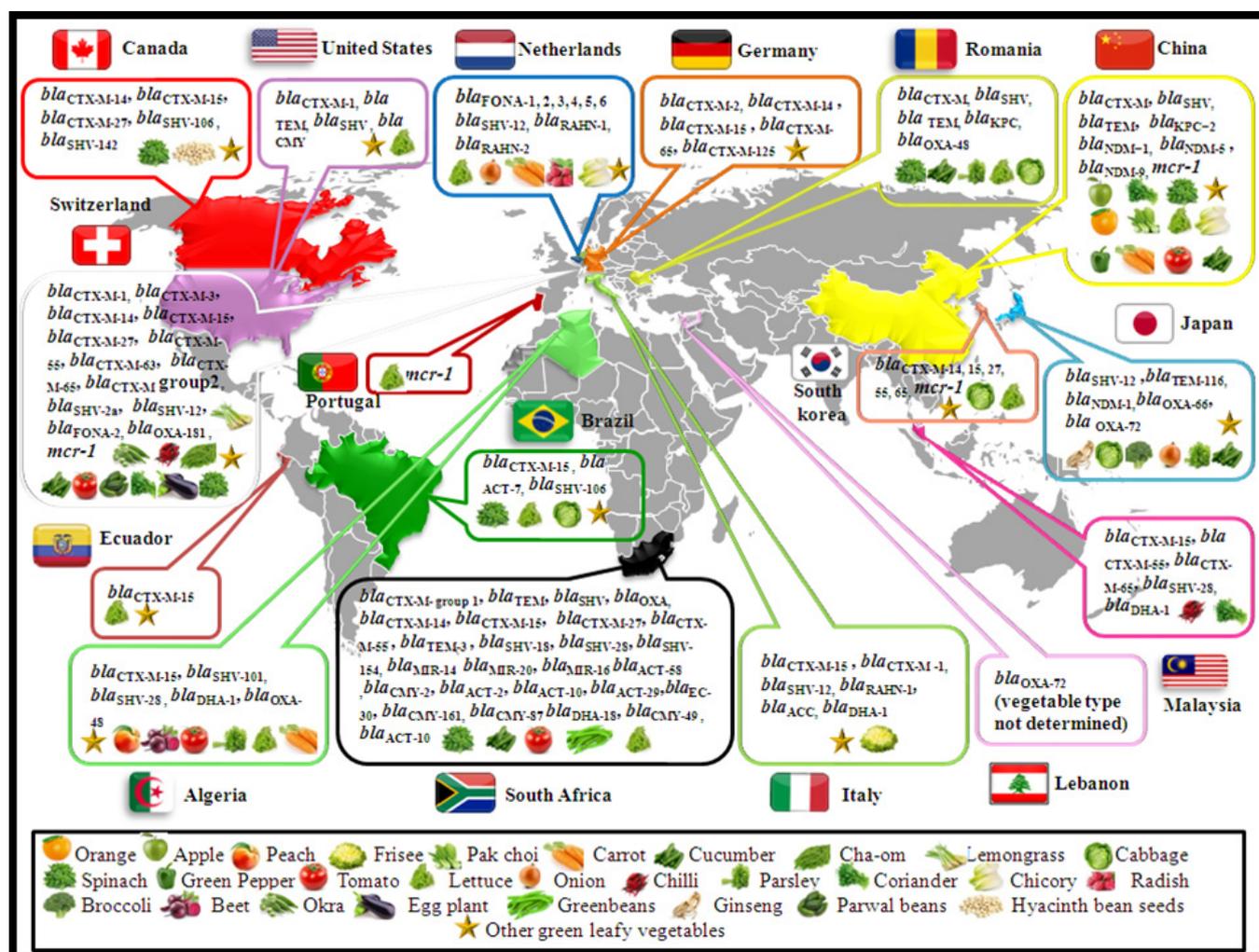
#### 4. Literature Search Strategy and Data Collection

The dissemination of extended-spectrum  $\beta$ -lactamase, cephalosporinase, carbapenemase and MCR-producing Gram-negative bacteria in fresh produce is a major public health threat, since they are a very suitable pathway for the spread of antibiotic-resistant bacteria from farm to fork. Until June 2021, thirty-three molecular studies have revealed the isolation of Gram-negative bacteria producing  $\beta$ -lactamase and *mcr* genes on fresh vegetables and fruit. They have been used and are accessible through the PubMed database using the following keywords: “ESBL”, “AmpC”, “KPC”, “VIM”, “NDM”, “IMP”, “OXA-48”, “mcr”, “carbapenem resistance”, “fresh vegetables”, “vegetables” and “fruit”.

#### 5. Vegetable and Fruit Isolates with ESBL and Cephalosporinase Genes

A total of nineteen molecular studies reporting the isolation of ESBL-producing Gram-negative bacteria and AmpC genes from vegetables and fruit have been described (Figure 1, Table 1). The first report of ESBL-producing GNB isolates from vegetables and fruit was reported in 2014 in The Netherlands. These bacteria were reported on six vegetable types that are consumed raw (bunched carrots, blanched celery, endive, chicory, iceberg lettuce and radish), and from iceberg lettuce farms. In that study, the *bla*<sub>FONA-5</sub> gene was detected among *Serratia fonticola* isolates on iceberg lettuce from a farm. In addition, 35 *Rahnella aquatilis* strains harboring the *bla*<sub>RAHN</sub> gene were identified. Of the 35 isolates, 34 strains were producing the *bla*<sub>RAHN-1</sub>, and only one *R. aquatilis* strain carried the *bla*<sub>RAHN-2</sub> gene [42]. After this publication, this level of resistance has been reported in Europe, Africa, Asia and America. Like isolates from humans, animals and the environment, the CTX-M family is the most prevalent type of ESBL-producing Enterobacteriaceae found in vegetables. Similarly, in an Italian study carried out on fresh vegetables, the authors refer to the detection of different ESBL enzymes, including CTX-M-15, CTX-M-1, SHV-12 and RAHN-1 in twenty isolates (the *bla*<sub>CTX-M-15</sub> gene in *C. freundii*, *E. coli* and *Pantoea agglomerans*, the *bla*<sub>CTX-M-1</sub> gene in *Enterobacter cloacae*, the *bla*<sub>SHV-12</sub> in *E. coli* and *bla*<sub>RAHN-1</sub> in *R. aquatilis*). Whereas only four isolates displayed AmpC production, among the four strains obtained, two *Hafnia alvei* isolates carried a *bla*<sub>ACC</sub> gene and two *E. cloacae* harbored a *bla*<sub>DHA-1</sub> gene [43]. A study in The Netherlands investigated the prevalence of third-generation cephalosporin (3GC) resistant Gram-negative bacteria on fresh vegetables. A total of 27 *Serratia* spp. isolates with an ESBL phenotype harboring a *bla*<sub>FONA</sub> variant were obtained, including *bla*<sub>FONA-1</sub> (18.5%), *bla*<sub>FONA-2</sub> (37.0%), *bla*<sub>FONA-3</sub> (7.4%), *bla*<sub>FONA-4</sub> (7.4%), *bla*<sub>FONA-5</sub> (18.5%) and *bla*<sub>FONA-6</sub> (11.1%). The *bla*<sub>SHV-12</sub> gene was detected in one *E. coli* and two *Enterobacter* spp. strains; however, one *R. aquatilis* strain harbored the *bla*<sub>RAHN-1</sub> gene [3]. In Switzerland, two studies reported the detection of *bla*<sub>ESBL</sub> genes on vegetable samples. In the first study, the authors evaluated the presence of ESBL-producing Enterobacteriaceae in 68 vegetables imported from the Dominican Republic, India, Thailand and Vietnam via the national airport in Zürich, and 101 samples were purchased in the city of Zürich. In total, 60 ESBL producers were retrieved, including *bla*<sub>CTX-M</sub> and *bla*<sub>SHV</sub>-producing *E. coli* (*bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-55</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-65</sub>, *bla*<sub>CTX-M-1</sub> and *bla*<sub>SHV-12</sub>) and *K. pneumoniae* strains (*bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-3</sub>, *bla*<sub>CTX-M-27</sub>, *bla*<sub>CTX-M-63</sub>, *bla*<sub>SHV-2</sub>, *bla*<sub>SHV-2a</sub>, and *bla*<sub>SHV-12</sub>). Moreover, *bla*<sub>CTX-M-15</sub> and *bla*<sub>SHV-2</sub> genes were identified in *E. cloacae*, *E. aerogenes* and *C. sakazakii*, respectively [10]. The second study reported the detection of CTX-M group 2, CTX-M-15 and FONA-2 in *Kluyvera ascorbata*, *E. cloacae* and *S. fonticola* isolates from diced tomato, chopped chives and spinach, respectively [44]. A study from Germany described the isolation of seven ESBL-producing *E. coli* isolates collected by food safety inspectors during 2011–2013 from markets, producers and supermarkets. Of the seven isolates, two strains were positive for *bla*<sub>CTX-M-14</sub> and two other isolates harbored *bla*<sub>CTX-M-15</sub> genes. However, three remaining strains were positive for *bla*<sub>CTX-M-65</sub>, *bla*<sub>CTX-M-125</sub> and *bla*<sub>CTX-M-2</sub> genes, respectively [45]. In addition, the *bla*<sub>TEM</sub>,

*bla<sub>SHV</sub>*, *bla<sub>CTX-M</sub>* and *bla<sub>DHA</sub>* genes were also reported in Romania in different Enterobacteriaceae species (*S. marcescens*, *E. cloacae*, *E. coli*, *Klebsiella oxytoca* and *Proteus vulgaris*) [46].



**Figure 1.** Worldwide distribution of extended-spectrum β-lactamase, cephalosporinase, carbapenemase and mcr-producing GNB on fresh vegetables and fruit.

In Africa, the first recorded ESBL and/or cephalosporinase-positive GNB was observed in 2019 in South Africa. In this report, 545 vegetable samples including spinach, cucumbers, tomatoes, green beans and lettuce, were collected from street-trading green-grocers, mobile trolley vendors, formal retailers and vendors at two farmers markets from September 2017 to May 2018. ESBL genes were detected in 39 strains, while AmpC production was observed in 20 strains belonging to 10 genera of Enterobacteriaceae including *S. fonticola*, *Serratia marcescens*, *E. coli*, *E. cloacae*, *Enterobacter asburiae*, *Enterobacter cowanii*, *Enterobacter ludwigii*, *R. aquatilis*, *K. pneumoniae*, *Klebsiella oxytoca*, *Citrobacter freundii*, *Proteus mirabilis* and *Proteus penneri*. Different *bla<sub>CTX-M</sub>* genes were obtained, including *bla<sub>CTX-M-14</sub>* ( $n = 15$ ), *bla<sub>CTX-M-15</sub>* ( $n = 6$ ), *bla<sub>CTX-M-27</sub>* ( $n = 4$ ) and *bla<sub>CTX-M-55</sub>* ( $n = 3$ ). In addition, the *bla<sub>TEM-3</sub>* gene ( $n = 3$ ), as well as *bla<sub>SHV</sub>* genes encoding *bla<sub>SHV-18</sub>* ( $n = 6$ ), *bla<sub>SHV-28</sub>* ( $n = 1$ ), and *bla<sub>SHV-154</sub>* ( $n = 1$ ) were detected. Three isolates carried more than one ESBL gene; two strains (*E. cowanii* and *E. coli*) harbored the *bla<sub>TEM-3</sub>* gene in association with *bla<sub>SHV-18</sub>* and *bla<sub>CTX-M-14</sub>* genes, respectively, while one *E. coli* isolate carried *bla<sub>CTX-M-14</sub>*, *bla<sub>SHV-18</sub>* and *bla<sub>TEM-3</sub>* genes. AmpC genetic determinants were observed in 18 of 58 (31%) isolates, 17 strains carried only one pAmpC gene, including *bla<sub>MIR-20</sub>* ( $n = 4$ ), *bla<sub>MIR-16</sub>* ( $n = 3$ ), and *bla<sub>ACT-58</sub>* ( $n = 2$ ), and one isolate each harbored *bla<sub>MIR-14</sub>*, *bla<sub>CMY-2</sub>*, *bla<sub>ACT-2</sub>*,

*bla*<sub>ACT-10</sub>, *bla*<sub>ACT-29</sub>, *bla*<sub>EC-30</sub>, *bla*<sub>CMY-161</sub> or *bla*<sub>CMY-87</sub>, respectively. As well, one *P. penneri* isolate harbored three AmpC genetic determinants (*bla*<sub>DHA-18</sub>, *bla*<sub>CMY-49</sub> and *bla*<sub>ACT-10</sub>). Among these 17 isolates, five strains (*Enterobacter* spp. (*n* = 2), *R. aquatilis* (*n* = 1), *E. coli* (*n* = 1) and *S. fonticola* (*n* = 1)) also carried ESBL genes [9]. Another report from South Africa described the detection of twenty enterobacterial isolates, identified as *E. asburiae*, *E. coli*, *K. pneumoniae*, *R. aquatilis* and *S. fonticola*, harboring different ESBL and AmpC genes, including *bla*<sub>CTX-M-group1</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub> and *bla*<sub>CIT</sub> genes [47]. In Algeria, Mesbah Zekar et al. reported the identification of multi-drug resistant *K. pneumoniae* isolates in fresh fruit and vegetables purchased in Bejaia city. In this study, eleven *K. pneumoniae* isolates harbored multiple ESBL genes, and *bla*<sub>CTX-M-15</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>SHV-101</sub> and *bla*<sub>SHV-28</sub> were described. In addition, two *K. pneumoniae* strains coharbored *bla*<sub>DHA-1</sub> with ESBL genes [48].

In Asia, Usui et al. analyzed 130 samples of fresh vegetables collected from seven supermarkets in Japan, 10 out of the 130 samples contained ESBL-producing *Pseudomonas* spp. including; *P. humanensis*, *P. putida*, *P. parafulva*, *P. beteli*, *P. mosselii*, *P. paralactis* and *P. arsenicoxydans*. These isolates harbored the *bla*<sub>SHV-12</sub> or *bla*<sub>TEM-116</sub> ESBL gene [49]. In China, a nationwide survey investigated the prevalence of ESBL-producing Enterobacteriaceae from retail food, where four isolates were obtained. Three were identified as *E. coli* and one as *C. freundii* isolated from retail vegetables, including tomatoes, cucumber and coriander. The *C. freundii* isolate carried *bla*<sub>CTX-M</sub> and *bla*<sub>OXA</sub> genes, while two *E. coli* isolates harbored *bla*<sub>CTX-M</sub> and *bla*<sub>SHV</sub> genes and one other *E. coli* strain carried *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub> and *bla*<sub>TEM</sub> genes [7]. In Malaysia, ESBL or AmpC genes were detected in two *E. coli* (*bla*<sub>CTX-M-55</sub> and *bla*<sub>CTX-M-65</sub>) and two *K. pneumoniae* isolates (*bla*<sub>CTX-M-15</sub>, *bla*<sub>SHV-28</sub> and *bla*<sub>DHA-1</sub>) from coriander and chili pepper respectively [50]. In addition, different CTX-M variants were described from *E. coli* isolates in South Korea including CTX-M-14, CTX-M-15, CTX-M-27, CTX-M-55 and the CTX-M-65 variant [51].

On the American continent, different Enterobacteriaceae isolates harbored *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub> and *bla*<sub>CTX-M-1</sub> as well as *bla*<sub>CTX-M</sub> and *bla*<sub>CMY</sub> genes and were detected from iceberg lettuce and leafy greens, respectively in the United States [52,53]. Moreover, seven *E. coli* isolates carrying the *bla*<sub>CTX-M-15</sub> gene were reported from leaf lettuce, alfalfa and parsley/cilantro in Ecuador [54], while *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-27</sub>, *bla*<sub>SHV-106</sub> and *bla*<sub>SHV-142</sub>-positive Enterobacteriaceae were reported in Canada from imported vegetable samples [55], and the *bla*<sub>CTX-M-15</sub> gene in Brazil [56].

**Table 1.** ESBL and cephalosporinase genes reported in Gram-negative bacteria isolates from vegetables and fruit worldwide.

Vegetable Type	ESBL/AmpC Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	References
Lettuce	<i>bla</i> <sub>FONA-5</sub>	2011	<i>Serratia fonticola</i>	1	The Netherlands	ND	ND	[42]
	<i>bla</i> <sub>RAHN-2</sub>		<i>Rahnella aquatilis</i>	1				
	<i>bla</i> <sub>CTX-M-15</sub>	2013–2014	<i>Klebsiella pneumoniae</i>	1	Algeria	aph(3')-Ia, aadA2, strB, strA, qnrS1, oqxB, oqxA, fosA, mph(A), catA2, sul1, sul2, tet(A), dfrA12	ST219	[48]
	<i>bla</i> <sub>DHA-1</sub> <i>bla</i> <sub>SHV-101</sub>		<i>K. pneumoniae</i>	1		<i>bla</i> <sub>OXA-1</sub> , aac(6')Ib-cr, aph(3')-Ia, aac(6')Ib-cr, qnrB4, oqxB, oqxA, fosA, mph(A) catB3, ARR-3, sul1	ST882	
	<i>bla</i> <sub>SHV-28</sub> , <i>bla</i> <sub>CTX-M-15</sub>		<i>K. pneumoniae</i>	1		<i>bla</i> <sub>OXA-1</sub> , aac(6')Ib-cr, aac(3)-Iia, aac(6')Ib-cr, qnrB66, oqxB, oqxA, fosA, catB3, dfrA14.	ST14	
	<i>bla</i> <sub>CTX-M-15</sub>	2015	<i>Escherichia coli</i>	1	Ecuador	dfrA1, aadA5	ST44	[54]
	<i>bla</i> <sub>CTX-M-15</sub>			1		None	ST44	
	<i>bla</i> <sub>CTX-M-14</sub>	2017–2018	<i>S. fonticola</i>	1	South Africa	ND	ND	[9]
	<i>bla</i> <sub>SHV-154</sub>		<i>S. marcescens</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-15</sub>	2018	<i>E. coli</i>	1	South Korea	ND	ST2509	[51]

Table 1. Cont.

Vegetable Type	ESBL/AmpC Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	References
	<i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>TEM</sub>	2019	<i>Proteus vulgaris</i>	1	Romania	ND	ND	[46]
	<i>bla</i> <sub>CTX-M-15</sub>	ND	<i>K. pneumoniae</i>	1	Brazil	<i>bla</i> <sub>OXA-1</sub> , <i>bla</i> <sub>SHV-110</sub> , <i>aac</i> (3)IIa, <i>aac</i> (6′)-Ib-cr, <i>opxB</i> , <i>drfA14</i> , <i>catA1</i> , <i>tet</i> (A), <i>fosA</i> , <i>opxB</i>	ST198	[56]
Butterhead lettuce	<i>bla</i> <sub>FONA-1</sub> (1–6)	2012–2013	<i>S. fonticola</i>	ND	The Netherlands	ND	ND	[3]
	<i>bla</i> <sub>RAHN-1</sub>	2011	<i>R. aquatilis</i>	ND	The Netherlands	ND	ND	[42]
Iceberg lettuce	<i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>TEM</sub>	2011–2012	<i>K. pneumoniae</i>	2	United States	ND	ND	[52]
	<i>bla</i> <sub>CTX-M-1</sub>		<i>S. marcescens</i>	1		ND	ND	
	<i>bla</i> <sub>FONA-1</sub> (1–6)	2012–2013	<i>S. fonticola</i>	ND	The Netherlands	ND	ND	[3]
	<i>bla</i> <sub>CTX-M</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>TEM</sub>	2011–2014	<i>E. coli</i>	1	China	ND	ND	[7]
	<i>bla</i> <sub>SHV-28</sub> , <i>bla</i> <sub>CTX-M-15</sub>			1		<i>aac</i> (3)-Iia, <i>qnrB66</i> , <i>oqxB</i> , <i>oqxA</i> , <i>fosA</i>	ST14	
	<i>bla</i> <sub>SHV-28</sub> , <i>bla</i> <sub>CTX-M-15</sub>	2013–2014	<i>K. pneumoniae</i>	1	Algeria	<i>bla</i> <sub>OXA-1</sub> , <i>aac</i> (6′)Ib-cr, <i>aac</i> (3)-Iia, <i>aac</i> (6′)Ib-cr, <i>qnrB66</i> , <i>oqxB</i> , <i>oqxA</i> , <i>fosA</i> , <i>catB3</i> , <i>dfrA14</i>	ST14	[48]
	<i>bla</i> <sub>CMY-2</sub>		<i>Citrobacter freundii</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-14</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-55</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-14</sub>		<i>E. coli</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-14</sub>			1		<i>bla</i> <sub>SHV-1</sub> , <i>bla</i> <sub>TEM-215</sub>	ND	
	<i>bla</i> <sub>SHV-18</sub>			1		ND	ND	
	<i>bla</i> <sub>MIR-14</sub>		<i>E. asburiae</i>	1		<i>bla</i> <sub>SHV-26</sub>	ND	
	<i>bla</i> <sub>ACT-29</sub>			1		ND	ND	
Tomato	<i>bla</i> <sub>CTX-M-27</sub> , <i>bla</i> <sub>CTX-M-15</sub>			1		<i>bla</i> <sub>SHV-26</sub>	ND	
	<i>bla</i> <sub>MIR-20</sub>		<i>E. cloacae</i>	1		ND	ND	
	<i>bla</i> <sub>TEM-3</sub> , <i>bla</i> <sub>ACT-2</sub> , <i>bla</i> <sub>SHV-18</sub>	2017–2018		1	South Africa	<i>bla</i> <sub>TEM-1</sub> , <i>bla</i> <sub>SHV-11</sub>	ND	[9]
	<i>bla</i> <sub>SHV-18</sub> , <i>bla</i> <sub>TEM-3</sub>		<i>E. cowanii</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-15</sub>		<i>K. pneumoniae</i>	1		ND	ND	
	<i>bla</i> <sub>ACT-10</sub>		<i>K. oxytoca</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-55</sub>		<i>Proteus mirabilis</i>	1		<i>bla</i> <sub>TEM-215</sub>	ND	
	<i>bla</i> <sub>ACT-10</sub> , <i>bla</i> <sub>DHA-18</sub> , <i>bla</i> <sub>CMY-49</sub>		<i>Pseudomonas penneri</i>	1		ND	ND	
	<i>bla</i> <sub>SHV-18</sub>		<i>R. aquatilis</i>	1		<i>bla</i> <sub>TEM-215</sub>	ND	
	<i>bla</i> <sub>MIR-16</sub>		<i>R. aquatilis</i>	1		ND	ND	
	<i>bla</i> <sub>SHV-18</sub> , <i>bla</i> <sub>MIR-16</sub>	2017–2018	<i>E. asburiae</i>	1	South Africa	<i>bla</i> <sub>TEM-1</sub> , <i>bla</i> <sub>OXA-1</sub>	ND	[9]
Diced tomato	<i>bla</i> <sub>CTX-MGroup2</sub>	2014	<i>Kluyvera ascorbata</i>	1	Switzerland	ND	ND	[44]
	<i>bla</i> <sub>FONA-2</sub>	2014	<i>S. fonticola</i>	1	Switzerland	ND	ND	[44]
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub>		<i>E. asburiae</i>	1		ND	ND	
Spinach	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>OXA</sub>	2017	<i>E. coli</i>	2	South Africa	ND	ND	[47]
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>OXA</sub>		<i>K. pneumoniae</i>	3		ND	ND	

Table 1. Cont.

Vegetable Type	ESBL/AmpC Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	References
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub>		<i>E. asburiae</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>OXA</sub>		<i>E. coli</i>	2		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>OXA</sub>		<i>K. pneumoniae</i>	3		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub>		<i>R. aquatilis</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>OXA</sub>		<i>R. aquatilis</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub>		<i>R. aquatilis</i>	2		ND	ND	
	<i>bla</i> <sub>CIT</sub>			3		ND	ND	
	<i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub>		<i>S. fonticola</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>OXA</sub>			2		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>OXA</sub> , <i>bla</i> <sub>CIT</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>SHV</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-group1</sub> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>CIT</sub>			2		ND	ND	
	<i>bla</i> <sub>CTX-M-27</sub>			2		ND	ND	
	<i>bla</i> <sub>MIR-20</sub>			1		ND	ND	
	<i>bla</i> <sub>SHV-18</sub> , <i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>TEM-3</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-14</sub> , <i>bla</i> <sub>TEM-3</sub>		<i>E. coli</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-14</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-15</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-55</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-14</sub> , <i>bla</i> <sub>ACT-58</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-14</sub>	2017–2018		2	South Africa	ND	ND	[9]
	<i>bla</i> <sub>CTX-M-14</sub>			2		<i>bla</i> <sub>TEM-215</sub>	ND	
	<i>bla</i> <sub>ACT-58</sub>		<i>E. asburiae</i>	1		<i>bla</i> <sub>TEM-215</sub>	ND	
	<i>bla</i> <sub>CMY-87</sub>		<i>E. ludwigii</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-27</sub> , <i>bla</i> <sub>EC-30</sub>		<i>R. aquatilis</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-15</sub>			1		<i>bla</i> <sub>SHV-11</sub>	ND	
	<i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>SHV-28</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-14</sub> , <i>bla</i> <sub>SHV-28</sub>		<i>S. fonticola</i>	1		ND	ND	
	<i>bla</i> <sub>MIR-16</sub>			1		<i>bla</i> <sub>TEM-1</sub> , <i>bla</i> <sub>OXA-1</sub>	ND	
	<i>bla</i> <sub>CTXM-15</sub>			1		<i>bla</i> <sub>TEM-215</sub>	ND	
	<i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>TEM</sub>	2019	<i>S. marcescens</i>	1	Romania	ND	ND	[46]
	<i>bla</i> <sub>CTXM</sub>		<i>E. cloacae</i>	1		ND	ND	
	<i>bla</i> <sub>CTXM-15</sub>	ND	<i>E. cloacae</i>	1	Brazil	<i>bla</i> <sub>OXA-1</sub> , <i>bla</i> <sub>TEM-1B</sub> , <i>bla</i> <sub>ACT-7</sub> , <i>aac</i> (3)-IIa, <i>aac</i> (6')Ib-cr, <i>ant</i> (3'')Ia, <i>strA</i> , <i>strB</i> , <i>qnrB</i> , <i>sul2</i> , <i>tet</i> (A), <i>fosA</i> .	ST927	[56]
	<i>bla</i> <sub>CTXM-15</sub>	ND	<i>E. coli</i>	1		<i>bla</i> <sub>TEM-1B</sub> , <i>aac</i> (3)IIId, <i>aadA5</i> , <i>strA</i> , <i>strB</i> , <i>tet</i> (A)	ST14012	

Table 1. Cont.

Vegetable Type	ESBL/AmpC Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	References
Chopped Spinach	<i>bla</i> <sub>CTXM14</sub> , <i>bla</i> <sub>SHV-142</sub>	2017	<i>K. pneumoniae</i>	1	Canada	ND	ST261	[55]
	<i>bla</i> <sub>CTXM-27</sub>		<i>E. cloacae</i>	1		qnrB2, qnrS1, aac(6')Ib cr	ND	
	<i>bla</i> <sub>CTXM-27</sub>		<i>E. aerogenes</i>	1		aac(6') Ib cr	ND	
Ceylon spinach	<i>bla</i> <sub>CTXM-14</sub>	2014	<i>K. pneumoniae</i>	1	Switzerland	ND	ST37	[10]
Water spinach	<i>bla</i> <sub>CTXM-15</sub>		<i>K. pneumoniae</i>	1		ND	ST16	
Cucumber	<i>bla</i> <sub>CTX-M</sub> , <i>bla</i> <sub>SHV</sub>	2011–2014	<i>E. coli</i>	2	China	ND	ND	[7]
	<i>bla</i> <sub>CTXM-15</sub>	2014	<i>E. cloacae</i>	1	Switzerland	ND	ND	[10]
	<i>bla</i> <sub>CTXM-15</sub>		<i>E. coli</i>	1		ND	ST410	
	<i>bla</i> <sub>TEM-116</sub>	2015–2016	<i>P. mosselii</i>	1	Japan	ND	ND	[49]
	<i>bla</i> <sub>MIR-20</sub>	2017–2018	<i>E. cloacae</i>	1	South Africa	ND	ND	[9]
	<i>bla</i> <sub>SHV-18</sub>		<i>R. aquatilis</i>	1		<i>bla</i> <sub>OXA-1</sub>	ND	
	<i>bla</i> <sub>CTXM</sub> , <i>bla</i> <sub>TEM</sub>	2019	<i>E. coli</i>	1	Romania	ND	ND	[46]
<i>bla</i> <sub>DHA</sub>	<i>E. cloacae</i>		1	ND				
Bitter cucumber	<i>bla</i> <sub>CTXM-15</sub>	2014	<i>E. coli</i>	1	Switzerland	ND	ST131	[10]
Coriander	<i>bla</i> <sub>CTXM-55</sub>	2011–2014	<i>E. coli</i>	2	China	ND	ST48, ST4680	[7]
	<i>bla</i> <sub>CTX-M</sub> , <i>bla</i> <sub>OXA</sub>		<i>Citrobacter freundii</i>	1		ND	ND	
	<i>bla</i> <sub>CTX-M-55</sub>	2018	<i>E. coli</i>	1	Malaysia	<i>bla</i> <sub>TEM-1B</sub> , aph(3 0)-Ia, aph(300)-Ib, aph(6)-Id, mdf(A), floR, ARR-2, sul2, tet(A), dfrA14	ST155	[50]
	<i>bla</i> <sub>CTX-M-65</sub>		<i>E. coli</i>	1		aac(3)-IV, aadA5, aph(4)-Ia, oqxA, oqxB, mdf(A), floR, sul1, sul2, tet(A), dfrA17	ST479	
Parsley	<i>bla</i> <sub>SHV-28</sub> , <i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>OXA-1</sub>	2013–2014	<i>K. pneumoniae</i>	1	Algeria	aac(6')Ib-cr, aac(3)-IIa, aac(6')Ib-cr, qnrB66, oqxB, oqxA, fosA, catB3, dfrA14	ST14	[48]
	<i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>OXA-1</sub>			1		aac(6')Ib-cr, aac(3)-IIa, strB, strA, aac(6')Ib-cr, oqxB, oqxA, fosA, catB3, sul2, tet(A), dfrA14	ST45	
	<i>bla</i> <sub>SHV</sub>	2019	<i>K. oxytoca</i>	1	Romania	ND	ND	[46]
Water parsley	<i>bla</i> <sub>CTX-M-55</sub>			1		ND	ND	
	<i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>TEM-1</sub>	2018	<i>E. coli</i>	1	South Korea	ND	ST101	[51]
	<i>bla</i> <sub>CTX-M-14</sub> , <i>bla</i> <sub>TEM-1</sub>			1		ND	ST354	
	<i>bla</i> <sub>CTX-M-14</sub>			1		ND	ST38	
Parsley/cilantro	<i>bla</i> <sub>CTX-M-15</sub>	2015	<i>E. coli</i>	1	Ecuador	None	ST410	[54]
				1		dfrA1, aadA5	ST44	
Soy sprouts	<i>bla</i> <sub>CTX-M-65</sub>	2011–2013	<i>E. coli</i>	1	Germany	floR, aac(6')-Ib3, sul2, tet(A), fosA3	ST10	[45]
	<i>bla</i> <sub>CTX-M-125</sub>			1		aph(3')-II, tet(A), fosA3	ST542	
	<i>bla</i> <sub>CTX-M-14</sub>			1		catA1, floR, aac(6')Ib-cr, aph(3')-Ia, aadA5, sul1, sul2, tet(A), dfrA17, fosA3	ST527	
	<i>bla</i> <sub>CTXM-14</sub>	2014	<i>K. pneumoniae</i>	1	Switzerland	ND	ST208	[10]
Sprouts-mixture	<i>bla</i> <sub>CTX-M-15</sub>	2011–2013	<i>E. coli</i>	1	Germany	<i>bla</i> <sub>TEM-1</sub> , qnrS1, strA, strB, sul2, tet(A), dfrA14	ST847	[45]
Alfalfa	<i>bla</i> <sub>CTX-M-15</sub>			1		dfrA1, aadA5	ST410	
	<i>bla</i> <sub>CTX-M-15</sub>	2015	<i>E. coli</i>	1	Ecuador	None	ST44	[54]
	<i>bla</i> <sub>CTX-M-15</sub>			1		None	ST44	

Table 1. Cont.

Vegetable Type	ESBL/AmpC Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	References
	<i>bla</i> <sub>CTX-M-14</sub> , <i>bla</i> <sub>CMY-2</sub>		<i>S. fonticola</i>	1		<i>bla</i> <sub>TEM-215</sub>	ND	
	<i>bla</i> <sub>CTX-M-14</sub> , <i>bla</i> <sub>CMY-161</sub>	2017–2018	<i>S. fonticola</i>	1		<i>bla</i> <sub>TEM-215</sub>	ND	
Alfalfa sprouts	<i>bla</i> <sub>CTX-M-15</sub>	2011–2013	<i>E. coli</i>	1	Germany	<i>bla</i> <sub>TEM-1</sub> , <i>qnrS1</i> , <i>strA</i> , <i>strB</i> , <i>sul2</i> , <i>tet(A)</i> , <i>dfrA14</i>	ST410	[45]
Greenbeans	<i>bla</i> <sub>CTX-M-14</sub>	2017–2018	<i>E. coli</i>	2	South Africa	ND	ND	[9]
Curry leaves	<i>bla</i> <sub>CTXM-15</sub>	2014	<i>K. pneumoniae</i>	1	Switzerland	ND	ST307	[10]
	<i>bla</i> <sub>CTXM-14</sub>		<i>E. coli</i>	1		ND	ST38	
	<i>bla</i> <sub>CTXM-15</sub>		<i>K. pneumoniae</i>	1		ND	ST1742	
	<i>bla</i> <sub>SHV-12</sub>		<i>E. coli</i>	1		ND	ST1656	
	<i>bla</i> <sub>CTXM-15</sub>		<i>K. pneumoniae</i>	4		ND	ST1739, ST1741, ST1881, ST1740	
	<i>bla</i> <sub>CTXM-1</sub>			1		ND	ST1555	
	<i>bla</i> <sub>CTXM-15</sub>		<i>E. coli</i>	1		ND	ST4681, ST152	
	<i>bla</i> <sub>CTXM-14</sub>			1		ND	ST4679	
	<i>bla</i> <sub>CTXM-55</sub>			1		ND	ST10	
Mint	<i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>SHV-28</sub>	2013–2014	<i>K. pneumoniae</i>	1	Algeria	<i>bla</i> <sub>OXA-1</sub> , <i>aac(6')</i> Ib-cr, <i>aac(3)-Iia</i> , <i>aac(6')</i> Ib-cr, <i>qnrB66</i> , <i>oqxB</i> , <i>oqxA</i> , <i>fosA</i> , <i>catB3</i> , <i>dfrA14</i>	ST14	[48]
	<i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>SHV-28</sub>			1		<i>bla</i> <sub>OXA-1</sub> , <i>aac(6')</i> Ib-cr, <i>aac(3)-Iia</i> , <i>aac(6')</i> Ib-cr, <i>qnrB66</i> , <i>oqxB</i> , <i>oqxA</i> , <i>fosA</i> , <i>catB3</i> , <i>dfrA14</i>	ST14	
Chili	<i>bla</i> <sub>CTXM-15</sub>	2014	<i>E. coli</i>	1	Switzerland	ND	ST405	[10]
	<i>bla</i> <sub>CTXM-15</sub>		<i>E. cloacae</i>	1		ND	ND	
Green chili	<i>bla</i> <sub>CTXM-15</sub>		<i>K. pneumoniae</i>	2		ND	ST1740, ST37	
	<i>bla</i> <sub>CTXM-27</sub>			1		ND	ST458	
Small chili	<i>bla</i> <sub>CTXM-65</sub>		<i>E. coli</i>	1		ND	ST167	
Chili pepper	<i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>SHV-28</sub>	2018	<i>K. pneumoniae</i>	1	Malaysia	<i>bla</i> <sub>TEM-1B</sub> , <i>bla</i> <sub>OXA-1</sub> , <i>aac(3)-IIa</i> , <i>aac(6 0)-Ib-cr</i> , <i>aph(300)-Ib</i> , <i>aph(6)-Id</i> , <i>aac(6 0)-Ib-cr</i> , <i>oqxA</i> , <i>oqxB</i> , <i>qnrB1</i> , <i>fosA</i> , <i>catB3</i> , <i>sul2</i> , <i>tet(A)</i> , <i>dfrA14</i>	ST307	[50]
	<i>bla</i> <sub>DHA-1</sub> , <i>bla</i> <sub>SHV-28</sub>		<i>K. pneumoniae</i>	1		<i>oqxA</i> , <i>oqxB</i> , <i>qnrS1</i> , <i>fosA</i> , <i>sul1</i> , <i>tet(A)</i> , <i>dfrA1</i>	ST101	
Hyacinth bean seeds	<i>bla</i> <sub>CTXM-15</sub>	2017	<i>E. coli</i>	1	Canada	ND	ST189	[55]
				1		<i>bla</i> <sub>TEM-1</sub>	ST226	
Ginseng	<i>bla</i> <sub>TEM-116</sub>	2015–2016	<i>Pseudomonas paralactis</i>	1	Japan	ND	ND	[49]
	<i>bla</i> <sub>TEM-116</sub>		<i>P. arsenicoxydans</i>	1		ND	ND	
	<i>bla</i> <sub>TEM-116</sub>			1		ND	ND	
Beets	<i>bla</i> <sub>CTX-M-15</sub>	2013–2014	<i>K. pneumoniae</i>	1	Algeria	<i>aph(3')</i> -Ia, <i>aadA2</i> , <i>strB</i> , <i>strA</i> , <i>nrS1</i> , <i>oqxB</i> , <i>oqxA</i> , <i>fosA</i> , <i>mph(A)</i> , <i>catA2</i> , <i>sul1</i> , <i>sul2</i> , <i>tet(A)</i> , <i>dfrA12</i>	ST219	[48]
Carrot	<i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>OXA-1</sub>	2013–2014	<i>K. pneumoniae</i>	1		<i>bla</i> <sub>TEM-1B</sub> , <i>aac(6')</i> Ib-cr, <i>aac(3)-IIa</i> , <i>strB</i> , <i>strA</i> , <i>ac(6')</i> Ib-cr, <i>qnrB66</i> , <i>oqxB</i> , <i>oqxA</i> , <i>fosA</i> , <i>catB3</i> , <i>sul2</i> , <i>dfrA14</i> .	ST45	[48]
	<i>bla</i> <sub>RAHN-1</sub>	2011	<i>R. aquatilis</i>	ND	The Netherlands	ND	ND	[42]

Table 1. Cont.

Vegetable Type	ESBL/AmpC Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	References		
Bunched carrot	<i>bla</i> <sub>FONA</sub> (1–6)	2012–2013	<i>S. fonticola</i>	ND	The Netherlands	ND	ND	[3]		
Arugula	<i>bla</i> <sub>RAHN-1</sub>	2015–2016	<i>R. aquatilis</i>	4	Italy	ND	ND	[43]		
	<i>bla</i> <sub>CTX-M-15</sub>		<i>C. freundii</i>	4		ND	ND			
	<i>bla</i> <sub>ACC</sub>		<i>Hafnia alvei</i>	2		ND	ND			
Egg plant	<i>bla</i> <sub>CTXM-15</sub>	2014	<i>K. pneumoniae</i>	1	Switzerland	ND	ST2739	[56]		
	<i>bla</i> <sub>CTXM-15</sub>			1					Brazil	<i>bla</i> <sub>OXA-1</sub> , <i>bla</i> <sub>TEM-1B</sub> , <i>aac</i> (6)Ib-cr, <i>strA</i> , <i>strB</i> , <i>qnrB1</i> , <i>opxAB</i> , <i>gyrA</i> , <i>parC</i> , <i>tet</i> (A), <i>fosA</i> .
	<i>bla</i> <sub>CTXM-15</sub>			1						
Chinese chive	<i>bla</i> <sub>SHV-12</sub>	2015–2016	<i>P. parafulva</i>	1	Japan	ND	ND	[49]		
Chopped chives	<i>bla</i> <sub>CTX-M-15</sub>	2014	<i>E. cloacae</i>	1	Switzerland	ND	ND	[44]		
Onion	<i>bla</i> <sub>FONA-1</sub> (1–6)	2012–2013	<i>S. fonticola</i>	ND	The Netherlands	ND	ND	[3]		
	<i>bla</i> <sub>TEM-116</sub>	2015–2016	<i>Pseudomonas beteli</i>	1	Japan	ND	ND	[49]		
Broccoli	<i>bla</i> <sub>TEM-116</sub>	2015–2016	<i>P. hunanensis</i>	1	Japan	ND	ND	[49]		
Cabbage	<i>bla</i> <sub>TEM-116</sub>	2015–2016	<i>P. hunanensis</i>	1	Japan	ND	ND	[49]		
	<i>bla</i> <sub>CTX-M</sub> , <i>bla</i> <sub>SHV</sub>	2019	<i>E. cloacae</i>	1	Romania	ND	ND	[46]		
	<i>bla</i> <sub>CTXM-65</sub>	2018	<i>E. coli</i>	1	South Korea	ND	2847	[51]		
	<i>bla</i> <sub>CTXM-15</sub>	ND	<i>E. coli</i>	1	Brazil	<i>bla</i> <sub>OXA-1</sub> , <i>bla</i> <sub>TEM-1B</sub> , <i>aac</i> (3)IIa, <i>aac</i> (6')Ib-cr, <i>aadA5</i> , <i>gyrA</i> , <i>parC</i> , <i>sul1</i> .	ST648	[56]		
<i>bla</i> <sub>CTXM-15</sub>	ND	<i>E. coli</i>	1	<i>bla</i> <sub>OXA-1</sub> , <i>aac</i> (6')Ib-cr, <i>aadA5</i> , <i>aac</i> (6')Ib-cr, <i>sul1</i> , <i>drfA17</i> , <i>tet</i> (B), <i>mph</i> (A)			ST38			
Cut cabbage	<i>bla</i> <sub>SHV-12</sub>	2015–2016	<i>P. hunanensis</i>	1	Japan	ND	ND	[49]		
Bean sprout	<i>bla</i> <sub>SHV-12</sub>		<i>P. putida</i>	1		ND	ND			
Yard long beans	<i>bla</i> <sub>CTXM-55</sub>	2015–2016	<i>E. cloacae</i>	1	Japan	ND	ND	[49]		
	<i>bla</i> <sub>SHV-12</sub>	2014	<i>Cronobacter sakazakii</i>	1	Switzerland	ND	ST3696			
	<i>bla</i> <sub>CTXM-14</sub>		<i>E. coli</i>	1		ND	ND			
Holy Basil	<i>bla</i> <sub>CTXM-15</sub>		<i>K. pneumoniae</i>	1		ND	ST36			
	<i>bla</i> <sub>CTXM-65</sub>		<i>E. coli</i>	1		ND	ST58			
Okra (marrow)	<i>bla</i> <sub>CTXM-14</sub>		<i>E. coli</i>	1		ND	ST38			
	<i>bla</i> <sub>CTXM-15</sub>		<i>E. coli</i>	2		ND	ST155, ST443			
Okra	<i>bla</i> <sub>CTXM-15</sub>		<i>K. pneumoniae</i>	2		ND	ST997, ST244	[10]		
	<i>bla</i> <sub>CTXM-15</sub>		<i>E. aerogenes</i>	1		ND	ND			
	<i>bla</i> <sub>CTXM-15</sub>		<i>E. cloacae</i>	2		ND	ND			
	<i>bla</i> <sub>CTXM-15</sub>		<i>E. coli</i>	2		ND	ST4682, ST4684			
Parwal beans	<i>bla</i> <sub>CTXM-15</sub>	2014	<i>E. coli</i>	1	Switzerland	ND	ST641			
Peppermint	<i>bla</i> <sub>CTXM-3</sub>		<i>K. pneumoniae</i>	1		ND	ST15			
Cha-om (acacia)	<i>bla</i> <sub>SHV-12</sub>		<i>K. pneumoniae</i>	1		ND	ND			
	<i>bla</i> <sub>CTXM-55</sub>		<i>E. coli</i>	2		ND	ST167, ST393			
	<i>bla</i> <sub>CTXM-14</sub>		<i>E. coli</i>	1		ND	ST58			

Table 1. Cont.

Vegetable Type	ESBL/AmpC Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	References
Garlic chives	<i>bla</i> <sub>CTXM-63</sub>		<i>K. pneumoniae</i>	1		ND	ST1743	
	<i>bla</i> <sub>CTXM-55</sub>		<i>E. coli</i>	1		ND	ST226	
Lemongrass	<i>bla</i> <sub>CTXM-14</sub>		<i>K. pneumoniae</i>	1		ND	ST1530	
Sweet basil	<i>bla</i> <sub>SHV-2a</sub>		<i>K. pneumoniae</i>	1		ND	ST76	
Basil leaves	<i>bla</i> <sub>CTXM-65</sub>		<i>E. coli</i>	1		ND	ST4683	
Celery	<i>bla</i> <sub>RAHN-1</sub>	2011	<i>R. aquatilis</i>	34	The Netherlands	ND	ND	[42]
	<i>bla</i> <sub>SHV-60, bla</sub> <sub>DHA-1</sub>	2013–2014	<i>K. pneumoniae</i>	1	Algeria	<i>bla</i> <sub>TEM-1D</sub> , <i>aadA1</i> , <i>strB</i> , <i>strA</i> , <i>qnrB4</i> , <i>oqxB</i> , <i>oqxA</i> , <i>fosA</i> , <i>sul1</i> , <i>tet(A)</i> , <i>dfrA1</i>	ST236	[48]
Lollo rosso leaves	<i>bla</i> <sub>CTX-M-14</sub>		<i>E. coli</i>	1		<i>strA</i> , <i>strB</i> , <i>sul1</i> , <i>dfrA1</i>	ST973	
Lollo rosso and Lollo bionda leaves	<i>bla</i> <sub>CTX-M-2</sub>	2011–2013	<i>E. coli</i>	1	Germany	<i>bla</i> <sub>TEM-1</sub> , <i>strA</i> , <i>strB</i> , <i>aadA5</i>	ST120	[45]
Blanched celery	<i>bla</i> <sub>SHV-12</sub>	2012–2013	<i>E. coli</i>	1	The Netherlands	ND	ND	[3]
	<i>bla</i> <sub>FONA-1</sub>		<i>S. fonticola</i>	ND		ND		
Radish	<i>bla</i> <sub>RAHN-1</sub>	2012–2013	<i>R. aquatilis</i>	1	The Netherlands	ND	ND	[3]
	<i>bla</i> <sub>FONA(1–6)</sub>		<i>S. fonticola</i>	ND		ND		
Chicory	<i>bla</i> <sub>RAHN-1</sub>	2011	<i>R. aquatilis</i>	ND	The Netherlands	ND	ND	[42]
	<i>bla</i> <sub>RAHN-1</sub>	2011	<i>R. aquatilis</i>	ND		ND	ND	
Endive	<i>bla</i> <sub>FONA-1(1–6)</sub>	2012–2013	<i>S. fonticola</i>	ND	The Netherlands	ND	ND	[3]
Iceberg lettuce + arugula	<i>bla</i> <sub>SHV-12</sub>	2015–2016	<i>E. coli</i>	3	Italy	ND	ND	[43]
	<i>bla</i> <sub>CTX-M-15</sub>		<i>E. coli</i>	1		ND	ND	
Mixed green vegetables	<i>bla</i> <sub>CTXM-15</sub>	2017	<i>E. cloacae</i>	1	Canada	<i>bla</i> <sub>TEM-1</sub> , <i>qnrB1</i> , <i>aac(6')</i> Ib cr	ND	[55]
Sambhar vegetables	<i>bla</i> <sub>CTXM15, bla</sub> <sub>SHV-106</sub>		<i>K. pneumoniae</i>	1		ND	ST101	
Aster scaber	<i>bla</i> <sub>CTX-14, bla</sub> <sub>TEM-1</sub>			1		ND	ST69	
Perilla leaf	<i>bla</i> <sub>CTX-M-27, bla</sub> <sub>TEM-1</sub>			1		ND	ST349	
Sweet potato stalk	<i>bla</i> <sub>CTX-M-15</sub>	2018	<i>E. coli</i>	1	South Korea	ND	ST224	[51]
Pepper leaf	<i>bla</i> <sub>CTX-M-55, bla</sub> <sub>TEM-1</sub>			1		ND	ND	
Mapleleaf ainsliaea	<i>bla</i> <sub>CTX-M-27</sub>			1		ND	ST10	
Leafy greens	<i>bla</i> <sub>CTX-M</sub>	2015–2016	<i>Enterobacterale</i>	1	United States	ND	ND	[53]
	<i>bla</i> <sub>CMY</sub>		<i>Enterobacterale</i>	6		ND	ND	
Frisee salad	<i>bla</i> <sub>CTX-M-1, bla</sub> <sub>DHA-1</sub>	2015–2016	<i>E. cloacae</i>	2	Italy	ND	ND	[43]
Frisee salad + carrot	<i>bla</i> <sub>CTX-M-15</sub>		<i>Pantoea agglomerans</i>	6		ND	ND	
Peach	<i>bla</i> <sub>CTX-M-15</sub>	2013–2014	<i>K. pneumoniae</i>	1	Algeria	<i>aadA2</i> , <i>strB</i> , <i>strA</i> , <i>qnrS1</i> , <i>oqxB</i> , <i>oqxA</i> , <i>fosA</i> , <i>mph(A)</i> , <i>catA2</i> , <i>sul1</i> , <i>sul2</i> , <i>dfrA12</i>	ST219	[48]

## 6. Vegetables and Fruit Isolates with Carbapenemase Genes

Only eight reports have revealed the isolation of Gram-negative bacteria producing carbapenemase genes from vegetables and fruit (Figure 1, Table 2). The first study describing carbapenemase-producing Gram-negative bacteria from fresh vegetable samples

was published in 2015. Samples were purchased from different retail shops specializing in Asian food from three different cities in Switzerland, imported from Vietnam, Thailand, and India. In this study, only one *Klebsiella variicola* strain carrying the *bla*<sub>OXA-181</sub> gene was isolated from a coriander sample from Thailand/Vietnam, and the obtained isolate harbored a quinolone resistant gene (*qnrS1*). These data suggest that the international production of imported fresh vegetables constitutes a possible reservoir for the spread of carbapenemase-producing Gram-negative bacteria, especially Enterobacteriaceae [57].

Since its first report, carbapenemase genes have been identified in bacteria from different vegetable samples in only five countries across the Asian, African and European continents. Indeed, OXA-72-producing *Acinetobacter calcoaceticus* strains have been found in two vegetable samples purchased from the same market in Beirut, Lebanon [58]. In Japan, two *K. pneumoniae* and one *Acinetobacter baumannii* isolate were collected from vegetable samples in the city of Higashi-Hiroshima. Both *K. pneumoniae* isolates carried *bla*<sub>NDM-1</sub> with other genes conferring resistance to  $\beta$ -lactams (*bla*<sub>CTX-M-15</sub>, *bla*<sub>OXA-9</sub>, and *bla*<sub>TEM-1A</sub>), aminoglycosides (*aac(6')-Ib*, *aadA1*, and *aph(3')-VI*), quinolones (*qnrS1*) and fluoroquinolones (*aac(6')-Ib-cr*). While the obtained *A. baumannii* isolate harbored *bla*<sub>OXA-66</sub>, *bla*<sub>OXA-72</sub> and genes conferring resistance to sulfonamides (*sul2*), tetracycline (*tet(B)*), and streptomycin (*strAB*) [59]. In China, Wang et al., identified an *Escherichia coli* strain coproducing *bla*<sub>KPC-2</sub> and *bla*<sub>NDM-1</sub> genes in fresh lettuce from a market in Guangzhou. In addition, this multidrug resistant *E. coli* strain harbored fosfomycin resistance genes (*fosA3* and *floR*). This study represents the first report of either *bla*<sub>KPC</sub> or *bla*<sub>NDM</sub> genes in bacteria obtained from vegetables [60]. Additionally, two other studies from China reported the detection of carbapenemase-positive enterobacterial isolates. The first reported the isolation of twelve carbapenem-resistant isolates obtained from vegetable samples, where the highest detection rate was found in curly endive samples. The authors identified two *K. pneumoniae* isolates carrying the *bla*<sub>KPC-2</sub> gene, while five of each *E. coli* and *C. freundii* strains harbored the *bla*<sub>NDM</sub> gene, including four *E. coli* with the *bla*<sub>NDM-5</sub> gene and five *C. freundii* with *bla*<sub>NDM-1</sub>. Notably, one *E. coli* strain from a cucumber sample harbored *bla*<sub>NDM-5</sub> and *bla*<sub>KPC-2</sub> genes simultaneously. All *C. freundii* and *E. coli* isolates carried fosfomycin resistance genes (*fosA3* and *floR*), and all *K. pneumoniae* and *C. freundii* isolates harbored the *floR* gene. However, one strain of *E. coli* and *C. freundii* harbored the aminoglycoside resistance gene (*rmtB*). Quinolone resistance genes including *oqxAB* and *qnrB*, were found in four and eight isolates, respectively [61]. The second report signaled the detection of two *E. coli* isolates carrying *bla*<sub>NDM</sub> genes in leaf rape and spinach recovered from two supermarkets in Shandong province; one isolate concomitantly harbored *bla*<sub>NDM-9</sub>, *mcr-1* and *fosA3*, while the second isolate carried *bla*<sub>NDM-5</sub>, *mcr-1* and *fosA3* genes [4].

From the African continent only one study from Algeria has been reported. The authors identified three *K. pneumoniae* isolates harboring the *bla*<sub>OXA-48</sub> gene from lettuce, tomatoes and parsley in Béjaia city [11]. In Europe and more precisely from Romania, the *bla*<sub>OXA-48</sub> and *bla*<sub>KPC</sub> genes were detected in *E. cloacae* and *K. oxytoca* isolates from parsley samples [46].

**Table 2.** Carbapenemases and *mcr* genes reported in Gram-negative bacteria isolates from vegetables and fruit worldwide.

Vegetables Type	Carbapenemase/ <i>mcr</i> Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	Plasmid Type	Reference
Coriander	<i>bla</i> <sub>OXA-181</sub>	2015	<i>Klebsiella variicola</i>	1	Switzerland	<i>qnrS1</i>	ND	IncX3	[57]
	<i>bla</i> <sub>KPC-2</sub> and <i>bla</i> <sub>NDM-1</sub>	2015	<i>Escherichia coli</i>	1	China	<i>bla</i> <sub>DHA-1</sub> , <i>fosA3</i> , <i>floR</i> , <i>aacA4</i> , <i>tet(D)</i> , <i>sul1</i> , <i>armA</i> , <i>mph(E)</i> , <i>msr(E)</i> , <i>erm(B)</i> , <i>strA</i> , <i>strB</i>	ST877	IncA/C ( <i>bla</i> <sub>NDM-1</sub> ), Untypable ( <i>bla</i> <sub>KPC-2</sub> )	[60]
Lettuce	<i>bla</i> <sub>OXA-48</sub>	2016	<i>K. pneumoniae</i>	1	Algeria	<i>bla</i> <sub>TEM-1</sub>	ST391	ND	[11]
	<i>bla</i> <sub>NDM-5</sub>	2017	<i>E. coli</i>	1	China	<i>bla</i> <sub>CTX-M-1G</sub> , <i>fosA3</i> , <i>floR</i> , <i>oqxAB</i>	ST4762	X3	[61]
	<i>bla</i> <sub>NDM-1</sub>			1			ND	ND	

Table 2. Cont.

Vegetables Type	Carbapenemase/mcr Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	Plasmid Type	Reference
Parsley	<i>bla</i> <sub>NDM-1</sub>	2015	<i>K. pneumoniae</i>	1	Japan	<i>bla</i> <sub>SHV-28</sub> , <i>bla</i> <sub>SHV-1</sub> , <i>bla</i> <sub>TEM-1A</sub> , <i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>CTX-M-14b</sub> , <i>bla</i> <sub>TEM-1A</sub> , <i>bla</i> <sub>OXA-9</sub> , <i>fosA</i> , <i>oqxAB</i> , <i>tet</i> (D), <i>aac</i> (69)-Ib, <i>aadA1</i> , <i>aph</i> (39)-VI, <i>aph</i> (6)-Id, <i>aph</i> (39)-VIb, <i>aph</i> (39')-Ib, <i>aac</i> (69)-Ib-cr, <i>qnrS1</i>	ST15	ND	[59]
	<i>bla</i> <sub>OXA-48</sub>	2016	<i>K. pneumoniae</i>	1	Algeria	None	ND	ND	[11]
	<i>bla</i> <sub>OXA-48</sub>	2019	<i>E. cloacae</i>	1	Romania	ND	ND	ND	[46]
<i>bla</i> <sub>KPC</sub>	<i>K. oxytoca</i>		1	ND		ND			
Baby leaf mix	<i>bla</i> <sub>NDM-1</sub>	2015	<i>K. pneumoniae</i>	1	Japan	<i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>OXA-9</sub> , <i>bla</i> <sub>TEM-1A</sub> , <i>bla</i> <sub>SHV-28</sub> , <i>bla</i> <sub>CTX-M-14b</sub> , <i>fosA</i> , <i>oqxAB</i> , <i>aac</i> (69)-Ib, <i>aadA1</i> , <i>aph</i> (39)-VI, <i>aac</i> (69)-Ib-cr, <i>qnrS1</i> , <i>aph</i> (6)-Id, <i>aph</i> (39)-VIb, <i>aph</i> (39')-Ib.	ST15	ND	[59]
	<i>bla</i> <sub>OXA-66</sub> , <i>bla</i> <sub>OXA-72</sub>		<i>A. baumannii</i>	1	<i>bla</i> <sub>ADC-25</sub> , <i>bla</i> <sub>OXA-66</sub> , <i>bla</i> <sub>OXA-72</sub> , <i>sul2</i> , <i>tet</i> (B), <i>aac</i> (3)-Ia, <i>aac</i> (69)-Ib, <i>aph</i> (39')-Ib, <i>aph</i> (6)-Id	ST2	GR2 ( <i>bla</i> <sub>OXA-72</sub> )		
Cucumber	<i>bla</i> <sub>KPC-2</sub>	2017	<i>K. pneumoniae</i>	1	China	<i>qnrB</i> , <i>oqxAB</i>	ST23	F35:A-B1	[61]
	<i>bla</i> <sub>NDM-5</sub>			1		<i>bla</i> <sub>CTX-M-1G</sub> , <i>qnrB</i> , <i>oqxAB</i>	ST23	F35:A-B1	
			<i>bla</i> <sub>NDM-5</sub> and <i>bla</i> <sub>KPC-2</sub>	1		<i>fosA3</i> , <i>floR</i> , <i>qnrB</i>	UT	ND	
	1			<i>bla</i> <sub>CTX-M-1G</sub> , <i>fosA3</i> , <i>floR</i> .		ST4762	ND		
Curly endive	<i>bla</i> <sub>NDM-5</sub>	2017	<i>E. coli</i>	1	China	<i>bla</i> <sub>CTX-M-1G</sub> , <i>fosA3</i> , <i>floR</i> , <i>rmtB</i>	ST167	ND	[61]
	<i>bla</i> <sub>NDM-1</sub>			1		<i>fosA3</i> , <i>floR</i> , <i>qnrB</i>	ND	ND	
			<i>bla</i> <sub>NDM-1</sub>	1		<i>fosA3</i> , <i>floR</i> , <i>oqxAB</i> , <i>qnrB</i> , <i>rmtB</i>	ND	ND	
	1			<i>fosA3</i> , <i>floR</i> , <i>qnrB</i>		ND	X3		
Tomato	<i>bla</i> <sub>OXA-48</sub>	2016	<i>K. pneumoniae</i>	1	Algeria	None	ST1877	ND	[11]
	<i>bla</i> <sub>NDM-1</sub>	2017	<i>C. freundii</i>	1	China	<i>bla</i> <sub>CTX-M-1G</sub> , <i>fosA3</i> , <i>floR</i> , <i>qnrB</i>	ND	X3	[61]
Leaf rape	<i>bla</i> <sub>NDM-5</sub>	2017–2018	<i>E. coli</i>	1	China	<i>mcr-1</i> , <i>fosA3</i>	ST156	X3	[4]
Spinach	<i>bla</i> <sub>NDM-9</sub>			1		<i>mcr-1</i> , <i>fosA3</i>	ST2847	Untypable	
	Vegetables (ND)	<i>bla</i> <sub>OXA-72</sub>	ND	<i>Acinetobacter calcoaceticus</i>	2	Lebanon	ND	ND	ND
Lettuce	<i>mcr-1</i>	2013	<i>E. coli</i>	1	Portugal	<i>bla</i> <sub>TEM-1</sub> , <i>aadA1y</i> , <i>aph</i> (4)-Ia, <i>estX-12</i> , <i>floR</i> , <i>sat2</i> , <i>strA</i> , <i>strB</i> , <i>sul2</i> , <i>tetA</i>	ST1716	ND	[62]
		2013–2014	<i>E. coli</i>	1		<i>bla</i> <sub>TEM-1</sub> , <i>aac</i> (3)-Iv, <i>aadA1</i> , <i>aph</i> (4)-Ia, <i>aph</i> (6)-Id, <i>mdf</i> (A)-type, <i>tetA</i> , <i>sul2</i> , <i>floR</i>	ST1716	ND	[63]
		2016	<i>E. coli</i>	1	<i>bla</i> <sub>CTX-M-14</sub> , <i>floR</i> , <i>fosA3</i> , <i>oqxAB</i>	ST795	IncHI2		
				1	<i>bla</i> <sub>CTX-M-55</sub> , <i>floR</i>	ST2505	ncI2		
			<i>E. coli</i>	1	<i>bla</i> <sub>CTX-M-55</sub> , <i>rmtB</i> , <i>floR</i> , <i>fosA3</i> .	ST156	IncI2		
				1	<i>floR</i>	ST48	IncX4		
		2015	<i>Raoultella ornithinolytica</i>	2	<i>bla</i> <sub>CTX-M-14</sub> , <i>floR</i> , <i>fosA3</i> , <i>oqxAB</i>	NA	IncHI2		
		2018	<i>E. coli</i>	1	South Korea	<i>bla</i> <sub>TEM-1</sub> and <i>bla</i> <sub>CTX-M-55</sub>	ST10	ND	[65]
2017–2018	<i>E. coli</i>	1	China	ND	ST10	X4	[66]		
Tomato	<i>mcr-1</i>	2016	<i>E. coli</i>	1	China	<i>bla</i> <sub>CTX-M-14</sub> , <i>floR</i> , <i>fosA3</i> , <i>oqxAB</i>	ST69	IncHI2	[64]
						2	<i>floR</i>	ST206	
	<i>mcr-1</i>	2017–2018	<i>E. coli</i>	1	China	ND	ST713	X4	[66]
						1	ND	UT	

Table 2. Cont.

Vegetables Type	Carbapenemase/mcr Gene	Isolation Period	Species	Isolates Number	Country	Other Antibiotic Resistance Genes	Sequence Type	Plasmid Type	Reference
Leaf rape	mcr-1	2017–2018	<i>K. pneumoniae</i>	1	China	<i>bla</i> <sub>NDM-5</sub> , <i>fosA3</i>	ST156	X4	[4]
Green Pepper	mcr-1	2017–2018	<i>E. coli</i>	1	China	ND	ST744	X4	[66]
			<i>E. cloacae</i>	1	China	ND	ND	ND	[66]
Spinach	mcr-1	2017–2018	<i>E. coli</i>	1	China	<i>bla</i> <sub>NDM-9</sub> , <i>fosA3</i>	ST2847	I2	[4]
				1		ND	ST2253	I2	[66]
Cha-om	mcr-1	2014	<i>E. coli</i>	1	Switzerland	<i>bla</i> <sub>CTX-M-55</sub>	ST167	ND	[67]
Basil leaves	mcr-1	2014	<i>E. coli</i>	1		<i>bla</i> <sub>CTX-M-65</sub>	ST4683	ND	
Cucumber	mcr-1	2017–2018	<i>E. coli</i>	2	China	ND	ST744	X4	[66]
				2		ND	ST1115	I2	
Carrot	mcr-1	2017–2018	<i>E. coli</i>	1	China	ND	ST5539	X4	[66]
			<i>E. coli</i>	1		ND	ST13	I2	
Curly endive	mcr-1	2017–2018	<i>E. coli</i>	1	China	ND	ST13	X4	
Pak choi	mcr-1	2017–2018	<i>E. coli</i>	1	China	ND	ST648	I2	
Apple	mcr-1	2016	<i>E. coli</i>	1	China	<i>aadA2</i> , <i>aadA1</i> , <i>floR</i> , <i>cmlA1</i> , <i>sul2</i> , <i>sul3</i> , <i>tetA</i> , <i>tetM</i> , <i>dfrA12</i> , <i>mdfA</i>	ST189	IncFIA	[68]
Orange	mcr-1		<i>K. pneumoniae</i>	1		<i>bla</i> <sub>SHV-110</sub> , <i>qnrS1</i> , <i>oqxA</i> , <i>oqxB</i> , <i>fosA6</i> , <i>sul1</i> , <i>tetA</i> , <i>dfrA1</i>	ST442	IncHI1	

## 7. Vegetables and Fruit Isolates with the mcr Gene

To date, eight studies have reported mcr-producing Gram-negative bacteria, especially isolates of Enterobacteriaceae species, from fresh produce that mostly originated from China (Figure 1, Table 2). The mcr-1 gene was first reported in 2016 in Switzerland in two out of sixty isolates. The two *E. coli* isolates carried the mcr-1 gene and coharbored *bla*<sub>CTX-M-55</sub> and *bla*<sub>CTX-M-65</sub> genes, respectively [67]. After this first description in fresh produce, mcr-1-producing GNB isolates on fresh produce were reported in China, where seven *E. coli* and two *Raoultella ornithinolytica* isolates were recovered from tomato and lettuce samples between May 2015 and August 2016 in Guangzhou. All the obtained mcr-1-positive strains harbored the florfenicol resistance gene (*floR*). Of the nine isolates, six strains carried the *bla*<sub>CTX-M</sub> gene (four *bla*<sub>CTX-M-14</sub> and two *bla*<sub>CTX-M-55</sub>), with five and four strains harboring the *fosA3* and *oqxAB* efflux pump gene, respectively [64]. Moreover, the mcr-1 gene was described in China from a total of 528 fresh vegetable samples, including 18 different types purchased from 53 supermarkets and farmers markets from 23 districts or cities in nine provinces between May 2017 and April 2018. Of the 528 samples analyzed, only 19 samples harbored one or more mcr-positive isolates, and the three highest detection rates were noted in carrots (14.3%), pakchoi (13.3%) and green pepper (7.7%), followed by leaf lettuce (5.6%), leaf rape (4.9%), romaine lettuce (4.3%), tomato (3.5%), spinach (3.2%), cucumber (3.1%), and curly endive (2.4%). In the above study, twenty-four mcr-1-positive isolates were obtained; twenty-three strains were identified as *E. coli* and one as *E. cloacae*. Fourteen mcr-1-positive strains coproduced the *bla*<sub>CTX-M</sub> gene, nine strains harbored the *bla*<sub>CTX-M-9G</sub> gene and three strains carried *bla*<sub>CTX-M-1G</sub>. However, the remaining two strains harbored both *bla*<sub>CTX-M-9G</sub> and *bla*<sub>CTX-M-1G</sub> genes. In addition, eight and two isolates harbored *fosA3* and *rmtB* genes, respectively. Plasmid-mediated resistance to quinolones (PMQR), including *oqxAB*, *qnrS* and *qnrB* genes, were also detected in this study [66]. Additionally, in the same country, two *E. coli* isolates carrying the mcr-1 gene were isolated from leaf rape and spinach in Shandong province. These isolates coharbored metallo-β-lactamase and *fosA3* genes; the first carried *bla*<sub>NDM-5</sub>, while the second harbored the *bla*<sub>NDM-9</sub> gene [4]. In 2018, one *E. coli* isolate carrying the mcr-1 gene recovered from lettuce was reported in South Korea. The obtained isolate coharbored *bla*<sub>TEM-1</sub> and *bla*<sub>CTX-M-55</sub> genes [65]. In Portugal, the mcr-1 gene was reported by Manageiro et al. in 2020, and they

documented the presence of this gene in an *E. coli* strain isolated from a lettuce sample. In silico analysis showed the presence of additional antibiotic resistance genes including *bla*<sub>TEM-1</sub>, *aac*(3)-Iv, *aadA1*, *aph*(4)-Ia, *aph*(6)-Id, *mdf*(A)-type, *tetA*, *sul2* and *floR*-type [63]. Another report from Portugal revealed the detection of the *mcr-1* gene in an *E. coli* strain from conventionally produced lettuce. The isolate co-carried *bla*<sub>TEM-1</sub>, *aph*(4)-Ia, *floR*, *sat2*, *strA*, *strB*, *sul2* and *tetA* genes, while no conventional and organic fruit were positive for the *mcr-1* gene [62].

On fruit samples, the *mcr-1* gene was detected in *E. coli* and *K. pneumoniae* isolates from apple and orange samples recovered in China [68].

## 8. Contamination Pathways and Genetic Characteristics of $\beta$ -Lactamases and *mcr*-Producing Gram-Negative Bacteria

The high diversity of global clones illustrates the extensive spread of ESBL-producing *K. pneumoniae* and *E. coli* isolates on vegetables around the world (ST45, ST219, ST15 and ST147 found in *K. pneumoniae* isolates, and ST410-A, ST44, ST405, ST131 and ST38 in *E. coli* isolates). In Algeria, sequence type 14, ST45, ST219, ST236, and ST882 have been identified among *K. pneumoniae* strains recovered on fresh fruit and vegetables carrying ESBL or cephalosporinase genes, including *bla*<sub>CTX-M-15</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>SHV-101</sub>, *bla*<sub>SHV-28</sub> and *bla*<sub>DHA-1</sub> genes that were mostly (11 of 13) located on the IncFII plasmid, while the IncR plasmid replicon was identified in only one isolate [48]. In Switzerland, twenty-two different sequence types identified in *E. coli*-positive ESBL have been described on imported vegetables, including ST4684 and ST4683, four of them belonging to the epidemiologically important sequence types ST405 ( $n = 1$ ), ST131 ( $n = 2$ ) and ST38 ( $n = 2$ ) [10]. Similarly, the ST131 *E. coli* clone is known for its role in the global spread of ESBLs, especially CTX-M-15, and this clone has had an inevitable clinical impact on antibiotic resistance and pathogenicity [69]. In the same study conducted in Switzerland, high clonal diversity was observed among *K. pneumoniae* strains, with two isolates belonging to the epidemic clones ST15 and ST147 [10]. In Quito, Ecuador, the hyper epidemic clones ST410 and ST44 harboring the *bla*<sub>CTX-M-15</sub> gene have been identified in *E. coli* isolates from leaf lettuce, alfalfa and parsley/cilantro, and three of them were found on the same integron 1 variable region (*dfrA1* and *addA5*). The five remaining isolates presented *bla*<sub>CTX-M-15</sub> downstream of an insertion sequence element p1 (ISEcp1) [54]. In a survey in Germany, a high diversity of global clones was identified in ESBL-producing *E. coli* isolates from different vegetable samples. These ESBL determinants were detected on different plasmids as follows: IncHI2 and IncK (*bla*<sub>CTX-M-14</sub>, ST527, ST973), IncN (*bla*<sub>CTX-M-65</sub>, ST10), IncFIB (*bla*<sub>CTX-M-15</sub>, ST410), IncHI2 (*bla*<sub>CTX-M-125</sub>, ST542) and IncFIA-FIB (*bla*<sub>CTX-M-2</sub>, ST120) [45]. The IncFIB and IncFIC plasmid replicons were found in *Pseudomonas humanensis*, and *P. putida* carried the *bla*<sub>SHV-12</sub> respectively. The plasmid IncK/B was reported in *P. paralactis* harboring the *bla*<sub>TEM-16</sub> gene [49].

The dissemination of carbapenemase-producing *E. coli* is polyclonal, where multiple STs have been reported. The sequence type 877 was reported in an *E. coli* strain coproducing *bla*<sub>KPC-2</sub> and *bla*<sub>NDM-1</sub> genes isolated from fresh lettuce. These genes were located on 64 and 118 kb plasmids, designated as plasmids pHNTS79-KPC and pHNTS79-NDM, respectively [60]. Furthermore, ST1877 and ST391 have been detected in OXA-48-positive *K. pneumoniae* isolates from lettuce, tomatoes and parsley in Algeria, where the ST391 clone is considered as an emergent carbapenemase-producing lineage of clinical importance [11]. The *bla*<sub>OXA-181</sub> detected in a *Klebsiella variicola* isolate from a coriander sample was mediated by the IncX3-type plasmid of 51-kb [57]. Indeed, in Japan the epidemic clones ST15 and ST2 were reported among *K. pneumoniae* that carried the *bla*<sub>NDM-1</sub> gene and the *A. baumannii* strain coharbored *bla*<sub>OXA-66</sub>, and *bla*<sub>OXA-72</sub> genes, respectively [59]. ST15 is a relatively common NDM-positive *K. pneumoniae* lineage, and it has been found in various countries across different continents, almost all of which were isolated from humans [70]. IncX3 plasmids carrying the *bla*<sub>NDM</sub> gene have been identified in *E. coli* and *C. freundii* strains isolated from cucumbers, and the identified IncX3 plasmid was identical or highly similar (99%) to the IncX3 plasmids identified from patients in other countries. In addition,

similar F35:A-:B1 plasmids were described in two *bla*<sub>KPC-2</sub>-producing-*K. pneumoniae* isolates belonging to ST23 obtained from different cities. Two *E. coli* isolates carrying the *bla*<sub>NDM-5</sub> gene isolated from cucumber and romaine lettuce samples in different cities in China shared an identical PFGE pattern and sequence type (ST4762); however, one *E. coli* strain belonged to ST167 [61].

Among *E. coli* isolates, the *mcr-1* gene was found in multiple STs from different countries, ST10 in South Korea, and ST167 and ST4683 in Switzerland [65,67]. ST156 and ST2847 have been identified in China from leaf rape and spinach samples. In the latter, *mcr-1* genes were located on the ~60-kb IncI2 plasmid or the ~33-kb IncX4 plasmid; even as *bla*<sub>NDM-5</sub> was on the ~46-kb IncX3 plasmid while *bla*<sub>NDM-9</sub> was on the ~120-kb untypeable plasmid. The detected plasmids were highly similar to those from patients and animals described in different countries [4]. In a Chinese study, six sequence types, including ST795, ST2505, ST69, ST156, ST48 and ST206, were described in seven *E. coli* isolates recovered from lettuce and tomato samples. For the four *E. coli* isolates, *mcr-1* genes were located on IncHI2, IncI2 or IncX4 plasmids, while for the two *Raoultella ornithinolytica* isolates, the *mcr-1* gene was located on the IncHI2 plasmid [64]. Moreover, in China, sixteen STs along with a new ST type were identified, while the most prevalent STs were ST744 and ST224. In this study, different plasmid replicons were detected, including IncX4, IncI2 and IncHI2; where IncX4 was the most detected and shared highly similar RFLP profiles, although they were from different cities and fresh vegetables. The *mcr-1* gene was located on the IncX4 plasmid of ~33 kb in size [66].

In addition to the above results, the study found that patients and animals shared identical or highly similar plasmids with vegetables [4,61]. Various other studies reported that vegetables may become contaminated with multidrug resistant bacteria from soil, manure fertilization, irrigation water or through direct contamination by humans [2]. In this context, the major way in which antibiotic resistance enters the soil is through the use of animal manure [71]. In Australia, Zhang et al. explored the impact of cattle and poultry manure application on the resistome in lettuce and soil microbiomes, including the rhizosphere, root endosphere, leaf endosphere and phyllosphere. In addition, they identified potential transmission routes of antibiotic resistance genes in the soil–plant system. The authors reported that poultry manure application increased antibiotic resistance genes in the rhizosphere, root endophyte and phyllosphere, while cattle manure use increased the abundance of antibiotic resistance genes only in the root endophyte, suggesting that poultry manure may have a stronger impact on lettuce resistomes. Moreover, the authors also identified an overlap of antibiotic resistance gene (ARG) profiles between lettuce tissues and soil, which indicates that plant and environmental resistomes are interconnected, and confirmed the transmission of antibiotic resistance genes from manured soil to vegetables. Two main transmission pathways were reported: an internal pathway through plant tissues and an external pathway via aerosol from the atmosphere to the plant surface. Thus, in the external pathway, sixty-nine ARGs were shared between poultry manure-amended soil and the phyllosphere of lettuce, while in the internal pathway 47 genes were common between rhizosphere soil and the root endophyte [72]. This finding was consistent with previous studies which reported that the phyllosphere resistome was significantly more abundant and diverse than the endophytic resistome in organic vegetables [73,74]. Another report from China described the impact of the long-term use of inorganic (chemical) and organic (manure) fertilizers on antibiotic resistance genes in greenhouse soils growing vegetables. The results showed that both inorganic and organic use increased the abundance and the diversity of soil ARGs, with a difference in the dominant ARG types. ARG abundance and diversity were both higher in organic fertilizer [75]. These data confirmed those of previous reports indicating that fertilizer application, especially organic fertilizer such as animal manure, raised ARG abundance and diversity in soil compared to soil without fertilization [75–77].

Several studies have reported food-borne human outbreaks linked to the consumption of fresh vegetables and fruit irrigated with wastewater and indicated that the type of irrigation

practice plays a vital role in the contamination of farm produce [78]. In this context, Araújo et al. characterized the presence of *E. coli* isolates on vegetables and in irrigation water sampled from 16 household farms in Portugal. In this later study, different commonly acquired genes such as *bla*<sub>TEM</sub>, *tetA* and *tetB* and plasmids (IncFIC, IncFrep and IncFIB) were detected in isolates in both water and vegetable samples. In addition, rep-PCR typing results detected the same STs and identical clones in vegetables and water, suggesting cross-contamination. These results suggest that irrigation groundwater is a reservoir of antibiotic resistant *E. coli* and may enter the food chain via vegetable consumption [79]. Makkaew et al. evaluated the contamination of lettuce by *E. coli*, grown under four diverse methods of wastewater irrigation: open spray, open drip, spray under plastic sheet cover, and drip under plastic sheet cover. *E. coli* contamination was reported in all lettuce samples in both open and covered spray beds in all types of spray beds. An equal level of microbial quality of spray bed lettuce and submersed lettuce irrigated with wastewater containing 1299.7 *E. coli* MPN/100 mL was detected, and this result was similar in both laboratory and experimental investigations [80]. In Ghana, Antwi-Agyei et al. reported that irrigation with partially treated and untreated wastewater is a key risk factor for the observed contamination of 80% of produce samples, with a median concentration ranging from 0.64 to 3.84 log *E. coli*/g produce, while ready-to-eat salad was the most contaminated with 4.23 log *E. coli*/g [81].

## 9. Conclusions

This review provides a reference for an enhanced understanding of the global risk of fresh vegetables and fruit in the transmission of multidrug resistant Gram-negative bacteria and emphasizes the necessity of paying close attention to these products as a future public health issue. Given that fresh produce is often consumed raw, this allows the transfer of these antibiotic resistance genes to human gut bacteria. It is now even more important that more investigations should be performed in order to survey the emergence and transmission of these genes to humans from farm to fork. In addition, suitable measures, including the improvement of water quality and agricultural practices, need to be considered to ensure consumer safety worldwide.

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