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1 **Extended-spectrum β -lactamase-producing-*Enterobacteriaceae* in vegetables imported from**
2 **the Dominican Republic, India, Thailand and Vietnam.**

3

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15 Running Head: ESBL-producing *Enterobacteriaceae* in imported vegetables

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23

24 **Abstract**

25 To examine to what extent fresh vegetables imported to Switzerland represent carriers of ESBL-
26 producing *Enterobacteriaceae*, 169 samples of different types of fresh vegetables imported to
27 Switzerland from the Dominican Republic, India, Thailand and Vietnam were analyzed. Overall,
28 25.4% of the vegetable samples yielded one or more ESBL-producing *Enterobacteriaceae*, of
29 which 78.3% were multidrug resistant. Sixty isolates were obtained: 26 *Escherichia coli*, 26
30 *Klebsiella pneumoniae*, 6 *Enterobacter cloacae*, 1 *Enterobacter aerogenes* and 1 *Cronobacter*
31 *sakazakii*.

32 Twenty-nine CTX-M-15, 8 CTX-M-14, 7 CTX-M-55, 3 CTX-M-65, one each of CTX-M-1, CTX-
33 M-3 and CTX-M-63, 5 SHV-2, 3 SHV-12 and one SHV-2a were found.

34 Four of the *E. coli* isolates belonged to epidemiologically important clones: CTX-M-15-producing
35 B2:ST131 (1 isolate), D:ST405 (1 isolate), and D:ST38 (2 isolates). One of the D:ST38 isolates
36 belonged to the extraintestinal enteroaggregative *E. coli* (EAEC) D:ST38 lineage. Two of the *K.*
37 *pneumoniae* isolates belonged to the epidemic clones ST15 and ST147.

38 Occurrence of antibiotic-resistant pathogenic and commensal *Enterobacteriaceae* in imported
39 agricultural foodstuffs constitutes a source of ESBL genes and a concern for food safety.

40

41 **Introduction**

42

43 The production of extended spectrum β -lactamases (ESBLs) is one of the most important
44 mechanisms of antibacterial resistance in *Enterobacteriaceae*. Most ESBLs can be divided into 4
45 groups: TEM, SHV, OXA, and CTX- M types (1). Currently, CTX-Ms are the most prevalent type
46 of ESBLs described (2, 3). The last decade has seen a rapid and massive global spread driven
47 primarily by their carriage on resistance plasmids and by the spread of extraintestinal pathogenic *E.*
48 *coli* clones (4, 5). Important clonal lineages include *E. coli* strains belonging to multilocus sequence
49 types (MLST) ST131 (often associated with CTX-M-15), and enteroaggregative *E. coli* (EAEC)

50 ST38 (6). In addition to these widespread ESBLs, less frequently occurring ESBLs have been
51 detected on regional scales, e.g. GES, PER, VEB types (7).

52 In recent years it has become widely recognized that the dissemination of ESBL-producing bacteria
53 is an issue that is no longer restricted to the medical health care system, but represents a growing
54 problem involving food safety and environmental integrity. There is increasing evidence that
55 antimicrobial drug use in the livestock sector plays an important role in the contamination of food
56 with ESBL-producing bacteria (8, 9), but little is still known about the burden of ESBL-producing
57 *Enterobacteriaceae* on fresh vegetables. In the crop production sector, products can be
58 contaminated through application of manure (animal origin) or sewage sludge (human origin) to the
59 soil, or through application of treated or untreated wastewater that is used for irrigation of crops
60 (10).

61 In Switzerland as in most industrialized countries, pre-harvest intervals restrict the application of
62 manure to the soil, and wastewater is treated before reuse, with high ecological standards and levels
63 of hygiene applied to all stages of culture and harvesting (11). Hence, the bacteriological burden of
64 vegetable crops is low. By contrast, in many developing countries, most prominently Vietnam,
65 China and India, wastewater without or with insufficient treatment is commonly used for
66 agriculture, implicating negative effects on human health and the environment (12, 13).

67 Analyses of alimentary consumption trends in Switzerland record an increase in Asian and Latin
68 American cuisine and point towards a demand for fresh produce (14). Import trade statistics show
69 that imports to Switzerland of edible vegetables from India have doubled over the last decade, and
70 quadrupled from Socialist Republic of Vietnam. Over the last 4 years, Switzerland imported an
71 average of 701.25 metric tons per annum of edible vegetables from the Dominican Republic, India,
72 Thailand and Vietnam. (Swiss Federal Customs Administration FCA; <https://www.swiss-impex.admin.ch/>).

73
74 The aim of this study was to evaluate the presence of ESBL-producing *Enterobacteriaceae* in
75 vegetables imported from these countries and to characterize isolated strains by (i) antibiotic

76 susceptibility testing, (ii) identification of the *bla* genes, (iii) multi locus sequence typing (MLST)
77 of the *E. coli* and *K. pneumoniae* isolates, and (iv) identifying phylogenetic groups of *E. coli*
78 isolates.

79

80 **Material and Methods**

81

82 **Bacterial sampling**

83

84 In July and August 2014, 68 samples of raw vegetables imported via the national airport of Zürich
85 were collected by the food control authority of the Canton Aarau, Switzerland. Vegetables consisted
86 of cucumbers, beans, breadfruit, celery leaves, cha-om (climbing wattle, acacia), chilies, curry
87 leaves, dill, egg plants, garlic chives, lemongrass, onions, peppermint leaves, Pak-Choy (Chinese
88 cabbage), Ponnangani (Asiatic pennywort), several types of squash, water mimosa and water
89 spinach. Countries of origin were the Dominican Republic (49 samples), India (3 samples) and
90 Thailand (16 samples).

91 In addition, 101 different fresh vegetable types were purchased in the city of Zürich from 7 retail
92 shops specializing in Asian and South American food, and from 3 supermarket chains. The
93 vegetables included basil leaves, beans, celery, Ceylon spinach, chilies, coriander, cucumbers, curry
94 leaves, eggplant, lemon grass, moringa pods (fruits of the horseradish tree), okra (marrow), onions,
95 shallots, dill, soy sprouts and several types of squash.

96 Samples had been imported from the Dominican Republic (1 sample), India (36 samples), Thailand
97 (44 samples) and the Socialist Republic of Vietnam (20 samples).

98 In total, 169 vegetable samples were collected for analysis: 50 from the Dominican Republic, 39
99 from India, 60 from Thailand and 20 from Vietnam.

100

101 **Microbiological analysis**

102

103 Of each unwashed vegetable sample, 15-20 g were placed in a sterile Stomacher[®] bag. Samples
104 were homogenized using a Stomacher[®] sample blender and incubated at ratio 1:10 in
105 *Enterobacteriaceae* Enrichment (EE) broth (BD, Franklin Lakes, USA) at 37°C over night. For the
106 detection of ESBL-producers, chromogenic Brilliance ESBL agar plates (Oxoid, Hampshire, UK)
107 were inoculated with one loopful of each of the enrichment cultures. Plates were incubated at 37°C
108 for 24 h under aerobic conditions. Colonies with different chromaticity and morphology were
109 picked from the selective plates and subcultured on sheep blood agar (DifcoTM Columbia Blood
110 Agar Base EH, Becton Dickinson AG, Allschwil, Switzerland; 5 % sheep blood SB055, Oxoid AG,
111 Pratteln, Switzerland) at 37°C for 24 h. Identification of isolates was either outsourced and achieved
112 by protein profiling using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF-
113 MS) (Mabritec SA, Riehen, Switzerland), for the samples collected from import border control, or
114 obtained using API ID 32 E (bioMérieux, Marcy l'Etoile, France), for the samples collected from
115 retail stores. In cases of doubtful results, identification was verified by *rpoB* sequence analysis (15).
116 The identity of *Cronobacter sakazakii* was confirmed by *rpoB* based PCR as described previously
117 (16). To investigate putative enteroaggregative properties of *E. coli* ST38, isolates were tested by
118 PCR for the presence of the EAEC transport regulator gene (*aggR*), using primers and conditions
119 described previously (17).

120

121 **ESBL confirmation and antimicrobial susceptibility testing**

122

123 ESBL production was confirmed using Etest-ESBL strips containing cefotaxime, ceftazidime, and
124 cefepime alone and in combination with clavulanic acid (bioMérieux, Marcy l'Etoile, France)
125 according to the manufacturer's instructions. Additionally, the presence of β -lactamase was verified
126 with the colorimetric β LACTATM test kit (BioRad, Cressier, Switzerland), as described previously
127 (18), and according to the manufacturer's instructions.

128 Isolates were subjected to susceptibility testing against 13 antimicrobial agents by the disc diffusion
129 method according to CLSI protocols and evaluated according to CLSI criteria (19). The panel
130 included ampicillin (AM), amoxicillin-clavulanic acid (AMC), cephalothin (CF), cefotaxime
131 (CTX), nalidixic acid (NA), ciprofloxacin (CIP), gentamicin (GM), kanamycin (K), streptomycin
132 (S), sulfamethoxazole (SMZ), trimethoprim (TMP) tetracycline (TE), and chloramphenicol (C)
133 (Becton, Dickinson, Heidelberg, Germany).

134 Strains exhibiting resistance to three or more classes of antibiotics were defined as multidrug
135 resistant (MDR).

136

137 **Molecular biological analysis of β -lactamase genes**

138

139 Isolates identified as potential ESBL-producers were further analyzed by PCR. DNA was extracted
140 by a standard heat lysis protocol and analyzed by PCR for the presence of *bla* genes. Synthesis of
141 primers and DNA custom sequencing was carried out by Microsynth (Balgach, Switzerland).

142 Purification of amplicons was performed using a PCR purification kit (Qiagen Courtaboeuf,
143 France). Screening for *bla*_{TEM} and *bla*_{SHV} was carried out using primers described previously (20),
144 and resulting amplicons were custom sequenced. Screening for *bla*_{CTX-M} alleles belonging to CTX-
145 M groups 1, 2, 8, 9, and 25 was done as described by Woodford *et al.* (21). Amplicons for
146 sequencing individual open reading frames of belonging to groups 1, 2 and 9 were generated using
147 primers described previously (8). Group 8 *bla*_{CTX-M} genes were amplified using the newly designed
148 primers gr. 8 CTX-M-fw: 5'-ATG AGA CAT CGC GTT AAG CGG ATG-3', and gr. 8 CTX-M-
149 rev: CAC GAC GAC TTT CTG CCT TCT GC-3'.

150 The *Cronobacter sakazakii* isolate was additionally tested by PCR for the presence of *bla*_{VEB}. (22).

151 Nucleotide sequences were analyzed with CLC Main Workbench 7.0.2. Database searches were
152 performed using the BLASTN program of NCBI (<http://www.ncbi.nlm.nih.gov/blast/>).

153

154 **Phylogenetic classification of *E. coli* isolates**

155 DNA from *E. coli* isolates were subjected to triplex PCR targeting the *chuA* gene, the *yjaA* gene and
156 an unspecified DNA fragment termed TspE4.C2, as described previously (23). Each isolate was
157 assigned to one of the four phylogenetic groups designated A, B1, B2 or D. Group A and B1
158 typically contain commensal *E. coli* strains while groups B2 and D consist of virulent extra-
159 intestinal strains (24).

160

161 **Multilocus sequence typing of *Escherichia coli* and *Klebsiella pneumoniae***

162 Multilocus sequence types of *E. coli* isolates were determined as described by Wirth *et al.* (25).
163 Sequences were imported into the *E. coli* MLST database website ([http:](http://mlst.ucc.ie/mlst/dbs/Ecoli)
164 [//mlst.ucc.ie/mlst/dbs/Ecoli](http://mlst.ucc.ie/mlst/dbs/Ecoli)) to determine MLST types.

165 MLST of the *K. pneumoniae* isolates was performed according to previously described methods
166 (26). Sequence types were determined according to the MLST database
167 (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>).

168 Alleles and STs that had not been previously described were submitted to the curators of the
169 databases and were assigned new designations.

170

171 **Results**

172

173 **Prevalence of ESBL-producing *Enterobacteriaceae* in imported vegetables**

174 Overall, 43 (25.4%) of the 169 vegetable samples yielded ESBL-producing *Enterobacteriaceae*.
175 They included 11 (22%) of the 50 samples collected from the Dominican Republic, 13 (33.3%) of
176 the 39 samples from India, 11 (18.3%) of the 60 samples from Thailand and 8 (40%) of the 20
177 samples from Vietnam. ESBL-producers were detected in 25% of the samples collected at the
178 airport and in 25.7% of the retail store samples. Of the 43 contaminated vegetables, 14 (32.6%)

179 contained multiple isolates (two or more distinct ESBL-producers). The type of contaminated
180 vegetables, their origin and the number of isolates per sample is shown in Figure 1.

181 In total, 60 ESBL producers were retrieved. Thereof, 26 (43.3%) were identified as *E. coli*, 26
182 (43.3%) classified as *Klebsiella pneumoniae* subsp. *pneumoniae*, six (10%) were *Enterobacter*
183 *cloacae*, one (1.7%) was *Enterobacter aerogenes* and one (1.7%) was *Cronobacter sakazakii*
184 (Figure 1).

185

186 **ESBL genes**

187 All 60 isolates were characterized with respect to their ESBL genotype. Overall, *bla*_{CTX-M} genes
188 were detected in 51 (85%) strains. Thirty-eight of the *bla*_{CTX-M} genes belonged to CTX-M group 1
189 (74.5% of the *bla*_{CTX-M} genes), 12 (23.5%) belonged to CTX-M group 9. One representative of
190 CTX-M group 8 was detected (2%). No genes from CTX-M group 2 were found.

191 Nine strains were identified as SHV-type ESBL producers. Five (55.6%) were SHV-2, three
192 (33.3%) were SHV-12. One SHV-2a (11.1%)-producer was detected.

193 Overall, of the 26 *E. coli* isolates, 17 (65.8%) *E. coli* strains produced CTX-M group 1 ESBLs and
194 8 (30.8%) produced CTX-M group 9 ESBLs: Ten (38.5%) harbored *bla*_{CTX-M-15}. Six (23%) carried
195 *bla*_{CTX-M-55}, five (19.2%) *bla*_{CTX-M-14}, and three (11.5%) *bla*_{CTX-M-65}. One isolate (3.8%) tested
196 positive for *bla*_{CTX-M-1}. One *E. coli* (3.8%) harbored SHV-12.

197 Of the 26 *K. pneumoniae* isolates, 14 (53.8%) *K. pneumoniae* strains produced CTX-M group 1
198 ESBLs, five (19.2%) CTX-M group 9 ESBLs. One isolate (3.8%) produced a CTX-M group 8
199 ESBL: Thirteen (50%) harbored *bla*_{CTX-M-15}. Three (11.5%) carried *bla*_{CTX-M-14}. One isolate (3.8%)
200 harbored *bla*_{CTX-M-3}, one *bla*_{CTX-M-27} and one further *bla*_{CTX-M-63}.

201 Five *K. pneumoniae* (19.2%) harbored SHV-2, one (3.8%) carried SHV-2a and one further SHV-
202 12. As incidental findings it was noted that non-ESBL- genes *bla*_{SHV-26}, *bla*_{SHV-36}, *bla*_{LEN}-like and
203 *bla*_{OKP-5a}-like genes were present in several *K. pneumoniae* isolates (data not shown).

204 The *E. cloacae* isolates harbored the CTX-M group 1 gene *bla*_{CTX-M-15} in five cases (83.3%). One
205 isolate carried *bla*_{CTX-M-55}.
206 The *E. aerogenes* isolate harbored *bla*_{CTX-M-15}, and the *C. sakazakii* carried *bla*_{SHV-2}.
207 Regarding the geographical distribution of the ESBLs, CTX-M-group 1 enzymes were detected in 7
208 of 12 isolates (58.3%) from the Dominican Republic, and in 19 of 22 isolates (86.3%) from India.
209 In both countries, CTX-M-15 was the predominant enzyme. By contrast, CTX-M group 9 enzymes
210 were detected more frequently from isolates from Thailand and from Vietnam (5 of 17 isolates
211 (29.4%), and 4 of 9 isolates (44.4%) respectively.
212 Notably, none of the isolates originating from India contained any SHV-ESBLs.

213

214 **Antimicrobial susceptibility patterns**

215 Disc diffusion tests showed that all 60 isolates were resistant to ampicillin and to the narrow-
216 spectrum cephalosporin cephalothin. Resistance to cefotaxime was noted for 53 (88.3%) of the
217 isolates.

218 Disc diffusion tests performed for other categories of antibiotics revealed that 19 (31.7%) isolates
219 were resistant to the quinolone antibiotic nalidixic acid, and 18 (30%) were resistant to the
220 fluoroquinolone ciprofloxacin. Resistance to aminoglycosides was detected in 20 (33.3%) isolates
221 resistant to gentamicin, 12 (20%) to resistant kanamycin and 31(51.7%) resistant to streptomycin.
222 Resistance to folate pathway inhibitors sulfamethoxazole and trimethoprim was noted in 44 (73.3%)
223 isolates and 45 (75%) isolates, respectively. Tetracycline resistance was found in 39 (65%), and
224 chloramphenicol resistance in 28 (46.7%) isolates, respectively.

225 Multidrug resistance was detected in 47 (78.3%) of the isolates: Eleven isolates (91.6%) from the
226 Dominican Republic, thirteen (59%) of the 22 isolates from India, sixteen (94%) of the 17 isolates
227 originating from Thailand, and 7 (77.8%) of the 9 strains from Vietnam.

228

229 **Epidemiological characteristics of *E. coli* and *K. pneumoniae* isolates**

230 **Phylogenetic groups and MLST of *E. coli***

231 Phylogenetic typing allocated 21 (80.8%) of the *E. coli* isolates to groups A or B1 that typically
232 contain commensal *E. coli*. Five isolates (19.2%) belonged to extraintestinal pathogenic
233 phylogroups B2 and D (one and four isolates, respectively).

234 Multilocus sequence typing of the 26 *E. coli* isolates identified 22 different sequence types (see
235 Figure 1). Four new allelic combinations (isolates E37SK2.1, 37SK1, ESBL H241 B and ESBL
236 H239 V). Two isolates contained new allelic variants of the *fumC* and *recA* genes: Isolate 54SK2
237 with ST4684 (*fumC*: 604) and isolate 2SK1 ST4683 (*recA*: 326).

238 Among the pathogenic groups B2 and D, four isolates belonged to the epidemiologically important
239 sequence types ST131, ST405 and ST38: Isolate ESBL DR06 was assigned to the internationally
240 disseminated CTX-M-15-producing B2:ST131 clone. Isolate ESBL DR45 belonged to D:ST405,
241 which belongs to the clonal complex CC405. Two isolates, ESBL DR26 and E3SK2, were
242 identified as D:ST38 which belongs to the clonal complex CC38. Since this clone may include
243 enteroaggregative *E. coli* strains, these two isolates were tested by PCR for the EAEC-specific
244 marker gene *aggR*. The results revealed that one isolate (E3SK2) belonged to the extraintestinal
245 enteroaggregative *E. coli* (EAEC) D:ST38 lineage. One further isolate (ESBL H226 L) was
246 classified as D:ST393.

247

248 **MLST of *K. pneumoniae***

249 Multilocus sequence typing revealed high diversity among the 26 *K. pneumoniae* isolates. Five
250 isolates exhibited new sequence types (isolates ESBL H238T, E48T, 19SK1, ESBL DR47T and
251 ESBL H239T). Two isolates, 45SK1 and ESBL DR27, belonged to epidemic clones ST15 and
252 ST147, respectively. For isolate ESBL H226 T the ST could not be determined, because the *mdh*
253 gene could not be amplified.

254

255 **Discussion**

256

257 Recent studies indicate that fresh vegetables constitute a source of ESBL-producers and represent a
258 possible route for the dissemination of resistance genes via the consumer in the community (27-29).

259 Vegetable crops originating from most European and North American countries are farmed
260 according to regulations for applying manure/slurry to protect vegetables from contamination with
261 pathogenic microorganisms, in accordance to the recommendations of the World Health
262 Organization (WHO) (30). Consequently, carriers of *bla*_{ESBL} and multidrug resistance genes
263 associated with vegetables have been described as predominantly saprophytic and opportunistic
264 bacteria which are thought to constitute a background reservoir of antibiotic resistance genes (31),
265 and not *per se* a threat to human health.

266 In this study, we examine the presence of ESBL-producing *Enterobacteriaceae* in fresh vegetables
267 imported to Switzerland from countries with very different farming standards and where the food
268 production industry is to a certain extent underdeveloped.

269 The high rate of contamination (average of 25.4%) of the samples with ESBL-producers and the
270 very high rate of 78.3% MDR *Enterobacteriaceae* detected in this study give rise to concern. These
271 results contrast strongly with results from similar studies that reported lower prevalences (6% -
272 12%), of ESBL-producers in raw vegetables (27, 28, 32).

273 We found national variations among the CTX-M types identified in the samples. The predominance
274 of *bla*_{CTX-M-15} genes in isolates from India is in accordance with previous studies involving clinical
275 isolates originating from Delhi and South India, and the frequency of group 9 CTX-M types in
276 isolates from Thailand and Vietnam is reflective of reports from China and the Far East (3, 33). In
277 the isolates from Thailand analyzed in this study, CTX-M-55 outnumbered CTX-M-14. Originally
278 detected in clinical isolates of *E. coli* and *K. pneumoniae* from Thailand in 2005 (34), this particular
279 ESBL type has been found widely in food-producing animals and humans in China and appears to
280 be displacing CTX-M-14 as the most common CTX-M-variant (35). Our data indicate that this
281 epidemiological characteristic may hold true for Thailand and also for Vietnam. By comparison, the

282 CTX-M type distribution of ESBL producers isolated from healthy humans in Switzerland is
283 predominated by CTX-M-15 and CTX-M-1 (36).

284 The predominance of phylogenetic groups A and B1 among the *E. coli* isolates and the wide
285 diversity of MLST among the *E. coli* and *K. pneumoniae* isolates indicate that *bla*_{ESBL} and MDR
286 genes are well established in commensal strains. It is already recognized that commensal bacteria
287 constitute an important reservoir of antibiotic resistance genes in food animals (37). Our results
288 suggest that vegetables of the types and origins analyzed in this study represent another potent and
289 hitherto underappreciated source of antibiotic resistance genes.

290 The occurrence of pathogenic bacteria in food is a threat to public health. In this study, we found
291 a CTX-M-15-producing isolate belonging to the highly virulent pandemic *E. coli* strain B2:ST131,
292 which is associated with severe infections in humans (38). Furthermore, one *E. coli* D:ST405, two
293 *E. coli* D:ST393 and two *E. coli* D:ST38 were found in this study. These strains also belong to
294 lineages that cause extra-intestinal diseases, mainly urinary tract infections, in humans and
295 contribute to the global dissemination of ESBLs and MDR genes (6, 39). The detection of
296 enteroaggregative properties in one of the *E. coli* D:ST38 strains is of particular concern. EAEC are
297 associated with acute or persistent diarrhea in outbreak and non-outbreak settings worldwide. Its
298 association with CTX-M-14 has been described recently in Europe (40, 41) as well as in Asia (42)
299 and its detection in vegetables destined for human consumption raises questions concerning food
300 safety.

301 *Cronobacter sakazakii* is an opportunistic food-borne pathogen that can cause fatal necrotizing
302 enterocolitis, bacteremia and meningitis in infants and immuno-compromized adults (43) and its
303 detection, to the best of our knowledge for the first time, as an SHV-12-producer in a vegetable
304 sample from Thailand merits attention. Previously, a clinical isolate of *C. sakazakii* harboring
305 *bla*_{VEB-1}, a *bla*_{ESBL} found increasingly in Thailand, was reported (22). However, the isolate in this
306 study tested negative for this particular gene.

307 Among the *Klebsiella pneumoniae* isolates detected in this study, two (45SK1 and ESBL DR27)
308 belonged to epidemic clones associated with nosocomial infections in humans (44, 45), giving rise
309 to further concern for consumer health.

310 In conclusion, the results of this study suggest that the international production and trade of fresh
311 vegetables constitute a possible route for the spread of ESBLs and pathogenic *Enterobacteriaceae*.
312 Appropriate measures such as the improvement of agricultural practices and water quality need to
313 be taken and globally mandatory guidelines should be established in order to ensure consumer and
314 public health worldwide.

315

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321

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323

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458 **Figure 1:** Source data, identity, distribution of sequence types, clonal complexes, *bla* genes and
459 antibiotic susceptibility patterns of ESBL-producing Enterobacteriaceae isolated from fresh
460 vegetables imported from the Dominican Republic, India, Thailand and Vietnam. Colors of squares
461 categorizing ESBLs: Light orange, CTX-M-group 1; dark orange, CTX-M-group 9; red, CTX-M-
462 group 8; blue, SHV-enzymes. Colors of squares categorizing antibiotic resistance profiles: Pink,
463 resistant; yellow, intermediate; green, susceptible. Abbreviations: MLST, multilocus sequence type;
464 CC, clonal complex; AM, ampicillin; AMC, amoxicillin-clavulanic acid; CF, cephalothin; CTX,
465 cefotaxime; CIP, ciprofloxacin; GM, gentamicin; TE, tetracycline; S, streptomycin; C,
466 chloramphenicol; K, kanamycin; NA, nalidixic acid; SMZ, sulfamethoxazole; TMP, trimethoprim;
467 nd, not determined.

